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ORIGINAL ARTICLE

Identity of *Gymnophthalmus* (Squamata: Gymnophthalmidae) from northeastern Amazonia with evidence for *G. underwoodi* as invasive in Belém

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ABSTRACT

We report the occurrence of the lizard *Gymnophthalmus underwoodi* in the municipality of Belém, state of Pará, Brazil. This is the first record of that species south of the Amazonas River, probably because of an accidental introduction by ships that dock in Belém, the same pathway suggested for the recent introduction of another species of exotic lizard recently recorded in the city. We also determined the identity of some specimens of *Gymnophthalmus* from the states of Amapá and Pará through external morphology and molecular data, confirming that, until now, *G. vanzoi* is the only *Gymnophthalmus* occurring in the savanna enclaves of those states. Finally, we provide a new distribution map for the species of *Gymnophthalmus*, including the new occurrence record for *G. underwoodi* for the state of Pará, where it can be considered as an invasive species.

KEYWORDS: exotic fauna, lizards, South America, DNA, morphology

Identidade dos *Gymnophthalmus* (Squamata: Gymnophthalmidae) do nordeste da Amazônia, com evidência para *G. underwoodi* como invasora em Belém

RESUMO

Nós relatamos a ocorrência do lagarto *Gymnophthalmus underwoodi* no município de Belém, estado do Pará, Brasil. Este é o primeiro registro ao sul do Rio Amazonas, provavelmente como resultado de uma introdução acidental por navios que atracam em Belém, o mesmo caminho sugerido para a introdução de outra espécie exótica de lagarto recentemente encontrada na cidade. Nós também determinamos a identidade de alguns espécimes de *Gymnophthalmus* dos estados do Amapá e Pará, através de dados morfológicos externos e moleculares, confirmando que, até o momento, *G. vanzoi* é o único *Gymnophthalmus* ocorrendo nos enclaves de savana desses estados. Por fim, nós fornecemos um novo mapa de distribuição para as espécies de *Gymnophthalmus,* incluindo o novo registro de ocorrência de *G. underwoodi* para o estado do Pará, onde ela pode ser considerada como uma espécie invasiva.

PALAVRAS-CHAVE: fauna exótica, lagartos, América do Sul, ADN, morfologia

INTRODUCTION

Gymnophthalmus Merren, 1820 includes eight species of diminutive lizards that inhabit areas of open vegetation and the litter of forests in Central America, the Antilles, and northern South America, mainly north of the Amazonas River (Avila-Pires 1995; Ribeiro-Júnior and Amaral 2017; Recoder *et al.* 2018). In Brazil, there are three species known *sensu* Recoder *et al.* (2018), namely: *Gymnophthalmus leucomystax* Vanzolini and Carvalho, 1991, found in the northeastern part of the state of Roraima; *Gymnophthalmus underwoodi* Grant, 1958, present in the states of Amazonas (north of the Amazonas River) and Roraima; and *Gymnophthalmus vanzoi* Carvalho, 1999, found in the states of Amapá, Pará and Roraima.

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Two species have been taxonomically confusing herpetologists collecting in northeastern Amazonia (Recoder *et al.* 2018). The first, *G. underwoodi*, is a parthenogenetic species (Hardy *et al.* 1989; Cole *et al.* 1990) possibly originating from the hybridization between a maternal lineage closely related to *G. cryptus* and a paternal lineage of *G. speciosus* (Kizirian and Cole 1999). The second species, *G. vanzoi*, is a sexual species with disjunct populations throughout Amazonia that show some color variation, but with extremely low genetic divergence (Recoder *et al.* 2018).

Despite the extensive taxonomic review on *Gymnophthalmus* by Recoder *et al.* (2018), minor questions remain regarding the status of some specimens from geographically isolated areas of savanna in the states of Amapá and Pará (Brazil) that were mentioned with inaccurate identification by Avila-Pires *et al.* (2010) and Ribeiro-Júnior and Amaral (2017). There are also some surprising records of specimens of *Gymnophthalmus* recently collected in different points of the municipality of Belém, state of Pará, where no species of the genus was known to occur. Using molecular and morphological data, we evaluated the taxonomic status of specimens recently collected in northeastern Amazonia, including specimens from localities not reviewed by Recoder *et al.* (2018) and in Belém south of the Amazonas River.

MATERIAL AND METHODS

We analyzed eight specimens of *Gymnophthalmus* from the states of Amapá and Pará, Brazil, housed in the herpetological collection (Coleção Herpetológica Osvaldo Rodrigues da Cunha) of Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil. Three specimens are from enclaves of savanna located within the distribution of *G. vanzoi* reported by Recoder *et al.* (2018), but they were not analyzed morphologically nor molecularly by the latter authors. One of these three specimens (MPEG 27592), from Óbidos, Pará, was referred as *Gymnophthalmus* cf. *underwoodi* by Avila-Pires *et al.* (2010) and as an undescribed *Gymnophthalmus* by Ribeiro-Júnior and Amaral (2017). The other two (MPEG 29841 and 29844) from Mazagão, Amapá, were catalogued as *Gymnophthalmus* sp.

Five recently collected specimens are from four localities in the municipality of Belém, Pará, Brazil (Table 1; Figure 1), in two districts bordering the Guajará River, which is formed by the confluence of the Acará and Guamá Rivers. Four specimens were found in the district of Icoaraci. The first (MPEG 33550) was collected on October 2, 2012, inside a residence, 100 m from the river (1°18'21"S, 48°29'19"W), close to several small ports which receive passanger and product transport vessels from Marajó Island and other nearby regions. On August 27, 2019, two more specimens (MPEG 33157 and MPEG 33158) (Figure 2) were collected at a second locality (1°18'44"S; 48°27'53"W), 2.8 km further inland. The fourth specimen (MPEG 33546) was collected on August 16, 2020, at a third locality 2.9 km from the first and 2.7 km from the second locality (1°19'49.2"S; 48°28'37.4"W). Finally, a single specimen (MPEG 33549) was collected in the district of Mosqueiro Island (1°4'3.1"S, 48°21'15.8"W) on November 5, 2020, 29 km from the closest locality in Icoaraci and 700 m from the river margin. The latter four specimens were found among underbrush vegetation in backyards of houses.

We examined nine morphological characters to assess the variation in the studied specimens and confirm their identity (Table 1) by comparison with the parameter ranges presented

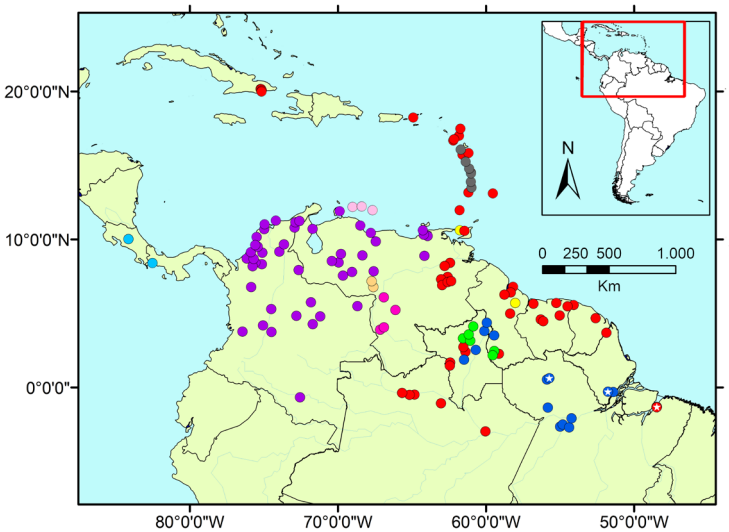

Figure 1. Geographic distribution of *Gymnophthalmus*: *G. speciosus* (light blue); *G.* cf. *speciosus* (purple); *G. marconaterai* (orange); *G. lineatus* (light pink); *G. cryptus* (dark pink); *G. underwoodi* (red); *G. pleei* (dark gray); *G. leucomystax* (green); *G. vanzoi* (blue); *G.* aff. *vanzoi* (yellow). Stars within the occurrence symbol indicate specimens sequenced in this study. Localities are mostly based on the map of Recoder *et al.* (2018) plus records of *G. underwoodi* for Cuba (Alfonso *et al.* 2012; Alfonso and Hernandez, 2017). This figure is in color in the digital version.

Table 1. Qualitative, morphometric (mm) and meristic data for specimens of *Gymnophthalmus underwoodi* and *G. vanzoi* from the states of Amapá and Pará analyzed in this study. See Material and Methods for acronym definition. na = data not available due to tail broken by autotomy.

Species	Locality	Voucher	Sex	SVL	DOR	VEN	GUL	SAM	LFT	LFF	SCA	SPL	Pores
Gymnophthalmus vanzoi	Óbidos, Pará	MPEG 27592	female	40	32	27	9	13	16	12	41	14	absent
Gymnophthalmus vanzoi	Mazagão, Amapá	MPEG 29841	male	35	32	24	9	13	14	11	na	14	present
Gymnophthalmus vanzoi	Mazagão, Amapá	MPEG 29844	male	35	31	23	9	13	14	11	na	14	present
Gymnophthalmus underwoodi	Icoaraci, Belém, Pará	MPEG 33157	female	37.5	31	24	9	13	18	13	na	14	absent
Gymnophthalmus underwoodi	Icoaraci, Belém, Pará	MPEG 33158	female	44	32	24	9	13	17	14	na	14	absent
Gymnophthalmus underwoodi	Icoaraci, Belém, Pará	MPEG 33546	female	20	32	24	10	13	15	13	27	14	absent
Gymnophthalmus underwoodi	Mosqueiro, Belém, Pará	MPEG 33549	female	19	32	22	9	13	15	14	24	13	absent
Gymnophthalmus underwoodi	Icoaraci, Belém, Pará	MPEG 33550	female	18.3	31	22	9	13	15	14	na	14	absent

in the Table 4 of Recoder *et al.* (2018). The acronyms of one morphometric and eight meristic data follow Recoder *et al.* (2018): snout-vent length (SVL), number of dorsal transversal rows (DOR); number of ventral transversal rows (VEN); gular transversal rows (GUL); scales around midbody (SAM); lamellae under the fourth toe (LFT); lamellae under the fourth finger (LFF); number of smooth subcaudal scales (SCA); and total number of supralabials on both sides (SPL). The sex of the specimens was identified by the presence (males) or absence (females) of femoral pores.

We extracted DNA from muscle samples preserved in 100% ethanol of four specimens (Table 2) using the Wizard® Genomics DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturer's recommended protocol for animal tissue (mouse tail) and quantified the extract in Qubit. We amplified the products with PCR Master Mix, 2X (Promega) with 10 µL reactions for two mitochondrial markers following a standard 4 min initial denaturation at 94 °C and a final extension of 6 min at 72 °C, with primers by Benavides *et al.* (2007) for 12S [95°(30") / 50°(60") / 72°(60") [35x]] and by Geurgas *et al.* (2008) and Whiting *et al.* (2003) for 16S [95°(30") / 45°(30") / 72°(60") [35x]]. We purified the PCR products using ExoSAP-IT, following the 5-min recommended protocol. We sequenced purified reactions using the specific primers and BigDye 3.1 cycle sequencing chemistry (Applied Biosystems) following the manufacturer's recommended protocol and cycling conditions on an Applied Biosystems 3100 automated capillary sequencer, producing two strands (forward and reverse). All procedures were conducted in the Molecular Biology Laboratory of MPEG. All sequences were deposited in Genbank. We also included in our analysis the sequences of other 29 specimens of seven species of *Gymnophthalmus* and one sequence of a *Micrablepharus maximiliani* used as outgroup (Table 2). All sequences were obtained from GenBank.

We edited the sequences using BioEdit (Hall 1999) and the alignments were made using MAFFT version 7 (Kuraku *et al.* 2013; Katoh *et al.* 2019). We choose the strategy Q-INS-I for both ribosomal 12S and 16S because this method considers the secondary structure of RNA (Katoh *et al.* 2005; Katoh and Toh 2008). Alignments were concatenated in Sequence Matrix version 1.7.8 (Vaidya *et al.* 2011), and the best scheme of partitioning and substitution models was chosen using PartitionFinder version 2 (Lanfear *et al.* 2017) in version 3.3 of the CIPRES web portal (Miller *et al.* 2010). For the phylogenetic positioning of our samples, we ran a maximum likelihood analysis in RAxML version 8 (Stamatakis 2014) using version 3.3 of the CIPRES web portal (Miller *et al.* 2010). The bootstrap analysis was implemented with 1000 pseudoreplicates. Finally, the bipartition support was drawn on the best likelihood tree.

Figure 2. Specimens of *Gymnophthalmus underwoodi* from Belém, Pará, Brazil [MPEG 33157 (top) and MPEG 33158 (bottom)]. This figure is in color in the digital version.

RESULTS

The phylogenetic analysis indicated that all specimens we analyzed from north of the Amazonas River (MPEG 27592, 29841 and 29844) are *Gymnophthalmus vanzoi* (Figure 3), while the specimen from Belém (MPEG 33517) was recovered as *Gymnophthalmus underwoodi* (Figure 3). In the morphological analysis, we found that in eight of the nine analyzed characters all northern specimens were very similar to the specimens from Belém (MPEG 33157, 33158, 33546, 33549, and 33550) (Table 1). *Gymnophthalmus underwoodi*

Table 2. List of specimens sampled for molecular analysis in this study. Codes in the columns 12S and 16S are GenBank accession numbers. Taxa in bold were sequenced exclusively for this study. na = data not available.

Species	Voucher number	Locality	Country	12S	16S
Gymnophthalmus vanzoi	**MPEG 27592**	**Óbidos, Pará**	**Brazil**	**MZ544008**	**na**
Gymnophthalmus vanzoi	**MPEG 29841**	**Mazagão, Amapá**	**Brazil**	**MZ544009**	**MZ544020**
Gymnophthalmus vanzoi	**MPEG 29844**	**Mazagão, Amapá**	**Brazil**	**MZ544010**	**MZ544021**
Gymnophthalmus vanzoi	MTR 33447	Santarém, Pará	Brazil	MH732644	MH732695
Gymnophthalmus vanzoi	APSJ 235	Santarém, Pará	Brazil	MH732646	MH732698
Gymnophthalmus vanzoi	APSJ 73	Santarém, Pará	Brazil	MH732647	MH732697
Gymnophthalmus vanzoi	UFOPA 0373	Santarém, Pará	Brazil	MH732645	MH732696
Gymnophthalmus vanzoi	MTR 946484	Boa Vista, Roraima	Brazil	MH732648	MH732699
Gymnophthalmus vanzoi	MTR 946487	Boa Vista, Roraima	Brazil	MH732649	MH732700
Gymnophthalmus vanzoi	MTR 946490	Boa Vista, Roraima	Brazil	MH732650	MH732701
Gymnophthalmus vanzoi	LSUMZ 12396	Boa Vista, Roraima	Brazil	MH732651	MH732702
Gymnophthalmus vanzoi	MTR 946529	Uiramutã, Roraima	Brazil	MH732653	MH732708
Gymnophthalmus vanzoi	MTR 946527	Uiramutã, Roraima	Brazil	MH732652	MH732707
Gymnophthalmus vanzoi	MTR 946534	Uiramutã, Roraima	Brazil	MH732654	MH732706
Gymnophthalmus vanzoi	MTR 946500	Normandia, Roraima	Brazil	MH732656	MH732705
Gymnophthalmus vanzoi	MTR 946501	Normandia, Roraima	Brazil	MH732657	MH732703
Gymnophthalmus vanzoi	MTR 946498	Normandia, Roraima	Brazil	MH732655	MH732704
Gymnophthalmus vanzoi	AMNH-R-138029	Northern Rupununi Savanna	Guyana	MH732658	na
Gymnophthalmus vanzoi	AMNH-R-138040	Northern Rupununi Savanna	Guyana	MH732659	na
Gymnophthalmus vanzoi	AMNH-R-138055	Northern Rupununi Savanna	Guyana	AF101368	AF101368
Gymnophthalmus aff. *vanzoi*	AMNH-R-128438	St. George	Trinidad & Tobago	MH732643	MH732709
Gymnophthalmus aff. *vanzoi*	FT1389/JC4823	St. George	Trinidad & Tobago	AF101365	AF101365
Gymnophthalmus aff. *vanzoi*	AMNH-R-140975	Berbice River	Guyana	AF101366	AF101366
Gymnophthalmus underwoodi	**MPEG 33517**	**Belém, Pará**	**Brazil**	**na**	**MZ544022**
Gymnophthalmus underwoodi	MTR 946601	Ilha de Maracá, Roraima	Brazil	MH732661	MH732711
Gymnophthalmus underwoodi	MTR 946590	Ilha de Maracá, Roraima	Brazil	MH732662	KT254406
Gymnophthalmus underwoodi	APL 21703	Manaus, Amazonas	Brazil	MH732663	MH732712
Gymnophthalmus underwoodi	JC/FT7054	Sam Lord's Castle, St. Phillip	Barbados	AF101369	AF101369
Gymnophthalmus underwoodi	NYSM 6432	Bottomless Ghaut	Montserrat	na	KX866265
Gymnophthalmus speciosus	MTR 33465	San José	Costa Rica	MH732660	MH732710
Gymnophthalmus cryptus	AMNH-R-138374	San Juan de Manapiare	Venezuela	AF101362	AF101362
Gymnophthalmus leucomystax	AMNH-R-139857	Southern Rupununi Savanna	Guyana	MH732666	MH732714
Gymnophthalmus pleei	AMNH-R-128428	Martinique	West indies	AF101364	AF101364
Micrablepharus maximiliani	LG 1017	Barra do Garças, Mato Grosso	Brazil	AF420657	AF420730

and *G. vanzoi* are known to be very similar in the traditionally used external morphological characters (Recoder *et al.* 2018), but we distinguished the northern specimens (*G. vanzoi*) from the Belém specimens (*G. underwoodi*) in the number of smooth subcaudal scales (SCA) (Table 1).

The number of SCA in *G. vanzoi* varies from 32 to 55 (Recoder *et al.* 2018), and we found 41 SCA in MPEG 27592, which is the single lizard with a whole tail among the three specimens from north of the Amazonas River. The SCA in *G. underwoodi* ranges from 23 to 26 (Recoder *et al.* 2018), and we found 24 and 27 SCA in MPEG 33549, and 33546, respectively. Therefore, although we cannot confirm indisputably the identity of all specimens based on morphology, at least we could confirm that all specimens from north of the Amazonas River are *G. vanzoi* in the molecular phylogeny. We confirmed the identity as *G. underwoodi* for three of the five specimens from Belém (one based on the molecular phylogeny and two by the number of SCA), and it is very likely that the other two individuals belong to the same species. Thus we assume that only *G. underwoodi* is present in Belém to date.

DISCUSSION

Our results confirm the designation as *Gymnophthalmus vanzoi* of Recoder *et al.* (2018) for *Gymnophthalmus* from north of the Amazonas River in the states of Amapá and Pará and show that the color differences reported as a differential characteristic of specimen MPEG 27592 from *G. vanzoi* by Avila-Pires *et al.* (2010) are not supported by molecular and meristic data, although future studies integrating more data

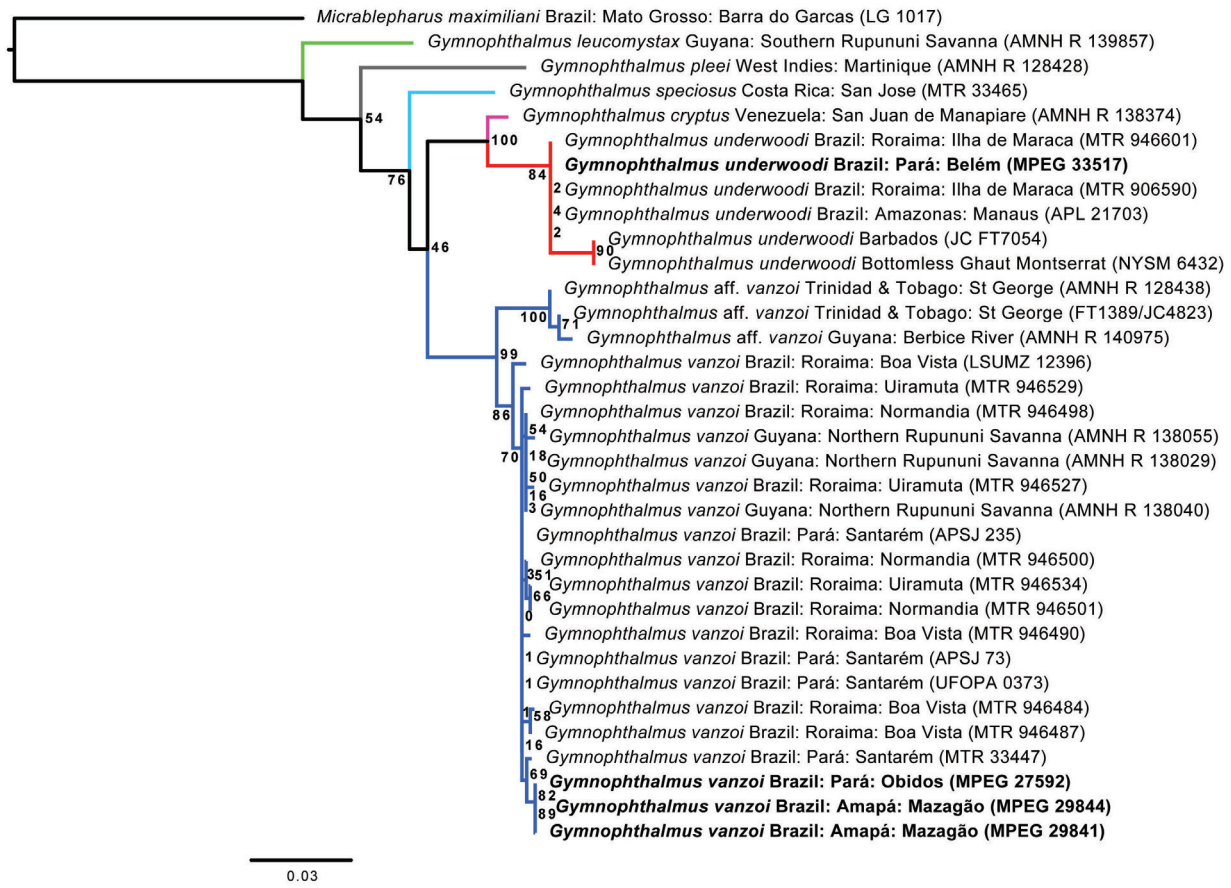

Figure 3. Maximum likelihood phylogram of *Gymnophthalmus* (1000 searches, loglikelihood = -2720.336686). Taxa labeled in bold were sequenced exclusively for this study. Colors correspond to the species codes in Figure 1. This figure is in color in the digital version.

can be important to reevaluate the taxonomic importance of color variation among the populations of this species.

The Belém region is one of the best studied areas in the Brazilian Amazon for herpetofauna, yet there existed no previous record of *G. underwoodi* in this area (Rand and Humphrey 1968; Avila-Pires 1995; Galati *et al.* 2007; Avila-Pires *et al.* 2018; Prudente *et al.* 2018), which is approximately 600 km away from the closest known occurrence of the species, in northeastern French Guiana. This strongly suggests that *G. underwoodi* does not occur naturally in the Belém municipality, but originates from an anthropic introduction. Further herpetological surveys in Belém and the wider Pará state, as well as refined molecular analyses involving larger samples from the entire distribution range of the species should elucidate the origin of the specimens found in the city.

Gymnophthalmus underwoodi was described from Barbados in the Lesser Antilles (Grant 1958) and was posteriorly found in the western and northern part of the Guiana region (Hoogmoed 1973; Avila-Pires 1995; Recoder *et al.* 2018). Kizirian and Cole (1999) suggested that *G. underwoodi* dispersed from South America to some Caribbean islands, since this species originated from the hybridization of two continental lineages and may have been transported through drift material from rivers that flow into the Caribbean Sea. Today, *G. underwoodi* is recognized to be invasive in several islands in the Lesser and Greater Antilles, where it can occur in sympatry with other native and non-native species of lizards (Orchard 2010; Turk *et al.* 2010; Breuil and Serre-Collet 2012; Questel and Boggio 2012; Alfonso and Hernandez 2017; Snyder *et al.* 2017).

The collection site of the *G. underwoodi* specimens in Belém (in an urban environment near a fluvial port) suggests the possibility of an introduction through a vessel docked at the port. Hoogmoed and Avila-Pires (2015) recently reported the presence in Belém of the lizard *Lepidodactylus lugubris* (Duméril and Bibron 1836), an Asiatic species of Gekkonidae introduced in several countries in the Caribbean region, and Central and South America. Discussing the origin of the *L. lugubris* specimens from Belém, the latter authors cited a shipping company that started to transport containers from Guadeloupe and Suriname to Belém in 2012 as a possible way for the introduction of the

species in the city. In this context, it is noteworthy that the first *G. underwoodi* was recorded in Belém in late 2012, but we cannot precisely define the entry pathway of the species in the city. Furthermore, *G. underwoodi* is also present in Guadeloupe (Breuil and Serre-Collet 2012) and Suriname (Kizirian and Cole 1999), reinforcing the alert for a possible route for the introduction of species in the Brazilian Amazon.

The reiterated record of individuals of *G. underwoodi* in different localities in Belém over nine years suggests that the species is establishing a population in the region. Future studies should confirm whether *G. underwoodi* can establish and expand its occurrence in the region of Belém. The most recent record (2020) was on Mosqueiro Island, which is connected to the mainland by a bridge and features no large harbor. The presence of *G. underwoodi* on Mosqueiro may represent a secondary introduction by small boats that transport products from the mainland or even by terrestrial vehicles from Icoaraci. D'Angiolella *et al*. (2021) recently reported new records of *L. lugubris* further inland in the state of Pará, which shows a fast territorial expansion of the species. Both *G. underwoodi*, and *L. lugubris* are parthenogenetic, which can be a great advantage for colonizing new areas, as a single individual is necessary to start reproduction (Cole *et al*. 1990).

The list of alien amphibians and reptiles in Brazil comprises 136 species, including native Brazilian species that are found outside their natural distribution range (Fonseca *et al*. 2019). We suggest that *G. underwoodi* should now be included in this list.

CONCLUSIONS

Our results on morphological and molecular data of isolated populations of *Gymnophthalmus* from Amazonian savanna enclaves in the states of Amapá and Pará confirmed that to date only *Gymnophthalmus vanzoi* is known to occur in these enclaves. *Gymnophthalmus underwoodi* is recorded for the first time south of the Amazonas River, likely as the result of an accidental introduction in the region of Belém, where the species had not been recorded before 2012, despite being one of the best known regions in Amazonia regarding herpetofauna. The probable introduction route is through ships coming from countries where *G. underwoodi* is historically known to occur.

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