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

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 (1º18’21”S, 48°29’19”W), close to several small ports which receive passenger 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 (1º18’44”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 (1º19’49.2”S; 48°28’37.4”W). Finally, a single specimen (MPEG 33549) was collected in the district of Mosqueiro Island (1º4’3.1”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...
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 species of 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.

### 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 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  | 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  | 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  | 14  | absent|

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.
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

**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   | MZ44008  | na       |
| *Gymnophthalmus vanzoi* | MPEG 29444     | Mazagão, Amapá         | Brazil   | MZ44009  | MZ44020  |
| *Gymnophthalmus vanzoi* | MTR 33447      | Santarém, Pará         | Brazil   | MH732644 | MH732650 |
| *Gymnophthalmus vanzoi* | APS1 235       | Santarém, Pará         | Brazil   | MH732646 | MH732666 |
| *Gymnophthalmus vanzoi* | APS1 73        | Santarém, Pará         | Brazil   | MH732647 | MH732691 |
| *Gymnophthalmus underwoodi* | UFOPA 0373   | Boa Vista, Roraima     | Brazil   | MH732645 | MH732696 |
| *Gymnophthalmus underwoodi* | MTR 946484   | Boa Vista, Roraima     | Brazil   | MH732648 | MH732699 |
| *Gymnophthalmus underwoodi* | MTR 946487   | Boa Vista, Roraima     | Brazil   | MH732649 | MH732700 |
| *Gymnophthalmus underwoodi* | MTR 946490   | Boa Vista, Roraima     | Brazil   | MH732650 | MH732701 |
| *Gymnophthalmus underwoodi* | LSUMZ 12396  | Boa Vista, Roraima     | Brazil   | MH732651 | MH732702 |
| *Gymnophthalmus underwoodi* | MTR 946529   | Uriramutá, Roraima     | Brazil   | MH732653 | MH732708 |
| *Gymnophthalmus underwoodi* | MTR 946527   | Uriramutá, Roraima     | Brazil   | MH732652 | MH732707 |
| *Gymnophthalmus underwoodi* | MTR 946534   | Uriramutá, Roraima     | Brazil   | MH732654 | MH732706 |
| *Gymnophthalmus underwoodi* | MTR 946500   | Normandia, Roraima     | Brazil   | MH732656 | MH732705 |
| *Gymnophthalmus underwoodi* | MTR 946501   | Normandia, Roraima     | Brazil   | MH732657 | MH732703 |
| *Gymnophthalmus underwoodi* | MTR 946498   | Normandia, Roraima     | Brazil   | MH732655 | MH732704 |
| *Gymnophthalmus underwoodi* | AMNH-R-138029 | Northern Rupununi Savanna | Guyana | MH732658 | na       |
| *Gymnophthalmus underwoodi* | AMNH-R-138040 | Southern Rupununi Savanna | Guyana | MH732659 | na       |
| *Gymnophthalmus underwoodi* | AMNH-R-138055 | Northern Rupununi Savanna | Guyana | AF101368 | AF101368 |
| *Gymnophthalmus aff. vanzoi* | ANHM-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 aff. vanzoi* | MPEG 33517   | Belém, Pará            | Brazil   | na       | MZ44022  |
| *Gymnophthalmus underwoodi* | MTR 946601   | Ilha de Maracá, Roraima | Brazil   | MH732661 | MH732711 |
| *Gymnophthalmus underwoodi* | MTR 946590   | Ilha de Maracá, Roraima | Brazil   | MH732662 | KT254066 |
| *Gymnophthalmus underwoodi* | APL 21703    | Manaus, Amazonas       | Brazil   | MH732663 | MH732712 |
| *Gymnophthalmus underwoodi* | JC/FT17054   | Sam Lord’s Castle, St. Phillip | Barbados | AF101369 | AF101369 |
| *Gymnophthalmus underwoodi* | NYSM 6432    | Bottomless Ghaut       | Montserrat | na       | KY966265 |
| *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 plesi* | AMNH-R-128428 | Martinique             | West Indies | AF101364 | AF101364 |
| *Microblepharus maximiliani* | LG 1017      | Barra do Garças, Mato Grosso | Brazil | AF420657 | AF420730 |
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, 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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