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## Urban growth threatens the lowland Amazonian Manaus harlequin frog which represents an evolutionarily significant unit within the genus Atelopus (Amphibia: Anura: Bufonidae)

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#### Abstract

The Manaus harlequin frog is an evolutionarily significant clade within the Atelopus hoogmoedi species complex. Analyses of 16S and COI concatenated sequences support Atelopus from the Manaus region as an evolutionary significant unit, sister of all species of a Guiana Shield clade. A previous study showed that subtle changes in stream characteristics influence the Manaus harlequin frog occurrence and density variation at local-scale in a reserve on the outskirts of Manaus. As deforestation is approaching areas where the Manaus harlequin frog occurs, we asked how site and landscape heterogeneity influence the geographic boundaries, occurrence patterns, and density variation of the Manaus harlequin frog. We searched for the frog in 80 plots that measured 250 m by 4 m on banks of first- to third-order streams during the rainy seasons in 2012-2013 and 2016-2019. The plot distribution covered all likely areas of occurrence of the Manaus harlequin frog and extended to the areas where it is substituted by its geographically closest relative on the Guiana Shield. Ecological drivers related to climate, flooding events, and forest structure apparently restrict the Manaus harlequin frog to a patchy distribution in a narrow portion of the interfluve between the Negro and Uatuma Rivers. Densities of individuals varied in response to subtle changes in floodplain and stream characteristics. The Manaus harlequin frog is associated with a very specific habitat that is directly affected by the growth of Manaus, the largest city in Amazonia. We conclude that it is endangered and urgent actions are required for its conservation.

#### KEYWORDS

Central Amazonia, environmental heterogeneity, extinction risk, habitat modification, harlequin frog

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## 1 | INTRODUCTION

The genus Atelopus is a clade of bufonid frogs that formerly occurred throughout the Andes highlands ranging eastwards into the Guiana Shield and central Amazonian lowlands and north in central America. However, most of the more than 96 species of Atelopus are now extinct or in decline, mainly because of infection by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) for those inhabiting mid to high elevations and due to habitat loss for lowland species (La Marca et al., 2005). Despite *Bd* being generally less severe in warmer lowland areas (Bacigalupe et al., 2019), these portions of the landscape in Amazonia are often deforested for human use and disturbed areas seem to be suitable for *Bd* invasion in central Amazonia (Becker, Rodriguez, Lambertini, Toledo, & Haddad, 2015), and other tropical regions (Bolom-Huet, Pineda, Días-Fleischer, Muñoz-Alonso, & Galindo-González, 2019).

About ten species of *Atelopus* are known from lowland Amazonia (Lötters, 1996), but the taxonomy of these species is not well established (Lötters, Hass, Schick, & Böhme, 2002; Lötters et al., 2011), and we will provide phylogenetic evidence that *Atelopus* from the Manaus region form an evolutionary significant unit (ESU), sister of all species of the Guiana Shield clade of *Atelopus*. The Manaus harlequin frog also differs considerably from its closest congener *Atelopus hoogmoedi* Lescure, 1974 (Hoogmoed harlequin toad—Uatuma River populations) in morphology (e.g., mean snout-vent length of 11 adult males from Manaus was 21.83 mm, compared to = 29.55 mm for 10 *A. hoogmoedi* adult males) and call traits (e.g., dominant frequency of calls of frogs from Manaus is 3088–3610 Hz, whereas it is 2614–2883 Hz in *A. hoogmoedi*).

Very little is known about local adaptations of the species of *Atelopus* that could affect their distributions. The distribution of the Manaus harlequin frog within Reserva Florestal Adolpho Ducke (hereafter Reserva Ducke), a protected area on the outskirts of Manaus, Amazonas, Brazil, is restricted to the eastern catchments, apparently because tadpoles of the species are sensitive to pH (Jorge, Simões, Magnusson, & Lima, 2016), but the limits to its distribution and the factors that determine its abundance at wider scales are unknown.

The fauna of the Manaus area has elements that do not occur further north on the Guiana Shield. For example, one species of primate, the Pied tamarin *Saguinus bicolor* Spix, 1823, is considered endangered because its distribution coincides almost completely with that of Manaus (Farias, Wagner, Gordo, & Hrbek, 2015). Manaus is the largest city in the Amazonia and has undergone rapid growth in recent years (Puppim de Oliveira et al., 2011). We studied the distribution and abundance of the Manaus harlequin frog to determine the factors that limit its distribution and to evaluate the threats to its effective conservation. We aimed at answering the following questions: What is the extent of the geographic range of the Manaus harlequin frog? How fragmented are concentrations of the Manaus harlequin frog within its occupancy area? Do the IUCN Red List Categories and Criteria (IUCN, 2012) used to assess species conservation status (extent of occurrence, occupancy area, quality of habitat, and number of subpopulations declining) indicate that the Manaus harlequin frog is endangered?

## 2 | MATERIAL AND METHODS

#### 2.1 | Study area

The Negro-Uatuma interfluve (Figure 1) is underlain by sedimentary rocks from the Alter do Chao Formation and Trombetas Group (a geological Group is composed of different formations), and crystalline base rocks from the Guiana Shield, which support heterogeneous soils (Quesada et al., 2010). This Amazonian landscape is covered mainly by submontane (Guiana Shield/Trombetas group) and low-land ombrophilous dense forests (Trombetas group/Alter do Chao; IBGE, 2004). The interfluve relief comprises alluvial floodplains, plateaus, and small hills ranging from 3 to 230 m a.s.l. (CPRM, 2010). Annual mean temperature ranged from 26.4 to 27.6°C in the period from 1970 to 2000, and rainfall varied from 2,028 to 3,006 mm between localities within the Negro-Uatuma interfluve in the same period (Fick & Hijmans, 2017). This interfluve has been subject to severe forest fragmentation since 1972 (Figure 2).

#### 2.2 | Biological Sampling

Surveys were undertaken in 250-m by 4-m plots extended along stream banks that were divided into 25 subplots (see Magnusson et al., 2013: 115-116). Eighty plots were installed on banks of firstto third-order streams distributed in four tributaries of the Negro River (the lower, middle, and upper courses of Taruma-Açu, Cuieiras and Apuau Rivers and the upper course of Camanau River) and four tributaries of the Amazon River (Puragueguara, Preto, Urubu, and Uatuma Rivers). We used acoustic and visual searches focused on leaf litter, roots, fallen trunks, and the bases of stemless palms and herbs at a maximum distance of four meters from the stream margin. When no individual was detected, recorded calls of the Manaus harlequin frog were played at the middle of each subplot for two minutes (three at 15-s intervals) because observations during previous studies (Jorge et al., 2016; Lima et al., 2006) indicated that Manaus harlequin frog males usually call when another male calls nearby. Only one individual per subplot was recorded in order to create an index of density (minimum = 0; maximum = 25). We searched for the frogs between 0700-1000 hr and 1500-1700 hr, the periods when males call and are more detectable. Sampling was undertaken during the rainy season (December-April) in 2012-2013, 2016-2017, 2017-2018, and 2018-2019.

### 2.3 | Phylogenetic assessment

To confirm the distinct taxonomic status of the Manaus harlequin frog, we used fragments of 16S ribosomal RNA gene (16S) and cytochrome c



**FIGURE 1** An altitudinal map (3–312 m a.s.l.) displaying the location of the investigated area in Brazil, the investigated area (green polygon), the study area (black polygon), and the Manaus- harlequin-frog geographic range (about 4,500 km<sup>2</sup>—white-and-black dashed polygon—minimum convex polygon). Manaus harlequin frog presences are represented by white circles (symbols are proportional to the density index for the Manaus harlequin frog); Manaus harlequin frog absences (black triangles); *Atelopus hoogmoedi* presences (black squares); Manaus city center (black star), the rivers, and a lake (blue lines and numbers) and the Trombetas group soils between the Guiana Shield and the Alter do Chao formation (black dashed polygon). Location of four individuals of the Manaus harlequin frog represented by white circles with \* (middle Cuieiras River and upper Taruma-Açu River) and two individuals of *A. hoogmoedi* from REBIO Uatumã represented by a black square with \*, collected for genetic analysis

oxidase subunit I gene (COI) from four specimens of the Manaus harlequin frog represented by white circles with \* in Figure 1 (two from the middle course of the Cuieiras River – 2°33'32.40" S, 60°13'48.36" W; two from the upper course of the Taruma-Açu River – 2°38'31.20" S, 60°5'45.6" W) and two of the closest populations from the sister clade, *A. hoogmoedi*, from the Reserva Biológica do Uatumã, Presidente Figueiredo, Amazonas, represented by a black square with \* in Figure 1 (1°46'48.07" S, 59°15'4.81" W). Marker sequences of these specimens were obtained in the course of the current study and are referred to by ID numbers one to six in Table 1. We also used 14 sequences of 10 species (four sequences of *A. hoogmoedi* from different localities), obtained from previously published data (ID numbers seven to 20 in



FIGURE 2 Degradation of the Manaus-harlequin-frog occurrence area (blue polygon) and occupancy habitats (blue circles) and borders of Reserve Florestal Adolpho Ducke (Reserva Ducke—black polygon), on the outskirts of Manaus, Amazonas, Brazil, from 1972 to 2019, where the species has been most intensively studied

Table 1), from the National Centre for Biotechnology Information's GenBank (Table 1 for accession number). Specimens were collected under permission number 56,759 from the Sistema de Autorização e Informação em Biodiversidade do Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO/ICMBIO) and were deposited in the herpetological section of the Zoological Collections of the Instituto Nacional de Pesquisas da Amazônia–INPA (Table 1 for specimens' vouchers–ID one to six). This study was approved by the INPA

ethics committee (registration number 002/2017–Comissão de Ética no Uso de Animais–CEUA/INPA).

For our samples (Table 1–ID one to six), we immersed 5 mg of muscle tissue in phenol/chloroform solution and left it for 24 hr in a thermocirculator for DNA extraction, followed by purification procedures using Qlamp® DNA FFPE Tissue Kit, following the manufacture's recommendation (Qiagen). 16S rDNA gene fragments were amplified and sequenced using 16Sar

**TABLE 1** Species used in the phylogenetic assessment, the accession number of 16S and COI sequences in GenBank, the voucher number, and the country where the individuals sequenced were collected. Sequences for specimens one to six was generated in the course of the current study

ID	Species	GenBank 165/COI	Voucher	Country
1	Manaus halerquin frog Atelopus sp.	MT176236/MT184269	INPA-H041289	Brazil
2	Manaus halerquin frog Atelopus sp.	MT176237/MT184270	INPA-H041290	Brazil
3	Manaus halerquin frog Atelopus sp.	MT176238/MT184271	INPA-H041291	Brazil
4	Manaus halerquin frog Atelopus sp.	MT176239/MT184272	INPA-H041292	Brazil
5	Atelopus hoogmoedi REBIO Uatumã	MT176240/MT184273	INPA-H041293	Brazil
6	Atelopus hoogmoedi REBIO Uatumã	MT176241/MT184274	INPA-H041294	Brazil
7	Atelopus hoogmoedi Lescure, 1974	JQ742148	IRSNB15781	Guyana
8	Atelopus hoogmoedi	JQ742149	IRSNB14477	Guyana
9	Atelopus barbotini Lescure, 1981	EU672971	-	French Guiana
10	Atelopus franciscus Lescure, 1974	JQ742150	PK3306	French Guiana
11	Atelopus (spumarius) hoogmoedi	DQ283260	BPN754UTA	French Guiana
12	Atelopus hoogmoedi	EU672972	-	French Guiana
13	Atelopus flavescens Duméril & Bibron, 1841	EU672970	-	French Guiana
14	Atelopus seminiferus Cope, 1874	EU672976	-	Peru
15	Atelopus spumarius Cope, 1971	EU672977	-	Peru
16	Atelopus pulcher Boulenger, 1882	EU672973	KU 211678	Peru
17	Atelopus bomolochos Peters, 1973	GU252227	KU 217468	Ecuador
18	Atelopus peruensis Gray & Cannatella, 1985	GU252229	KU 211631	Peru
19	Atelopus spurrelli Boulenger, 1914	EU672975/DQ502895	MHNUC 273	Colombia
20	Atelopus loettersi De la Riva, Castroviejo-Fisher, Chaparro, Boistel, & Padial, 2011	EU672980	-	Peru

Note: Sequences of specimens from seven to 20 were obtained from previously published data. GenBank accession number is referred to in Figure 3 after species names, and specimens "ID" are referred to by the "ID" column in Table S4.



FIGURE 3 Bayesian phylogenetic inference based on 16S (20 individuals of 11 species and the Manaus harlequin frog) and COI (seven individuals of two species and the Manaus harlequin frog) concatenated sequences (20 sequences of 11 species and the Manaus harlequin frog) showing the relationships between the Manaus harlequin frog and Atelopus species from the flavescens-spumarius clade (Guiana Shield, western Amazonia lowlands and eastern Andean foothills) and four species from the bomolochos-tricolor clade (eastern and western Andean foothills and Andean highlands). Posterior probabilities are shown on the right sides of nodes and divergence times in millions of years on the left. The scale on the bottom represents genetic distances in substitution per nucleotide, a temporal scale in million years ago (Mya), and the geologic epics represented by different colors

(forward, 5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (reverse, 5'-CCGGTCTGAACTCAGATCACGT-3') primers (Palumbi, 1996). COI gene fragments were amplified and sequenced using AnF1 (forward, 5'-ACHAAYCAYAAAGAYATYG-3') and AnR1 (reverse, 5'-CCRAARAATCARAADARRTGTTG-3') primers (Jungfer et al., 2013; Lyra, Haddad, & Azeredo-Espin, 2017), and M13F (forward, 5'-TGTAAAACGACGGCCAGT-3') and M13R (reverse, 5'-CAGGAAACAGCTATGAC-3') extension tails (Messing, 1983). PCR reactions were carried out with 20 µl final volume [3 µl DNA  $(2 \text{ ng/}\mu\text{l}) + 5 \mu\text{l} 4X$  Platinum buffer (Applied Biosystems) + 0.2  $\mu\text{l}$  Tag DNA polymerase (5 U/ $\mu$ I) + 5  $\mu$ I of each primer (2  $\mu$ M) + 1.8  $\mu$ I H<sub>2</sub>O] under the following amplifications conditions: for 16S-3 min hot start at 95°C (denaturation) followed by 35 cycles at 95°C for 30 s (heating), 50°C for 30 s (annealing), and 60°C for 1 min (extension), concluded by 72°C for 10 min (final extension); and for COI-3 min hot start at 95°C (denaturation), 5 cycles at 95°C for 30 s (heating), 48°C for 30 s (annealing), and 60°C for 1 min (extension), followed by 30 cycles at 95°C for 30 s (heating), 50°C for 30 s (annealing), and 60°C for 1 min (extension) with a final extension step at 60°C for 5 min. The 16S and COI PCR products (~600 and ~700 bp, respectively) were checked on 2% agarose gel electrophoresis to

evaluate PCR success and purified using the ExoSAP-IT kit (Ecoli) following the standard protocol. We used 10 µl final volume of purified PCR products [3 µl DNA + 3 µl of BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher) + 4  $\mu$ I H<sub>2</sub>O] for forward and reverse terminal reactions following manufacturer recommendations, with 25 cycles at the first stage (95°C for 10 s, 50°C for 5 min, and 60°C for 4 min) and 15°C until the end, following by precipitation according to the standard protocol. Forward and reverse sequences were resolved in an ABI 3,500 automatic sequencer (Applied Biosystems).

The extremities of the six alignments obtained in the course of the current study were trimmed to final alignment size of 567 (five sequences) and 512 bp (one alignment) for 16S and 653 bp for COI to avoid errors in phylogenetic reconstruction associated with missing data. All sequences were edited and aligned in Geneious 4.8.2 using its own algorithm (Kearse et al., 2012). The most probable evolutionary model and the best partition schema for the 20 concatenated and aligned 16S and COI sequences (1,274 bp-available in TreeBase under URL http://purl.org/phylo/treebase/phylows/study/TB2:S26036) were selected by Bayesian Information Criteria (BIC) and Akaike Information Criteria (AIC) using PartitionFinder2 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016), as follows: 16S = TRN + G + X; COI\1 = TRNEF; COI\2 = HKY + X; COI\3 = HKY + X. The gene tree from the 16S and COI concatenated sequences was inferred through Bayesian Inference (BI) using Beast 2.5 (Bouckaert et al., 2019). Four runs of 10 million generations were calculated with the Metropolis coupled Markov chain Monte Carlo algorithm (MCMC), and each run had four Markov chains. Probabilities were sampled every 1,000 generations. We used three priors for divergence times as normal distributions with means and 95% confidence intervals following Kok et al. (2017) to calibrate and estimate divergence times in our phylogeny, as follows: 18.7 (8.5-28.9) million years ago (Mya-Glossary of abbreviations available in Appendix 1) assigned to the root; 5.25 (0-10.5) Mya to the node splitting Atelopus spurrelli from A. bomolochos and A. peruensis; and 2.51 (0.1-4.9) Mya to the node splitting the two major clades within Guiana Shield. Runs convergence was assessed in Tracer 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). A consensus tree was calculated after discarding the first 25% of trees using LogCombiner v2 6.0 (Rambaut & Drummond, 2019a), and a Maximum Credibility Clade tree was built in TreeAnotator v2 6.0 (Rambaut & Drummond, 2019b).

#### 2.4 | Environmental variables

To represent environmental heterogeneity likely to influence occurrence patterns of the Manaus harlequin frog, we used seven raster layers as landscape-scale variables: (a) precipitation seasonality in percent (PS, 1-km<sup>2</sup> grain; Fick & Hijmans, 2017); (b) Walsh Index, which describes the duration and severity of the dry season (WI, 1-km<sup>2</sup> grain; Amaral, Costa, Arasato, Ximenes, & Rennó, 2013); (c) percentage soil silt content (SC, 1-km<sup>2</sup> grain; Hengl et al., 2014); (d) JERS in square kilometer, which describes flooded-area extent in the high-water seasons (100-m<sup>2</sup> grain; Hess et al., 2015); (e) live aboveground biomass (MG C ha<sup>-1</sup>)/forest structure (LAB, 500-m<sup>2</sup> grain; Baccini et al., 2012); (f) altitude (m a.s.l.) (AL, 90-m<sup>2</sup> grain; Amaral et al., 2013); and height above the nearest drainage (HAND, 30-m<sup>2</sup> grain; created from a SRTM raster layer using the Hydrology tools of the ArcMap 10.3.1 package Spatial Analyst). All landscape variables were cropped to the study area, projected as Datum WGS 84 and maintained at the original scale for subsequent extraction using the "dismo" R package "extract" tool (Hijmans, Phillips, Leathwick, & Elith, 2017). The values refer to the geographic coordinates obtained at the beginning (1.5 m from stream margin) of each plot using a Garmin 64s GPS (error ± 3 m/Datum WGS 1984).

To represent local heterogeneity of stream banks and streams, we used three variables: (a) pH determined using a calibrated digital pHmeter—the electrode was placed in the middle of the stream and water column, at the beginning of each plot; (b) width (W), depth (D), and water velocity (V) determined at the same point as pH, in order to calculate the stream discharge (*SD*), by applying the formula *SD* m<sup>3</sup>/s =  $W \times D \times V$ . Water velocity was estimated from the time a 0.5-g silicon ball took to travel one meter in the stream; and (c) canopy openness (CO) obtained at six equidistant points 1.5 m from the stream margin using a spherical densiometer, following Lemmon (1956).

The local and landscape variables were not highly correlated (Pearson's r < 0.7 in all cases), nor did they show multicollinearity (VIF < 3 in all cases). We detected spatial autocorrelation (Moran's Index) associated with CO at distances less than 1.5 km (p < .003), although it was not detected in the response variable (Manausharlequin-frog density) or in other local predictor variables (pH and stream discharge) at any distance class (p < .05). As autocorrelation induces type 1 errors in statistical tests only when it occurs in both response and predictor variables (Legendre et al., 2002), no autocorrelation adjustments were necessary. Descriptive statistics and the correlation matrix are given in Tables S1–S3. All variables, geographic coordinates, and metadata are available at https://ppbio data.inpa.gov.br/metacatui/data/Rafael Jorge.

#### 2.5 | Distribution and density-variation analyses

We recorded 22 presences and 58 absences in the surveys for the Manaus harlequin frog. Nevertheless, only 17 presences were included in a logistic model (LM), in order to maintain a minimum distance of 1.5 km among sampling locations. Sites separated by lesser distances are likely not to be independent because of movement of individuals. We defined the occurrence area of the Manaus harlequin frog as that enclosed in a minimum convex polygon containing the occupied sites. Sites outside this area might be unoccupied for biogeographic reasons unrelated to ecological suitability. We used the 30 sampling locations only within this area in a generalized linear model (GLM). As we observed a unimodal relationship between the Manaus harlequin frog's density with pH and SD gradients, we used quadratic transformation ( $y = a + b_1 + c_1^2 + e$ ) of the GLM standard equation, to best fit the data. We applied the LM to estimate how much of the variation in presence (n = 17)/absence (n = 58) of the Manaus harlequin frog could be explained by seven landscape-scale variables (Presence/Absence) =  $a + b_1 * PS + b_2 * WI + b_3 * SC + b_4$ \* LAB +  $b_5$  \* JERS +  $b_6$  \* AL +  $b_7$  \* HAND + e). We used the GLM, assuming a negative binomial distribution in order to estimate how much of the density variation was explained by stream-bank and stream characteristics (Relative density =  $a + b_1 * SD + c_1 * I(SD^2) + b_2$ \* pH +  $c_2$  \* I (pH<sup>2</sup>) +  $b_3$  \* CO + e).

#### 3 | RESULTS

#### 3.1 | Taxonomic status

Our phylogenetic tree inferred from 1,274 bp concatenated and aligned 16S (20 individuals) and COI (seven individuals) sequences supported the Manaus harlequin frog as an evolutionary significant unit (Posterior Probability = 1), which diverged 3 Mya from of all its sister species of a Guiana Shield clade, including A. *hoogmoedi* (Figure 3). This confirms that *Atelopus* from the Manaus region represents a candidate species waiting for formal description (Table S4 for genetic distances), but much more information is necessary to

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formally describe the species and some of the authors of this paper are in the process of doing so.

## 3.2 | Density variation and geographic range

The relative density index (number of segments occupied per plot) varied from 0 to 11. The Manaus harlequin frog was encountered only in the Cuieiras-Urubu interfluve, at intermediate altitudes in the Alter do Chao Formation. However, it was not recorded along the entire courses of the five tributaries within its narrow geographic range. It was recorded in the lower, middle, and upper course of the Puraquequara River, but only beside headwater streams. It occurred in middle and upper courses of the Preto, Urubu, and Cuieiras Rivers and the upper course of the Taruma-Açu River (Figure 1). Two Negro River tributaries (Curiau and Jauperi) were not sampled, as they are covered mainly by open forests and savanna-like vegetation from which no Manaus harlequin frogs have been recorded.

# 3.3 | Environmental influence on occurrence pattern and density variation

The logistic model explained 51% of occurrences (SE = 18.61, *df* = 67, *z* = 2.77, *p* = .005, *pseudo*  $R^2$  = 0.51; Figure 4). The Manaus harlequin frog was associated with a narrow portion of the Negro-Uatuma interfluve with low precipitation seasonality (SE<sub>b1</sub> = 0.30, *b*<sub>1</sub> = -0.79, *p*<sub>b1</sub> = 0.009) and less marked dry season (SE<sub>b2</sub> = 0.30, *b*<sub>2</sub> = -0.89, *p*<sub>b2</sub> = 0.003). The Manaus harlequin frog was only found in sections of streams and rivers with little flooding (SE<sub>b5</sub> = 0.09, *b*<sub>5</sub> = -0.16, *p*<sub>b5</sub> = 0.09) and in well-structured forests (SE<sub>b4</sub> = 0.009, *b*<sub>4</sub> = 0.01, *p*<sub>b4</sub> = 0.02). Silt content in soil (SE<sub>b3</sub> = 0.21, *b*<sub>3</sub> = 0.11, *p*<sub>b3</sub> = 0.6), HAND (SE<sub>b7</sub> = 0.05, *b*<sub>7</sub> = 0.02, *p*<sub>b7</sub> = 0.6) and altitude (SE<sub>b6</sub> = 0.01, *b*<sub>6</sub> = 0.01, *p*<sub>b6</sub> = 0.29) did not contribute significantly to the model.

The generalized linear model explained 41% of relative density variation (SE = 8.9, *df* = 24, *z* = -2.44, *p* = .01, *pseudo*  $R^2$  = 0.41). The Manaus harlequin frog was more abundant on banks of streams with pH ranging from 5 to 6 (SE<sub>b1</sub> = 3.19, *b*<sub>1</sub> = 7.53, *p*<sub>b1</sub> = 0.01; SE<sub>c1</sub> = 0.28,

 $c_1 = -0.65$ ,  $p_{c1} = 0.02$ ) and stream discharge between 0.2 and 0.5 m/s<sup>3</sup> (SE<sub>b2</sub> = 2.66,  $b_2 = 6.49$ ,  $p_{b2} = 0.01$ ; SE<sub>c2</sub> = 3.83,  $c_2 = -9.79$ ,  $p_{c2} = 0.01$ ). The lowest densities (0–3) were found at the extreme values of these gradients (Figure 5). Relative densities were higher in forests with more open canopies within ombrophilous dense forests (SE<sub>b3</sub> = 0.02,  $b_3 = 0.04$ ,  $p_{b3} = 0.05$ ).

## 4 | DISCUSSION

Our phylogenetic assessment shows that an undescribed species is already under extinction risk. The Manaus harlequin frog was considered to be part of a wide-spread species due to its superficial morphological similarity to an Andean species, the Pebas stubfoot toad (Atelopus spumarius Cope, 1871), which was attributed a Vulnerable conservation status due to its broad geographic distribution (Azevedo-Ramos et al., 2010). However, previous studies (Cocroft, Mcdiarmid, & Ruiz-Carranza, 1990; Lötters et al., 2002) suggested that A. spumarius should be considered a species complex (Frost, 2020), and our analyses show that the Manaus harleguin frog is more related to species distributed on the Guiana Shield that occupy distinct topographic, climatic and vegetational conditions. This clade seems to be isolated from its closest relatives by unsuitable environments underlain by Trombetas Group soils between the Guiana Shield and the Alter do Chao Formation. The divergence time between Manaus harlequin frog and its sister species from the Guiana Shield (3 Mya) coincides with the end of Miocene glaciations, which may be related to diversification events under a Disturbance-Vicariant hypothesis (Lötters et al., 2010) suggested to explain the diversification of Atelopus species from Guiana Shield during the last Glaciations (Noonan & Gaucher, 2005).

#### 4.1 | Environmental associations

FIGURE 4 Plots indicating the relationships of four environmental variables that significantly influence Manaus-harlequin-frog occurrence probability, as follows: (a) precipitation seasonality; (b) Walsh Index, representing the dry season severity; (c) JERS, representing the flooded-area extent in the high-water seasons; and (d) live aboveground biomass, representing forest structure



The Manaus harlequin frog is restricted to a narrow portion of the interfluve with little precipitation seasonality and a short dry season. Generally, *Atelopus* species are very sensitive to high temperatures

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**FIGURE 5** Plots indicating the relationships between local variables and Manaus-harlequin-frog density index variation: (a) stream pH; (b) stream discharge; and (c) canopy openness

(Peters, 1973), and severe dry seasons could presumably kill adults or limit egg and tadpole development. Many *Atelopus* species are exclusive to narrow altitudinal zones (Lötters, 1996). These are usually associated with climatic variables, but not in our study area. The Manaus harlequin frog was recorded at altitudes between 61 and 130 m a.s.l., which is a range too small to be associated with large climatic variation. More likely, it is because lower areas have more sandy soils (Schaefer et al., 2017) and more acid water.

The Manaus harlequin frog inhabits banks of streams with a wide range of pH, but higher densities were observed on banks of streams with less acid waters. Very acid pHs (< 4) are related to reduced growth or even death of larvae and adults of amphibians not adapted to those conditions (Barth & Wilson, 2010). The Manaus harlequin frog is more abundant on stream banks with intermediate discharges; very small streams may not have suitable habitats for its tadpoles, and high discharges of large streams may wash tadpoles away. The Manaus harlequin frog occurs only in well-structured forests, but the densities are low where the canopy is continuous.

#### 4.2 | Threats and conservation status

The conservation threats associated with a restricted distribution are exacerbated by the fact that the Manaus-harlequin-frog distribution coincides in great part with Manaus, the largest city in Amazonia. Manaus has experienced high rates of urban development and the area occupied by the city and surrounding deforested areas increased by 29% from 2000 to 2010 (IBGE, 2000; 2010). Direct loss of habitat is not the only threat to the Manaus harlequin frog. Pollution and forest-fragment border effects may reduce the capacity of individuals to resist infection by the pathogenic fungus *Bd* (Becker et al., 2015), which has been devastating for other *Atelopus* species (La Marca et al., 2005). Although *Bd* has not been found in the Manaus region, an ongoing study indicates that the Manaus harlequin frog shows high mortality rates in case of *Bd* infection (Lambertini et al., in prep.). There are no published studies of *Bd* distribution in central Amazonia.

Our results indicate that, unless action is taken to minimize threats due to urban expansion, the Manaus harlequin frog is likely to become extinct in the near future. Based on IUCN criteria (IUCN, 2012), it should be assigned the conservation status Endangered, but it is likely to be critically endangered in the near future. The extent of occurrence of this clade is less than 5,000 km<sup>2</sup> (~4,500 km<sup>2</sup>-minimum convex polygon); its occupancy area is severely fragmented by roads, human settlements, and unsuitable habitats; its extent of occurrence is inferred to have been reduced and may be undergoing continuing decline in the headwaters of the Urubu and Cuieiras Rivers due to human use; its occupancy area is inferred to be reduced and declining on the right bank of the upper Puraquequara and middle Cuieiras Rivers; the Manaus harlequin frog habitat quality is declining as urban areas are expanding over pristine forests of the headwaters of the catchments occupied by it without any environmental-protection plan. Some areas that we planned to sample repeatedly were deforested during the few years of this study. The right bank of the upper course of the Puraquequara River, which was almost certainly occupied by the Manaus harlequin frog in the past, now has only highly polluted streams within rural unplanned human settlements and land use. Most of the geographic range of the Manaus harlequin frog coincides with that of Saguinus bicolor, indicating that the frog is probably as endangered as the primate and should have similar conservation status. Both species are limited to the Alter do Chao Formation, and the Manaus harlequin frog has an even smaller distribution than that of S. bicolor.

Therefore, there is an urgent need to design protective measures for the conservation of the Manaus harlequin frog and the Negro-Uatuma Rivers interfluve landscape as a whole.

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#### REFERENCES

- Amaral, S., Costa, C. B., Arasato, L. S., Ximenes, A. C., & Rennó, C. D. (2013). AMBDATA: Variáveis ambientais para Modelos de Distribuição de Espécies (SDMs). In: Simpósio Brasileiro de Sensoriamento Remoto, 16, Foz do Iguaçu, Paraná, Brasil. Retrieved from http://www.dpi.inpe.br/ AmBdata.
- Azevedo-Ramos, C., Ron, S., Coloma, L. A., Bustamante, M. R., Salas, A., Schulte, R., ... Hoogmoed, M. (2010). Atelopus spumarius. In IUCN Red List of Threatened Species. Version 2012.1. Retrieved from http:// www.iucnredlist.org.
- Baccini, A., Goetz, S. J., Walker, W. S., Laporte, N. T., Sun, M., Sulla-Menashe, D., ... Houghton, R. A. (2012). Estimated carbon dioxide emissions from tropical deforestation improved by carbon-density maps. *Nature and Climate Change*, 2, 182–185. https://doi. org/10.1038/NCLIMATE1354
- Bacigalupe, L. D., Vasquez, I. A., Estay, S. A., Valenzuela-Sanchez, A., Alvarado-Rybak, M., Piñafiel-Ricaurte, A., ... Soto-Azat, C. (2019). The amphibian-killing fungus in a biodiversity hotspot: Identifying and validating high-risk areas and refugia. *Disease Ecology*, 10, e02724. https://doi.org/10.1002/ecs2.2724
- Barth, B. J., & Wilson, R. S. (2010). Life in acid: Interactive effects of pH and natural organic acids on growth, development and locomotor performance of larval striped marsh frogs (*Limnodynastes peronii*). Journal of Experimental Biology, 213, 1293–1300. https://doi. org/10.1242/jeb.028472
- Becker, C. G., Rodriguez, D., Lambertini, C., Toledo, L. F., & Haddad, C. F. B. (2015). Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography*, *39*, 954–960. https://doi.org/10.1111/ ecog.02055
- Bolom-Huet, R., Pineda, E., Díaz-Fleischer, F., Muñoz-Alonso, A. L., & Galindo-González, J. (2019). Known and estimated distribution in Mexico of Batrachochytrium dendrobatidis, a pathogenic fungus of amphibians. Biotropica, 51, 731–746. https://doi.org/10.1111/ btp.12697

Bouckaert, R., Timothy, G., Vaughan, J., Barido-Sottani, S., Duchêne, M. F., Alexandra, G., ... Alexei, J. D. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 15, e1006650. https://doi.org/10.1371/journ al.pcbi.1006650

- Cocroft, R. B., Mcdiarmid, R. O. Y. W., & Ruiz-Carranza, P. M. (1990). Vocalizations of eight species of *Atelopus* (Anura: Bufonidae) with comments on communication in the genus. *Copeia*, *3*, 631–643. https://doi.org/10.2307/1446428
- CPRM (Serviço Geológico do Brasil) (2010). *Mapa Geodiversidade do Amazonas*. Manaus, Brasil: CPRM Companhia de Pesquisa de Recursos Minerais. Retrieve from http://www.cprm.maps.arcgis. com.
- Farias, I. P., Santos, W. G., Gordo, M., & Hrbek, T. (2015). Effects of forest fragmentation on genetic diversity of the critically endangered primate, the Pied Tamarin (*Saguinus bicolor*): Implications for conservation. *Journal of Heredity*, 106, 512–521. https://doi.org/10.1093/ jhered/esv048
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-Km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302–4315. https://doi.org/10.1002/joc.5086
- Frost, D. R. (2020). Amphibian Species of the World: An online reference. Version 6.1. New York, NY: American Museum of Natural History. doi: https://doi.org/10.5531/db.vz.0001. Retrieve from https://amphi biansoftheworld.amnh.org/index.php.
- Hengl, T., de Jesus, J. M., MacMillan, R. A., Batjes, N. H., Heuvelink, G. B. M., Ribeiro, E., ... Gonzalez, M. R. (2014). SoilGrids1km – global soil information based on automated mapping. *PLoS ONE*, *9*, e105992. https://doi.org/10.1371/journal.pone.0105992
- Hess, L. L., Melack, J. M., Affonso, A. G., Barbosa, C., Gastil-Buhl, M., & Evelyn, M. L. M. (2015). Wetlands of the lowland Amazon basin: Extent, vegetative cover, and dual-season inundated area as mapped with JERS-1 Synthetic Aperture Radar. Wetlands, 35, 745–756. https://doi.org/10.1007/s13157-015-0666-y
- Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2017). dismo: Species Distribution Modelling. Retrieve from, https://CRAN.R-project.org/ package=dismo.
- IBGE (Instituto Brasileiro de Geografia e Estatística) (2000). Censo Demográfico 2000. Brasília, Brasil: IBGE (Instituto Brasileiro de Geografia e Estatística).
- IBGE (Instituto Brasileiro de Geografia e Estatística). (2004). *Mapa da Vegetação do Brasil* 1:5.000.000. Brasília, Brasil: IBGE (Instituto Brasileiro de Geografia e Estatística). Retrieve from http://mapas. ibge.gov.br/tematicos/vegetacao.
- IBGE (Instituto Brasileiro de Geografia e Estatística). (2010). XII Censo Brasil. Brasília, Brasil: IBGE (Instituto Brasileiro de Geografia e Estatística).
- IUCN (2012). IUCN Red List Categories and Criteria: Version 3.1, 2nd ed. Gland, Switzerland and Cambridge, UK: IUCN.
- Jorge, R. F., Simões, P. I., Magnusson, W. E., & Lima, A. P. (2016). Finescale habitat heterogeneity explains the local distribution of two Amazonian frog species of concern for conservation. *Biotropica*, 48, 694–703. https://doi.org/10.1111/btp.12333
- Jungfer, K.-H., Faivovich, J., Padial, J. M., Castroviejo-Fisher, S., Lyra, M. M., Berneck, B. V. M., ... Haddad, C. F. B. (2013). Systematics of spinybacked treefrogs (Hylidae: Osteocephalus): An Amazonian puzzle. Zoologica Scripta, 42, 351–380. https://doi.org/10.1111/zsc.12015
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. https://doi. org/10.1093/bioinformatics/bts199
- Kok, P. J. R., Ratz, S., MacCulloch, R. D., Lathrop, A., Dezfoulian, R., Aubret, F., & Means, D. B. (2017). Historical biogeography of the palaeoendemic toad genus *Oreophrynella* (Amphibia: Bufonidae)

<sup>10</sup> WILEY-

sheds a new light on the origin of the Pantepui endemic terrestrial biota. *Journal of Biogeography*, 45, 26–36. https://doi.org/10.1111/jbi.13093

- La Marca, E., Lips, K. L., Lötters, S., Puschendorf, R., Ibáñez, R., Rueda-Almonacid, J. V., ... Young, B. E. (2005). Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: Atelopus). Biotropica, 37, 190–201. https://doi. org/10.1111/j.1744-7429.2005.00026.x
- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773. https://doi. org/10.1093/molbev/msw260
- Legendre, L., Dale, M. R. T., Fortin, M.-J., Gurevitch, J., Hohn, M., & Myers, D. (2002). The consequences of spatial structure for the design and analysis of ecological field surveys. *Ecography*, 25, 601–615. https://doi.org/10.1034/j.1600-0587.2002.250508.x
- Lemmon, P. E. (1956). A spherical densiometer for estimating forest overstory density. *Forest Science*, 2, 314–320. https://doi.org/10.1093/ forestscience/2.4.314
- Lima, A. P., Magnusson, W. E., Menin, M., Erdtmann, L. K., Rodrigues, D. J., Keller, C., & Hödl, W. (2006). Guide to the frogs of Reserva Ducke - central Amazonia, 2nd ed. Manaus: Áttema Design Editorial.
- Lötters, S. (1996). The neotropical toad genus Atelopus. Checklist, biology and distribution, 1st ed. M. Vences, & F. Glaw Glaw. Köln, Germany: Verlags GBR.
- Lötters, S., Haas, W., Schick, S., & Böhme, W. (2002). On the systematics of the harlequin frogs (Amphibia: Bufonidae: Atelopus) from Amazonia. II: Redescription of Atelopus pulcher (Boulenger, 1882) from the eastern Andean versant in Peru. Salamandra, 38, 165–184.
- Lötters, S., van der Meijden, A., Rödder, D., Köster, T. E., Kraus, T., La Marca, E., ... Veith, M. (2010). Reinforcing and expanding the predictions of the disturbance vicariance hypothesis in Amazonian harlequin frogs: A molecular phylogenetic and climate envelope modelling approach. *Biodiversity and Conservation*, 19, 2125–2146. https://doi. org/10.1007/s10531-010-9869-y
- Lötters, S., Van Der Meijden, A., Coloma, L. A., Boistel, R., Cloetens, P., Ernst, R., ... Veith, M. (2011). Assessing the molecular phylogeny of a near extinct group of vertebrates: The Neotropical harlequin frogs (Bufonidae: Atelopus). Systematics and Biodiversity, 9, 45–57. https:// doi.org/10.1080/14772000.2011.557403
- Lyra, M. L., Haddad, C. F. B., & Azeredo-Espin, A. M. L. (2017). Meeting the challenge of DNA barcoding Neotropical amphibians: Polymerase chain reaction optimization and new COI primers. *Molecular Ecology*, 17, 966–980. https://doi.org/10.1111/1755-0998.12648
- Magnusson, W. E., Braga-Neto, R., Pezzini, F. F., Baccaro, F., Bergallo, H., Penha, J., ... Pontes, A. R. M. (2013). *Biodiversidade e Monitoramento Ambiental Integrado*, 1st ed. Manaus: Áttema Editorial: Assessoria e Design.
- Messing, J. (1983). New M13 vectors for cloning. *Methods in Enzymology*, 101, 20–78. https://doi.org/10.1016/0076-6879(83)01005-8
- Noonan, B. P., & Gaucher, P. (2005). Phylogeography and demography of Guianan harlequin toads (*Atelopus*): Diversification within a refuge. *Molecular Ecology*, 14, 3017–3031. https://doi. org/10.1111/j.1365-294X.2005.02624.x
- Palumbi, S. R. (1996). Nucleic acids II: The polymerase chain reaction. In D. M. Hillis, C. Moritz, & B. K. Mable (Eds.), *Molecular Systematics* (pp. 205–247). Sunderland, Massachusetts: Sinauer & Associates Inc.
- Peters, J. A. (1973). The frog genus Atelopus in Ecuador (Anura: Bufonidae). Smithsonian Contribution to Zoology, 145, 1-49. https:// doi.org/10.5479/si.00810282.145
- Puppim de Oliveira, J. A., Balaban, O., Doll, C. N. H., Moreno-Peñaranda, R., Gasparatos, A., Iossifova, D., & Suwa, A. (2011). Cities and

biodiversity: Perspectives and governance challenges for implementing the convention on biological diversity (*CBD*) at the city level. *Biological Conservation*, 144, 1302–1313. https://doi.org/10.1016/j. biocon.2010.12.007

- Quesada, C. A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T. R., Czimczik, C., ... Paiva, R. (2010). Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences*, 7, 1515–1541. https://doi.org/10.5194/bg-7-1515-2010
- Rambaut, A., & Drummond, A. J. (2019a). Logcombiner v2 6.0. Software development. Part of Beast 2.5.
- Rambaut, A., & Drummond, A. J. (2019b). Treeannotator v2 6.0 MCMC output analysis. Software development. Part of Beast 2.5.
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology, 67, 901–904. https://doi.org/10.1093/sysbio/ syy032
- Schaefer, C. E. G. R., de Lima, H. N., Teixeira, W. G., do Vale, J. F. Jr, de Souza, K. W., Corrêia, G. R., ... Ruivo, M. L. P. (2017). Solos da região amazônica. In N. Curi, J. C. Ker, R. F. Novais, P. Vidal-Torrado, & C. E. G. R. Schaefer (Eds.), *Pedologia Solos dos Biomas Brasileiros* (pp. 112–167). Viçosa, MG: Sociedade Brasileira de Ciência do Solo.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1**. Descriptive statistics of seven landscape-scale variables used in the logistic model to estimate the occurrence probability of the Manaus harlequin frog and of four local-scale variables used in the generalized linear model to estimate its density variation

Table S2. Pearson correlation matrix between landscape-scale variables used in the logistic model to predict the occurrence probability of the Manaus harlequin frog in 75 sampling sites in the study area. Table S3. Pearson correlation matrix between local-scale variables used in the generalized linear model to predict the density variation for the Manaus harlequin frog in 30 sampling sites within the species geographic range.

**Table S4**. Kimura 2-Parameter (lower diagonal) and uncorrected pdistances (upper diagonal) genetic distances among the taxonomic groups.

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## APPENDIX 1 GLOSSARY OF ABBREVIATIONS

AIC, Akaike Information Criteria; *Bd, Batrachochytrium dendrobatidis*; BIC, Bayesian Information Criteria; CEUA, Comissão de Ética no Uso de Animais; CPRM, Serviço Geológico do Brazil; ESU, Evolutionary significant unit; GLM, Generalized Linear Model; IBGE,

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Instituto Brasileiro de Geografia e Estatística; ICMBio, Instituto Chico Medes de Conservação da Biodiversidade; INPA, Instituto Nacional de Pesquisas da Amazônia; IUCN, International Union for Conservation of Nature; LM, Logistic Model; Mya, Million Years Ago; PCR, Polymerase Chain Reaction; SISBIO, Sistema de Autorização e Informação em Biodiversidade; SRTM, Shuttle Radar Topography Mission; VIF, Variance Inflation Factor.