



On the occurrence of *Hemiphractus scutatus* (Spix, 1824) (Anura: Hemiphractidae) in eastern Amazonia

^{1,2,3}Leandro João Carneiro de Lima Moraes and ^{2,4}Dante Pavan

¹Programa de Capacitação Institucional, Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus, AM, BRAZIL ²Ecosfera Consultoria e Pesquisa em Meio Ambiente Ltda., São Paulo, SP, BRAZIL

Abstract.—*Hemiphractus* Wagler, 1828 is part of Hemiphractidae Peters, 1862, a family that harbors species of frogs characterized by the deposition of eggs on the females' dorsum. Both the genus *Hemiphractus* and the species *Hemiphractus scutatus* (Spix, 1824) are only known to the Andean mountain range and western half of the upper Amazon Basin. Herein, we provide the first records of *H. scutatus* from the eastern Amazonia (middle Tapajós River region, Pará State, Brazil), which extends its geographic range ca. 1,000 km from nearest known occurrence record and are among the lowest known levels for the species elevational range. Comparisons of morphologic and molecular data with available voucher specimens and published information on the species revealed variation that we interpret as intraspecific polymorphism. Phylogenetic analysis of a fragment of the mitochondrial gene *16S* recovered the newly discovered specimens as most closely related to samples from Peru. These results add new evidence in the known biogeographic patterns of the genus and species, and ongoing plans to build hydroelectric plants in the middle Tapajós River region can negatively affect this unique population.

Keywords. Biogeography, conservation, geographic range, marsupial frogs, morphology, Pará State, phylogenetic relationships

Citation: Moraes LJCDL, Pavan D. 2018. On the occurrence of *Hemiphractus scutatus* (Spix, 1824) (Anura: Hemiphractidae) in eastern Amazonia. *Amphibian & Reptile Conservation* 12(1) [General Section]: 5–14 (e151).

Copyright: © 2018 Moraes and Pavan. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercialNoDerivatives 4.0 International License, which permits unrestricted use for non-commercial and education purposes only, in any medium, provided the original author and the official and authorized publication sources are recognized and properly credited. The official and authorized publication credit sources, which will be duly enforced, are as follows: official journal title *Amphibian & Reptile Conservation*; official journal website <amphibian-reptile-conservation.org>.

Received: 02 August 2017; **Accepted:** 28 September 2017; **Published:** 30 January 2018

Introduction

According to the latest phylogenetic revisions (Castroviejo-Fisher et al. 2015; Duellman 2015), the family Hemiphractidae Peters, 1862 is considered monophyletic and include the genera *Cryptobatrachus* Ruthven, 1916 (six species), *Gastrotheca* Fitzinger, 1843 (70 species), *Stefania* Rivero, 1968 (19 species), *Flectonotus* Miranda-Ribeiro, 1926 (two species), *Fritziana* Mello-Leitão, 1937 (five species), and *Hemiphractus* (six species). Members of this family inhabit humid Neotropical forests in different elevational zones: Central America, Chocó, Andes, mountainous Caribbean coast, the island of Trinidad and Tobago, Amazonia, and the Atlantic Forest (Castroviejo-Fisher et al. 2015; Duellman 2015). These frogs share a unique reproductive mechanism, with deposition of eggs on the females' dorsum (Duellman 2015). In Amazonia, this characteristic seems to be relevant to define hemiphractid geographic ranges, as they are more diverse and abundant in the west, which

may be a result of ideal climatic conditions for its life cycle in this region (Bernal and Lynch 2013; Duellman 2015), such as the lower seasonality and higher annual rainfall (Sombroek 2001).

The species of the genus *Hemiphractus* are terrestrial and arboreal frogs with well-modified and ornamented skulls (Trueb 1974), which are distributed throughout Central America, East of Andes and in the extreme western Amazon basin (Frost 2017): *H. bubalus* (Jimenez de la Espada, 1870), *H. fasciatus* Peters, 1862, *H. helioi* Sheil, and Mendelson III, 2001, *H. johnsoni* (Noble, 1917), *H. proboscideus* (Jimenez de la Espada, 1870), and *H. scutatus* (Spix, 1824). The latter is the type species of the genus and inhabits a wide elevational range along the western Amazon Basin and Andean mountain range, in Bolivia, Peru, Ecuador, and Brazil (Spix 1824; Myers and Carvalho 1945; Trueb 1974; Duellman and Lynch 1988; Rodríguez and Duellman 1994; Ruiz-Carranza et al. 1996; Sheil and Mendelson III 2001; Lehr 2001; Moravec et al. 2002; Coloma et al. 2004; Duellman 2005;

Correspondence. ³leandro.jclm@gmail.com (corresponding author); ⁴dtpavan@yahoo.com.br

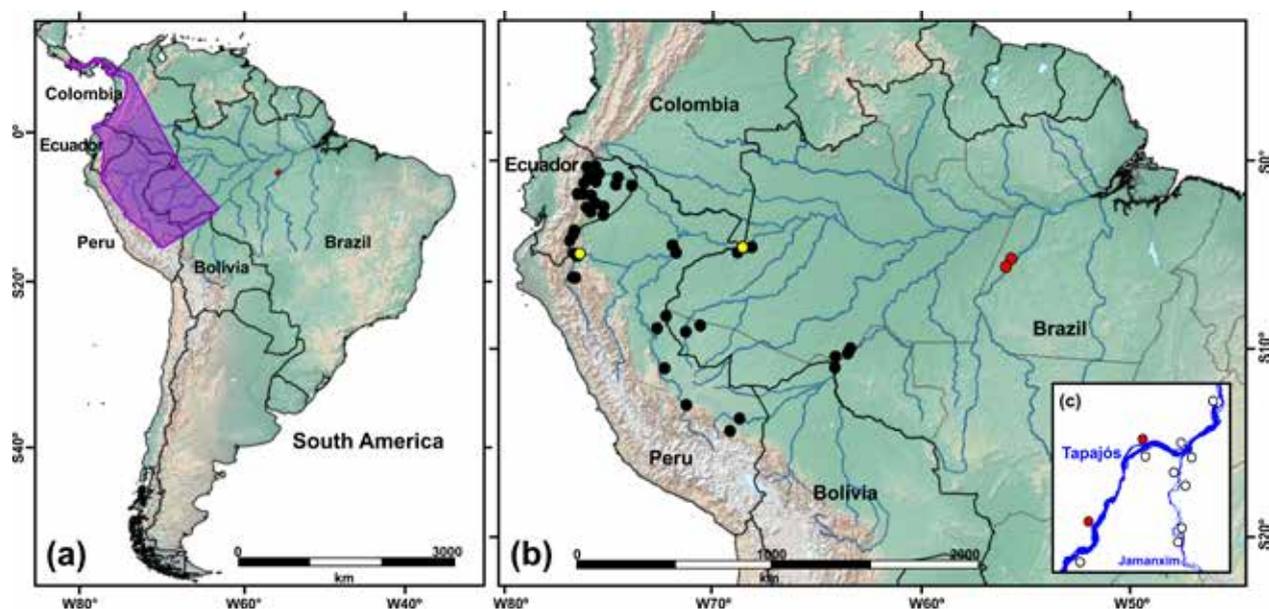


Fig. 1. Known distribution of (a) genus *Hemiphractus* (in purple) and (b) *Hemiphractus scutatus* (dots), highlighting new locality of occurrence in middle Tapajós River region, Pará State, Brazil (red dots) and localities of sequences included in molecular analysis (yellow dots). The region of new records is zoomed in (c), showing the sampling sites where *H. scutatus* was present (red) and not recorded (white).

Lynch 2005; Cisneros-Heredia 2006; Glaw and Franzen 2006; Muñoz-Saravia 2008; Souza 2009; von May et al. 2009; Beirne and Whitworth 2011; Bernarde et al. 2011; Catenazzi et al. 2013; Ortiz 2013; Almendáriz et al. 2014; Castroviejo-Fisher et al. 2015; Frost 2017; GBIF 2017; Rainforest Conservation Fund 2017; SpeciesLink 2017).

Herein we present the first records of *Hemiphractus scutatus* from the middle Tapajós River region, Pará State, Brazil. These records are the easternmost known localities of occurrence reported for this species and the genus, and are among the lowest known elevational levels for the species distribution. We also present a phylogenetic tree based on mtDNA gene *16S* for some *Hemiphractus* species, and discuss on the biogeographic implications of these records and conservation of this population.

Material and Methods

The amphibian survey was conducted on the middle Tapajós River region, Pará State, Brazil. This river is one of the largest tributaries of the Amazon River (Sioli 1968) and is located in eastern Amazonia. The climate in this region have a high seasonality (Sombroek 2001), with average annual temperature of 26 °C and total annual rainfall exceeding 2,400 mm (Wang et al. 2017), with driest months from June to August (Alvares et al. 2013). We survey for amphibians in 11 sampling sites with four km long, installed in both banks of the Tapajós River and its tributary the Jamanxim River. Each sampling site covered humid primary *Terra Firme* forests, which does not suffer the seasonal riverine flood pulse effect (Junk et al. 1989) and riparian forests (Fig. 1). We used complemen-

tary sampling methods (Heyer et al. 1994), such as pitfall traps (600 trap nights) and diurnal and nocturnal active searches (more than 340 days). Six field campaigns were conducted along July 2012 and November 2013.

Aiming to better understand the relevance of these records in the general context of the geographic and elevational distribution of the species, we survey for its occurrence data available in the literature (Spix 1824; Myers and Carvalho 1945; Trueb 1974; Duellman and Lynch 1988; Rodríguez and Duellman 1994; Ruiz-Carranza et al. 1996; Sheil and Mendelson III 2001; Lehr 2001; Moravec et al. 2002; Coloma et al. 2004; Duellman 2005; Lynch 2005; Cisneros-Heredia 2006; Glaw and Franzen 2006; Muñoz-Saravia 2008; Souza 2009; von May et al. 2009; Beirne and Whitworth 2011; Bernarde et al. 2011; Catenazzi et al. 2013; Almendáriz et al. 2014; Castroviejo-Fisher et al. 2015; Frost 2017; Rainforest Conservation Fund 2017; AP Lima, pers. comm.) and online databases (Ortiz 2013; GBIF 2017; SpeciesLink 2017), mostly with associated vouchers in zoological collections, obtaining a total of 77 georeferenced localities of occurrence.

Morphologic data survey

We analyzed morphologic data traditionally used in the taxonomy of the genus (Trueb 1974), obtaining qualitative characters of external morphology and quantitative characters using a caliper to the nearest 0.1 mm: snout-vent length (SVL); forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow (FAL); hand length from proximal edge of palmar tubercle to tip of finger III (HA); tibia length from proximal edge of



Fig. 2. Specimens of *Hemiphractus scutatus* from middle Tapajós River region, Pará State, Brazil. **(a)** Female, 76.1 mm SVL, INPA-H38116; **(b)** Male, 57.8 mm SVL, INPA-H38117; **(c)** Female, 61.7 mm SVL, INPA-H38118.

flexed knee to heel (TL); foot length from proximal edge of inner metatarsal tubercle to tip of Toe IV (FL); head width at level of angle of jaw (HW); head length from angle of jaw to tip of snout (HL); eye diameter (ED); internarial distance (IN); diameter of tympanum (DT); interorbital distance (IO) and thigh length (THL). We compared the measurements with information available from the literature and voucher specimens deposited at the Collection of Amphibians and Reptiles (INPA-H) of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil (Appendix S1), where the new specimens were also deposited (under accession numbers INPA-H 38116–38118).

Molecular data protocols

We extracted the genomic DNA from two specimens liver tissue samples conserved in absolute ethanol using the phenol-chloroform protocol (Sambrook and Russell 2001). The *16S* mtDNA gene, a standard marker for amphibians (Vences et al. 2012), was amplified via the Polymerase Chain Reaction (PCR). The PCR amplification used a mix with final volume of 25 μ l: 4 μ l of 1.25 M dNTPs, 2.5 μ l of 10X amplification buffer (75mM Tris HCl, 50 mM KCl, 20 mM $(\text{NH}_4)_2\text{SO}_4$), 1.0 μ l of 50 mM MgCl_2 , 1.0 μ l of DNA in a concentration of 250 ng/ μ l, 0.25 μ l of each primer (*16Sar* and *16Sbr* - Palumbi et al. 1991) in a concentration of 200 ng/ μ l, 0.25 μ l of Taq DNA polymerase 5 U/ μ l and 15.75 μ l of ddH₂O. Reaction conditions had an initial heating step at 94 °C for five minutes, 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 60 s, and extension at 72 °C for 120 s, followed by a final extension at 72 °C for seven minutes. PCR products were purified with ExoSAP-IT (USB Corporation) and submitted to a sequencing reaction following BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, EUA) protocols. The sequences were obtained in the automated sequencer ABI PRISM 3500 (Applied Biosystems, EUA) and deposited in GenBank (accession numbers MG011478, MG011479).

The sequences were aligned using the MUSCLE algorithm, implemented in MEGA 6.06 (Tamura et al. 2013) and corrected manually, obtaining a 524 bp alignment. Using the same software, we generated a maximum likelihood phylogenetic tree, constructed through a general

time reversible model with a gamma distribution of rate variation (GTR+G), selected as the best DNA evolution model for the alignment by Bayesian Information Criterion (BIC), as well as to calculate two inter and intra-specific genetic distances: uncorrected-pairwise and Kimura-2-Parameter (K2P) (Kimura 1980). Additional sequences were obtained in GenBank, including the two distinct lineages of *H. scutatus* identified by Castroviejo-Fisher et al. (2015) (Table 3). The statistical support for the tree nodes was estimated by bootstrapping (5,000 replicates).

Results

New records of *Hemiphractus scutatus*

We found three specimens of *H. scutatus* in two of the 11 sampling sites (Figs. 1–3). It was a rare species in the sampling, recorded at a ratio of one specimen in about each 300 days of sampling, while the most abundant syntopic terrestrial frogs were from genera *Adenomera* Steindachner, 1867, *Pristimantis* Jiménez de la Espada, 1870, *Allobates* Zimmermann and Zimmermann, 1988, and *Rhinella* Fitzinger, 1826, with 2,700 specimens recorded in this same sampling effort. The three specimens of *H. scutatus* were only recorded by the active searches, and exclusively in *Terra Firme* forests (Fig. 4).

On 28 September 2012 one female voucher specimen was collected by D. Pavan close to a large tree and under a palm leaf, on the left bank of Tapajós River, at 19:15 h (76.1 mm SVL; 05°02'S, 56°53'W, 62 m above mean sea level, hereafter referred as asl). On 16 October 2012 a male voucher specimen was collected on the same riverbank by LJCL Moraes hidden inside the leaf-litter at 21:05 h, distant ca. 51 km in straight line from the first record (57.8 mm SVL; 04°39'S, 56°37'W, 60 m asl). On 28 April 2013 a second female voucher specimen was collected also hidden inside the leaf-litter on the same riverbank by J. Cassimiro at 21:30 h (61.7 mm SVL; 04°40'S, 56°37'W, 83 m asl), distant ca. 50 km in straight line from the first record and 430 m from the second record. No evidence of reproductive activity or gaping posture (Trueb 1974) was observed.

These three records represent the easternmost known localities of occurrence of *H. scutatus*, extending the geo-



Fig. 3. Dorsal and ventral views of voucher specimens of *Hemiphractus scutatus* from middle Tapajós River region, Pará State, Brazil. (a) Female, 76.1 mm SVL, INPA-H38116; (b) Male, 57.8 mm SVL, INPA-H38117. Scale bar = 20 mm.

graphic range of the species and the genus *Hemiphractus*. They are distant ca. 1,000–1,500 km from the previously known easternmost points of the species occurrence, in Rondônia (INPA-H 15398, 15399) and Amazonas States, Brazil (GBIF 2017; SpeciesLink 2017) (Fig. 1). Considering only the Amazon Basin at South of Amazon River, these new records even extend to the East the geographic range of the family Hemiphractidae. Furthermore, the elevation level in which these specimens were recorded are among the lowest known elevation for the species (60, 62, and 83 m asl; Fig. 5), and two of them (60 and 62 m asl) also extend downwards the known elevational range of this species, since there are no documented records of individuals below 70 m asl.

Morphologic variation and molecular relationships

The morphologic data confirms the identification of our specimens in accordance to the literature (Trueb 1974) and voucher specimens. Qualitative characters include the triangular head, canthus rostralis rounded in section; tympanum large and vertically ovoid; oblique rows of tubercles on dorsal surfaces of forearm and hind limb (less pronounced in female specimens); small triangular fleshy proboscis, dorsoventrally flattened, on tip of

snout; eyelids granular with one (female specimens) or three (male specimen) prominent fleshy conical tubercles; single bony projection at the angle of the jaw; slightly enlarged tubercles at the knee and small tubercles at calcaneum (divergent from the absence of calcar projections reported by Trueb 1974 and Rodríguez and Duellman 1994); fingers and toes with vestigial adhesive discs, well-developed round subarticular tubercles and basal webbing; thenar tubercle elliptical and outer palmar tubercle diffuse, flat and cordiform; no evidence of nuptial pads in male specimen; toes also with well-developed round subarticular tubercles and about one-fourth webbed; inner metatarsal tubercle well-developed and elliptical, and outer metatarsal tubercle indistinct; shagreened skin on dorsum and granular on flanks, abdomen and ventral surfaces of thighs.

Dorsal coloration in life varies from reddish brown (INPA-H38116 and 38118) to pale tan background with dark mottling (INPA-H38117), with two dark vertebral spots; dark suborbital marks from the lower margin of the eye expanding posteroventrally but not reaching the lip (more pronounced in INPA-H38117 than in INPA-H38116 and 38118) and scattered dark spots in the tympanic region. Ventrally, gular coloration varies from uniformly brown (INPA-H38116 and 38118) to mottled (INPA-H38117), with a pale mid-ventral stripe reach-



Fig. 4. Aerial (a) and inside (b) view of the *Hemiphractus scutatus* habitat (Terra Firme forest) in middle Tapajós River region, Pará State, Brazil, also showing the BR-230 highway.

ing the pectoral region; same gular color reaches the pectoral region, and becomes less pigmented posteriorly. A finely dark venate pattern covers the flank areas above the forelimb; forelimbs and hind limbs varies from uniformly brown (INPA-H38116 and 38118) to tan (INPA-H38117), with dark transverse bands, reaching the dorsal surface of hands (more evident in INPA-H38117); iris bronze and darker ventrally, with a longitudinally crossing reddish area and pupil horizontal. Regarding quantitative characters, most of the measurements of the middle Tapajós River specimens agree with the known morphometric range of the species (Table 1), also showing the sexual dimorphism in body size. The only divergence is a small HW compared to SVL in female INPA-H38116.

The 16S mtDNA tree for *Hemiphractus* species shows, as the results presented by Castroviejo-Fisher et al. (2015), two distinct lineages of *H. scutatus*. The middle Tapajós River population is more related to the lineage from Peru (Figs. 6, 7), as the sequences have a higher genetic similarity (more than 97%) compared to sequence from Colombia, near the country's border with Brazil (93%) (Fig. 7).

Discussion

The presence of possible cryptic taxa under the name *H. scutatus* was suggested based on the results of a phylogeny

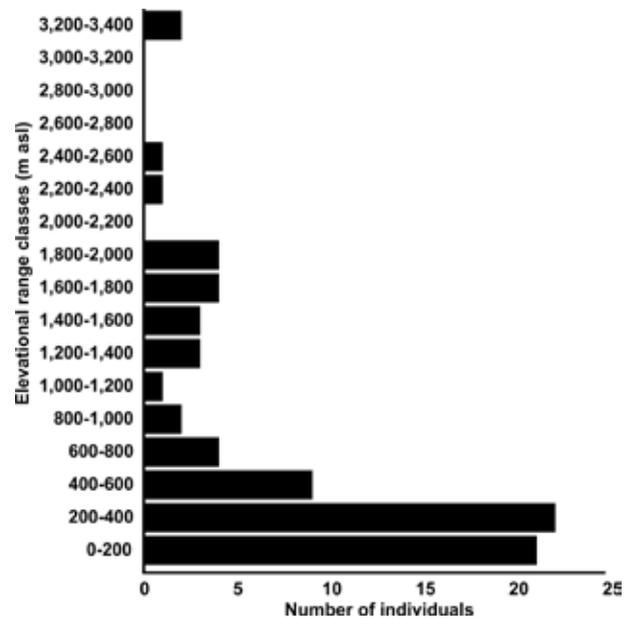


Fig. 5. Variation in number of individuals of *Hemiphractus scutatus* recorded along its known elevational range (60–3,300 m above mean sea level). The specimens from middle Tapajós River region, Pará State, Brazil are recorded among the lowest known elevation for the species.

of molecular and morphologic data (Castroviejo-Fisher et al. 2015), since two genetically distant intraspecific lineages were found. Although we initially worked with the hypothesis that specimens from middle Tapajós River were a new taxon, the morphologic and molecular analysis readily rejected this. Regarding the morphology, despite the possibility of strong variation due to large geographic distance to known distribution area, most of the qualitative and quantitative data of the specimens from middle Tapajós River were inside the known range for the species (Trueb 1974; Rodríguez and Duellman 1994) and other voucher specimens (Table 1). The slightly divergences in colors, shapes, and morphometric characters between this specimens and the known for the species may be part of intraspecific variation. Regarding the molecular data, despite the high geographic distance between the populations from middle Tapajós River and Peru (more than 2,300 km), there is a low genetic distance between the sequences from these regions (between 2% and 3%). As Castroviejo-Fisher et al. (2015) highlighted, the genetic distance between the sequences from Colombia and Peru, and now including the distance of Tapajós sequences, are high and may indicate cryptic speciation (more than 7%). As overall similarity in external morphology and pronounced morphologic variation are common events inside the genus *Hemiphractus* (Trueb 1974), further broader studies and integrative taxonomic revisions may indicate the extent of morphologic and molecular variability of this species and reveal the taxonomic status of these genetically distant lineages.

Table 1. Morphologic measurements (mm) of *Hemiphractus scutatus* specimens recorded in middle Tapajós River region (highlighted), compared to literature data and other voucher specimens from herpetological collection of Instituto Nacional de Pesquisas da Amazônia, Brazil (INPA-H). Literature: ^aTrueb 1974, *n* = 8 males and 15 females; ^bRodríguez and Duellman 1994.

Measurements	Literature	INPA-H38117	Literature	INPA-H15399	INPA-H15398	INPA-H38116	INPA-H38118
	Male	Male	Female	Female	Female	Female	Female
SVL	36.9–62 ^{a,b}	57.8	60.4–81 ^{a,b}	62.5	73.6	76.1	61.7
FAL	-	12.7	-	12.2	15.7	16.4	12.9
HA	-	16.3	-	18	21.3	19	17.6
TL	15.5–27.5 ^a	25.1	23.7–38.3 ^a	21.2	34.5	32	27.6
FL	-	26.2	-	25.5	32	30.1	26.7
HL	17.5–30 ^a	28.3	25.7–42.9 ^a	29	33	36.3	31
HW	20.8–37.5 ^a	34.2	34.4–52.8 ^a	36	43.3	41.6	37.5
ED	-	6	-	5.6	7.3	7	5.6
IN	-	4	-	3.8	4.5	4.8	4
DT	-	5.2	-	3.3	4.9	4.7	3.7
IO	-	14.6	-	14.4	17.6	17.8	17.3
THL	-	28.2	-	29.7	38.2	34.4	29.2
TL/SVL (%)	42.3–48 ^a	43	39.3–47.6 ^a	34	47	42	45
HL/SVL (%)	47.6–52.3 ^a	49	42.7–53.3 ^a	46	45	48	50
HW/SVL (%)	56.6–65.5 ^a	59	57.1–65.7 ^a	58	59	55	61

Biogeography

After more than 190 years since the original description of *H. scutatus* (Spix 1824) we recorded this species in the eastern Amazonia, emphasizing the lack of knowledge about the general biogeographic patterns of Amazonian amphibians, which can be mainly generated by sampling difficulties, especially in cases of secretive species. Large forested regions in the Amazonia remain unexplored and have the potential to harbor new species or expanding species distributions (Azevedo-Ramos and Galatti 2002). Therefore, the recognition of broader biogeographic patterns to Amazonian amphibians, as areas of endemism historically recognized in the biome to other vertebrates (e.g., Cracraft 1985; Boubli et al. 2014) depends on the continued expansion of the sampling effort and new analytical techniques that is currently being held in the biome.

Our new records for *H. scutatus* bring new information to a biogeographic idea historically recognized on the low representation of Hemiphractidae in the eastern Amazonia, probably due to increased seasonality in this region (Sombroek 2001; Duellman 2015). Species of this family that have greater environmental plasticity, as appears to be the case of *H. scutatus* (the species of the genus with the widest known geographic and elevational range) may reach the preserved forests in this region and establish viable populations, although in less abundance in relation to the more climatically constant (Wang et al. 2017) and humid environments of western Amazonia.

Regarding elevational occurrence, although *H. scutatus* has already been recorded in high elevations at the Andean mountain range (GBIF 2017), a greater number of individuals is known for the Amazonian lowlands, and

67% of 77 published localities of occurrence are below 600 m asl (Fig. 5). This wide elevational range reinforce the high environmental plasticity, as the life-history strategies of amphibian populations in high and lowlands may drastically differ (Morrison and Hero 2003). The knowledge on the drivers of elevational variation in the distribution of Amazonian amphibians is still incipi-

Table 2. Sequences from GenBank with accession numbers. In bold are sequences generated from this study.

Taxon	16S
<i>Hemiphractus bubalus</i>	DQ679412
<i>Hemiphractus fasciatus</i>	KC014899
<i>Hemiphractus fasciatus</i>	KC014900
<i>Hemiphractus fasciatus</i>	KC129336
<i>Hemiphractus fasciatus</i>	KC129337
<i>Hemiphractus fasciatus</i>	KC129338
<i>Hemiphractus fasciatus</i>	KC129339
<i>Hemiphractus fasciatus</i>	KC129340
<i>Hemiphractus fasciatus</i>	KC129341
<i>Hemiphractus fasciatus</i>	KC129342
<i>Hemiphractus fasciatus</i>	KC129343
<i>Hemiphractus helioi</i>	AY843594
<i>Hemiphractus helioi</i>	KR270431
<i>Hemiphractus proboscideus</i>	DQ679413
<i>Hemiphractus scutatus</i>	DQ679414
<i>Hemiphractus scutatus</i>	KR270432
<i>Hemiphractus scutatus</i>	MG011478
<i>Hemiphractus scutatus</i>	MG011479

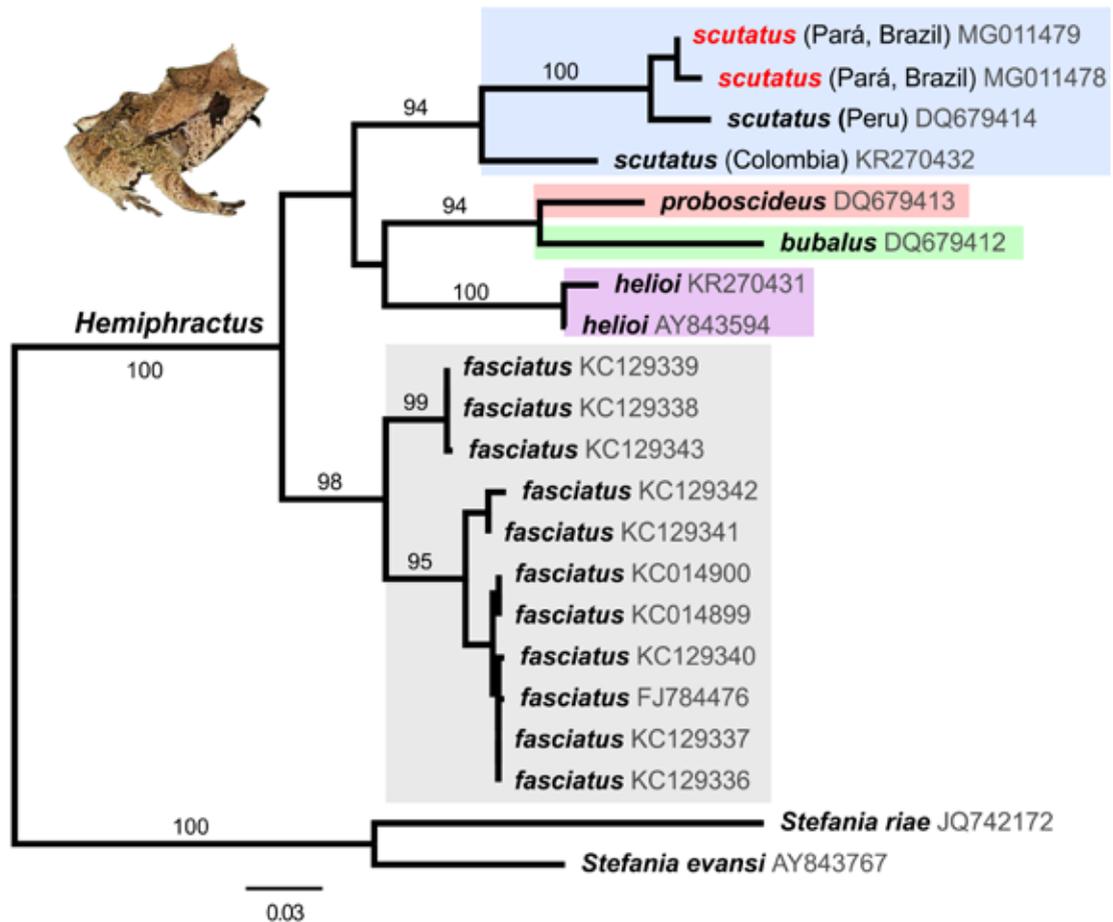


Fig. 6. Maximum likelihood phylogenetic tree of *Hemiphractus* species based in a fragment of the 16S mtDNA gene, with GenBank accession numbers. Only bootstrap values >80% are shown (5,000 replicates). For *Hemiphractus scutatus*, sample localities are in parentheses and specimens from middle Tapajós River region, Pará State, Brazil are highlighted.

ent (Siqueira and Rocha 2013) and the *H. scutatus* may be a target taxon for future studies testing this gradient.

Conservation

Hemiphractus scutatus is considered as “Least Concern” by IUCN due to its wide distribution and presumably large and stable populations (Coloma et al. 2004). However, this species is rarely recorded and have poorly known population dynamics to define its conservation status, that can even vary along its wide geographic and elevational range. As the Amazon region has suffered increasing anthropic impact through the advance of cities and highways, forests fragmentation and habitat loss (Fearnside 2015), the *H. scutatus* may have declining populations in most of its distribution, since they are dependents of undisturbed forests (Rodríguez and Duellman 1994).

The specimens of *H. scutatus* from middle Tapajós River region may represent a unique population, recorded near and within a federal conservation unit (Parque Nacional da Amazônia), same pattern already described for Peruvian populations (von May et al. 2009), reinforcing

the need to maintain large protected forest areas in the Amazonia and adequate land-use on the unprotected (Laurance et al. 2001). In addition to these threats, the biome has been target of dam construction in its larger rivers (Latrubesse et al. 2017), which can negatively affect the biodiversity of the surrounding forests (Moraes et al. 2016). The population of *H. scutatus* from Tapajós River is in the region affected by the construction of a large hydroelectric plant, part of a complex planned for the basin (Fearnside 2015), thus the implementation of this project may affect the viability of this population.

Conclusion

The discovery of the first specimens of *H. scutatus* from eastern Amazonia sheds new insights into ecology, biogeography, taxonomy, and conservation of these remarkable frogs. To better understand the population status and the total distribution range of this taxon in Amazonia, we need more long-term field studies, with standardized protocols, complementary sampling and broader approaches.

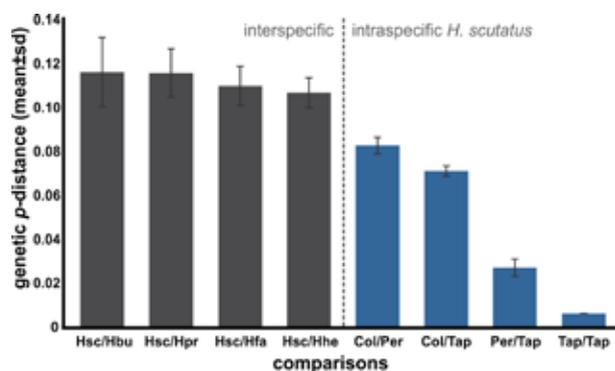


Fig. 7. Inter and intraspecific genetic distances (mean \pm standard deviation of pairwise and K2P distances) calculated for a fragment of *16S* mtDNA gene of *Hemiphractus* species and populations. (Hsc) *Hemiphractus scutatus*; (Hpr) *Hemiphractus proboscideus* (Hfa) *Hemiphractus fasciatus*; (Hhe) *Hemiphractus helioi*; (Col) Colombia; (Per) Peru; (Tap) middle Tapajós River region, Pará State, Brazil. GenBank accession numbers of sequences are in Table 2.

Acknowledgements.—We thank MC Barros and members of GENBIMOL Molecular Biology Laboratory of the Universidade Estadual do Maranhão - campus Caxias (UEMA) for assistance with the molecular data survey; LF Storti, J Cassimiro, JO Gomes, M Hoffman, TFD Rodrigues, JMB Ghelere, AB Barros, and ES Brito, for help in sampling; CNEC WorleyParsons Engenharia S.A., for financial and logistical support and FP Werneck, AAA Silva, and R Vogt, for allowing the specimens examination at the herpetological collection of INPA. The individuals were collected under permit 066/2012 provided by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) provided a scholarship to LJCL Moraes.

Literature Cited

Almendáriz A, Simmons JE, Vaca-Guerrero J, Brito J. 2014. Overview of the herpetofauna of the unexplored Cordillera del Cóndor of Ecuador. *Amphibian & Reptile Conservation* 8: 45–64 (e82).

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22(6): 711–728.

Azevedo-Ramos C, Galatti U. 2002. Patterns of amphibian diversity in Brazilian Amazonia: Conservation implications. *Biological Conservation* 103(1): 103–111.

Beirne C, Whitworth A. 2011. *Frogs of the Yachana Reserve*. Global Vision International, Exeter, United Kingdom. 109 p.

Bernal MH, Lynch JD. 2013. Thermal tolerance in anuran embryos with different reproductive modes: Relationship to altitude. *The Scientific World Journal* 2013: 1–7.

Bernarde PS, Machado RA, Turci LCB. 2011. Herpetofauna da área do Igarapé Esperança na Reserva Extrativista Riozinho da Liberdade, Acre – Brasil. *Biota Neotropica* 11(3): 117–144.

Boubli JP, Ribas CC, Lynch Alfaro J, Silva MNF, Pinho GM, Farias IP. 2015. Spatial and temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. *Molecular Phylogenetics and Evolution* 82: 400–412.

Catenazzi A, Lehr E, von May R. 2013. The amphibians and reptiles of Manu National Park and its buffer zone, Amazon basin and eastern slopes of the Andes, Peru. *Biota Neotropica* 13(4): 269–283.

Cracraft J. 1985. Historical biogeography and patterns of differentiation within the South American avifauna: Areas of endemism. *Ornithological Monographs* 36: 49–84.

Castroviejo-Fisher S, Padial Jr. JM, Silva HR, Rojas-Runjaic FJM, Medina-Méndez E, Frost DR. 2015. Phylogenetic systematics of egg-brooding frogs (Anura: Hemiphractidae) and the evolution of direct development. *Zootaxa* 4004: 1–75.

Cisneros-Heredia DF. 2006. La Herpetofauna de la Estación de Biodiversidad Tiputini, Ecuador: Diversidad & Ecología de los Anfibios & Reptiles de una Comunidad Taxonomicamente Diversa. B.Sc. Thesis, Universidad San Francisco de Quito, Quito, Ecuador. 129 p.

Coloma LA, Ron S, Azevedo-Ramos C. 2004. *Hemiphractus scutatus*. The IUCN Red List of Threatened Species 2004: e.T55371A11299534.

Duellman WE. 2005. *Cusco Amazónico: The Lives of Amphibians and Reptiles in an Amazonian Rainforest*. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, USA. 488 p.

Duellman WE. 2015. *Marsupial Frogs. Gastrotheca and Allied Genera*. Johns Hopkins University Press, Baltimore, Maryland, USA. 432 p.

Duellman WE, Lynch JD. 1988. Anuran amphibians from the Cordillera de Cutucú, Ecuador. *Proceedings of the Academy of Natural Sciences, Philadelphia* 140(2): 125–142.

Fearnside PM. 2015. Amazon dams and waterways: Brazil's Tapajós basin plans. *Ambio* 44(5): 426–439.

Frost DR. 2017. *Amphibian Species of the World: An Online Reference*. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 03 May 2017].

GBIF. 2017. *Hemiphractus scutatus* (Spix, 1824) species page. Available: <http://www.gbif.org/species/2429986> [Accessed: 03 May 2017].

Glaw F, Franzen M. 2006. Type catalogue of amphibians in the Zoologische Staatssammlung München. *Spixiana* 29: 153–192.

Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC,

- Foster MS. 1994. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- Junk WJ, Bayley PB, Sparks RE. 1989. The flood pulse concept in river-floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106: 110–127.
- Kimura MA. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2): 111–120.
- Latrubesse EM, Arima EY, Dunne T, Park E, Baker VR, d’Horta FM, Wight C, Wittmann F, Zuanon J, Baker PA, Ribas CC, Norgaard RB, Filizola N, Ansar A, Flyvbjerg B, Stevaux JC. 2017. Damming the rivers of the Amazon basin. *Nature* 546: 363–369.
- Laurance WF, Cochrane MA, Bergen S, Fearnside PM, Delamonica P, Barber C, D’Angelo S, Fernandes T. 2001. The future of the Brazilian Amazon. *Science* 291: 438–439.
- Lehr E. 2001. New records for amphibians and reptiles from Departamentos Pasco and Ucayali, Peru. *Herpetological Review* 32: 130–132.
- Lynch JD. 2005. Discovery of the richest frog fauna in the world—an exploration of the forests to the north of Leticia. *Revista de la Academia Colombiana de Ciencias* 29(113): 581–588.
- Morrison C, Hero JM. 2003. Geographic variation in life-history characteristics of amphibians: A review. *Journal of Animal Ecology* 72: 270–279.
- Moraes LJCL, Pavan D, Barros MC, Ribas CC. 2016. The combined influence of riverine barriers and flooding gradients on biogeographical patterns for amphibians and squamates in south-eastern Amazonia. *Journal of Biogeography* 43(11): 2,113–2,124.
- Moravec J, Tuanama IA, Burgos AM. 2002. Amphibians recently recorded from the surroundings of Iquitos (Departamento Loreto, Peru). I. Hylidae. *Časopis Národního Řada, přírodovědná* 171: 29–44.
- Muñoz-Saravia A. 2008. Geographic distribution: *Hemiphractus scutatus*. *Herpetological Review* 39: 233.
- Myers GS, Carvalho AL. 1945. Notes on some new or little-known Brazilian amphibians, with an examination of the history of the Plata salamander, *Ensatina platensis*. *Boletim do Museu Nacional, Nova Serie, Zoologia* 35: 1–24.
- Ortiz DA. 2013. *Hemiphractus scutatus*. In: AmphibiaWebEcuador. Version 2016.0. Editors, Ron SR, Guayasamin JM, Yanez-Muñoz MH, Merino-Viteri A, Ortiz DA, Nicolalde DA. 2016. Museo de Zoología, Pontificia Universidad Católica del Ecuador, Ecuador. Available: <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1275> [Accessed: 03 May 2017].
- Rainforest Conservation Fund. 2017. Species Data Sheets, Reptiles & Amphibians, *Hemiphractus scutatus*. Available: <http://www.rainforestconservation.org/species-data-sheets/frogs/hemiphractus-scutatus> [Accessed: 03 May 2017].
- Rodríguez LO, Duellman WE. 1994. *Guide to the Frogs of the Iquitos Region, Amazonian Peru*. Peruvian Field Guides Series No Sp 22 (Book 22). Asociación de Ecología y Conservación, Amazon Center for Environmental Education and Research and Natural History Museum, The University of Kansas, Lawrence, Kansas, USA. 89 p.
- Ruiz-Carranza PM., Ardila-Robayo MA, Lynch JD. 1996. Lista actualizada de la fauna de Amphibia de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 20: 77,365–77,415.
- Sambrook JD, Russel W. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA. 999 p.
- Sioli H. 1968. Hydrochemistry and geology in the Brazilian Amazon region. *Amazoniana* 1: 267–277.
- Siqueira CC, Rocha CFD. 2013. Altitudinal gradients: concepts and implications on the biology, the distribution and conservation of Anurans. *Oecologia Australis* 17: 282–302.
- Sombroek W. 2001. Spatial and temporal patterns of Amazon rainfall - Consequences for the planning of agricultural occupation and the protection of primary forests. *Ambio* 30: 388–396.
- Souza MB. 2009. *Anfibios: Reserva Extrativista do Alto Juruá e Parque Nacional da Serra do Divisor, Acre*. IFCH, Campinas, Brazil. 76 p.
- SpeciesLink. 2017. SpeciesLink. Centro de Referência em Informação Ambiental, CRIA. Available: <http://smlink.cria.org.br> [Accessed: 03 May 2017].
- Spix JBV. 1824. *Animalia nova sive Species novae Testudinum et Ranarum quas in itinere per Brasiliam annis MDCCCXVII–MDCCCXX jussu et auspiciis Maximiliani Josephi I. Bavariae Regis*. F.S. Hüb-schmann, München, Germany. 29 p.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2,725–2,729.
- Trueb L. 1974. Systematic relationships of Neotropical horned frogs, genus *Hemiphractus* (Anura: Hylidae). *Occasional Papers of the Museum of Natural History, the University of Kansas* 29: 1–60.
- Vences M, Nagy ZT, Sonet G, Verheyen E. 2012. DNA barcoding amphibians and reptiles. Pp. 79–107 In: *DNA Barcodes: Methods and Protocols*. Methods in Molecular Biology Series. Editors, Kress WJ, Erickson DL. Humana Press, Inc., New York, New York, USA. 470 p.
- von May R, Siu-Ting K, Jacob JM, Müller MM, Gagliardi G, Rodríguez LO, Donnelly MA. 2009. Species diversity and conservation status of amphibians in

Madre de Dios, southern Peru. *Herpetological Conservation and Biology* 4(1): 14–29.

Wang X, Edwards LR, Auler AS, Cheng H, Kong X,

Wang Y, Cruz FW, Dorale JA, Chiang HW. 2017. Hydroclimate changes across the Amazon lowlands over the past 45,000 years. *Nature* 541: 204–207.

Appendix S1.

Specimens examined. *Hemiphractus scutatus* ($n = 5$): BRAZIL: Rondônia: Abunã esquerda, Porto Velho (65°20'S 09°31'W), INPA-H15398, Jirau esquerda, Porto Velho, INPA-H15399 (64°44'S 09°20'W); Pará: Left bank of middle Tapajós River, Itaituba (05°02'S 56°53'W), INPA-H38116, Left bank of middle Tapajós River, Itaituba (04°39'S 56°37'W), INPA-H38117. Left bank of middle Tapajós River, Itaituba (04°40'S 56°37'W), INPA-H38118. INPA-H = Collection of Amphibians and Reptiles of the Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil.



Leandro J.C.L. Moraes has a B.S. in biology from the Universidade Federal de São Carlos (campus Sorocaba, Brazil) and a Master's degree in ecology at Instituto Nacional de Pesquisas da Amazônia–INPA, Manaus, AM, Brazil. Currently he is a researcher at the same institution. His research fields include diversity, taxonomy, biogeography, evolution, and conservation of Neotropical amphibians and reptiles.



Dante Pavan has a B.S. in biology from the Universidade de São Paulo – USP, São Paulo, Brazil and a Master's and Doctoral degrees in zoology at the same institution. He works mainly with environmental impact studies, analyzing and predicting the anthropic impacts on amphibians and reptiles from diverse Brazilian biomes.