A new Amazonian species of *Allobates* Zimmermann & Zimmermann, 1988 (Aromobatidae) with a trilled advertisement call ~~pattern~~

Leandro Alves da Silva[1], Ricardo Marques[1], Henrique Folly[2], Diego José Santana[2]

[1] Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil
[2] Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

Corresponding Author:
Leandro Alves da Silva
Universidade Federal da Paraíba, João Pessoa, Paraíba, 58051-900, Brazil
Email address: leandroherpeto@gmail.com

Abstract

Background. Currently, 57 species are assigned to the genus *Allobates*, with 70% of ~~which were~~this diversity described just in the last two decades. However, ~~while~~ many more species are still unnamed~~await formal description~~, and the ~~. The~~continuous description of these new species ~~is~~ are~~a~~ fundamental steps for the taxonomic resolution and conservation of the genus.

Methods. Based on molecular, acoustic, and morphological evidences, ~~herein~~ we describe a new species of *Allobates* from Teles Pires River region, southern Amazonia, ~~Teles Pires River,~~ and provide accounts on a sympatric putative new species ~~a~~ cryptic ~~lineage of~~to *A. tapajos* ~~found sympatrically with this new species~~.

Results. The ~~*Allobates paleci* sp. nov.~~new species is distinguished from its congeners by the coloration of thighs, venter, dorsum, and ventrolateral stripe. It has four types of calls, with advertisement calls formed by relatively long trills with a mean duration of 2.29 s ± 0.65, mean of 39.93 notes ± 11.18 emitted at a mean rate of 17.49 ± 0.68 notes per second, and mean dominant frequency of 5,717 Hz ± 220.81. The minimum genetic distance between the new species and ~~other~~ its ~~*Allobates*~~ congener~~species~~s in a fragment of the 16S mitochondrial gene ~~ranges~~ is~~from~~ 11.8% (compared to *A. carajas*) ~~to 18.3% (*A. niputidea*) in the 16S mitochondrial fragment~~. The sympatric putative ~~lineage~~ new species ~~associated~~cryptic to ~~with~~ *A. tapajos* ~~found in the present study~~ has overlapping features in morphology and calls ~~with~~ when compared to the nominal species ~~*A. tapajos*~~, but presents a relatively high genetic distance of nearly 6.5% in the 16S, suggesting that this case ~~it might~~may in fact represent ~~be representing until now~~ an example of ~~a classic case of~~ cryptic diversification~~ty~~.

Formatado: Fonte: Não Itálico
Formatado: Fonte: Não Itálico
Formatado: Fonte: Não Itálico
Formatado: Fonte: Não Itálico

Introduction

The number of species of *Allobates* Zimmermann & Zimmermann, 1988 has continuously increased ~~continuously~~ along the last two decades (e.g. Simões et al., 2018; Moraes, Pavan & Lima, 2019; Simões, Rojas & Lima, 2019; Souza et al., 2020; Jaramillo et al., 2021), but several phenotypically and molecularly distinct ~~unnamed~~ lineages remain unnamed (see Simões, Lima & Farias, 2010; Grant et al., 2017; Fouquet, Vidal & Dewynter, 2019; Lima, Ferrão & Silva, 2020; Réjaud et al., 2020). Currently, the genus includes 57 species allocated into four informal groups: the Atlantic Forest group [~~1~~ one species~~spp.~~, *A. olfersioides* (Lutz, 1925)], the trans-Andean group [two~~2~~ species~~p~~., *A. niputidea* Grant, Acosta & Rada, 2007 and *A. talamancae* (Cope, 1875)], the colorful *A. femoralis* group [~~4 spp~~four species~~.~~, *A. femoralis* (Boulenger, 1884), *A. hodli* Simões, Lima & Farias, 2010, *A. myersi* (Pyburn, 1981), and *A. zaparo* (Silverstone, 1976)], and the most diverse group, which presumably only includes species with 2n = 22 chromosomes [50 ~~spp~~species~~.~~, e.g. *A. brunneus* (Cope, 1887), *A. carajas* Simões, Rojas & Lima, 2019, *A. crombiei* (Morales, 2002), *A. grillisimilis* Simões, Sturaro, Peloso & Lima, 2013, and *A. tapajos* Lima, Simões & Kaefer, 2015 (Grant et al., 2017)].

Allobates tapajos was described based on individuals collected at Parque Nacional da Amazônia, Itaituba ~~Municipality~~municipality, Pará State, Brazil (Lima, Simões & Kaefer, 2015). This species is also known from ~~areas~~ other localities ~~adjacent~~close to the type locality, ~~on the right and left banks of the middle and low Tapajós River~~and its complete distribution covers both banks of the middle and lower Tapajós River and the limits of~~, in different sites of the~~ other two municipalities ~~of~~ (Aveiro and ~~,~~ Belterra)~~, and Itaituba~~, all in Pará State (Lima, Simões & Kaefer, 2015; Maia, Lima & Kaefer, 2017). Recently, the existence of unnamed ~~lineages~~ species associated with thi~~s~~e nominal *A. tapajos*~~*species*~~ has been proposed based on the molecular evidence (Réjaud et al., 2020) and large geographic distances among the populations of the putative new species and the known range of the nominal species, representing possible allopatric distributions , ~~large geographical distances between newly reported populations and the previously known range of the species~~ (Fouquet, Vidal & Dewynter, 2019; Réjaud et al., 2020). ~~and molecular evidence (Réjaud et al., 2020).~~

Recent studies highlighted the conservative morphology of *Allobates* species (e.g. Carvalho, Martins & Giaretta, 2016; Moraes & Lima, 2021), and taxonomic decisions considering pluralistic lines of evidence are therefore crucial to accurately describe the ~~biodiversity within the~~ genus diversity (Carvalho, Martins & Giaretta, 2016; Grant et al., 2017; Simões et al., 2018; Moraes, Pavan & Lima, 2019; Simões, Rojas & Lima, 2019). During field~~-~~work~~s~~ along the banks of the Teles Pires River, southern Amazonia, we found two sympatric, cryptically colored and similar lineages of *Allobates*. Analyzing their morphology, advertisement calls, and fragments of the gene 16S from mitochondrial DNA~~, and morphology~~, we diagnosed both lineages as representing unnamed species. One corresponds to a ~~previously reported cryptic lineage~~new species ~~related~~ cryptic to *A. tapajos*, ~~-~~previously reported in the literature (Réjaud et al., 2020), whereas the second represents a newly sampled lineage, which is also phenotypically

Comentado [r1]: Please check on the updates to the composition of these informal groups after Rejáud et al. 2020. I also missed the new insights from this reference along the text.

Formatado: Fonte: Itálico

Comentado [r2]: similar lineages in what sense? Please explain diagnosable from its congeners~~an unknown lineage~~. Therefore, here~~, which~~ we describe the latter lineage as a new species of *Allobates*, and ~~. We also~~ provide accounts on the sympatric cryptic ~~lineage~~ new species related to *A. tapajos*.

Material and Methods

Study area and sampling

We performed field~~-~~work on both ~~right and left~~ banks of the Teles Pires River, in Jacareacanga municipality (hereafter JAR), southern Pará State, and in Paranaíta ~~M~~municipality (hereafter PAR), northern Mato Grosso State, all in Brazil, between November 2015 and November 2019 (Figure 1). During diurnal surveys at different sites, we collected 24 specimens of *Allobates*, ~~10~~ ten ~~of them assigned~~ representing~~to~~ the ~~lineage~~ new species described below and 15 ~~specimens of~~representing the new species cryptic ~~lineage related~~ to ~~the nominal~~ *A. tapajos* (hereafter *A.* aff. *tapajos*). We ~~killed~~ euthanized specimens using a liquid solution of 2% lidocaine chlorhydrate, preserved them in 10% formalin, and posteriorly stored ~~specimens~~ them in 70% ethanol. Tissue samples ~~(~~from muscle~~s)~~ were taken before specimen preservation, preserved in 100% ethanol and stored at -20ºC. Collect permit was issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO #79127-1). We deposited specimens in Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande, MS, and in Coleção Herpetológica da Universidade Federal da Paraíba, João Pessoa, PB (UFPB). Specimens analyzed are listed in Appendix 1.

Bioacoustics Analysis

We recorded calls from nine males of the new species from JAR, and eight males of ~~*Allobates*~~ *A.* aff. *tapajos* from JAR (n = 7) and PAR (n = 1). All calls were recorded using a Tascam DR-40 digital recorder with built in microphones at 44.1 kHz with a 16-bit resolution. The recordings were made between 7:30 and 18:00 h, air temperature range was 24–30°C and air humidity 80–90%. We analyzed calls in Raven Pro v. 1.5 (Bioacoustics Research Program 2014). Temporal parameters were measured from oscillograms, whereas spectral parameters were measured from spectrograms. Remaining sets were: Hann window type, FFT size = 256, brightness 67%, and contrast 70%; to reduce background noise we applied a 2500-Hz high-pass filter before acoustic analyses.

We describe four call types for the new species: i) calls composed of single notes (voucher CHUFPB30245 [field number: AAGARDA12596], three calls); ii) warming-up short calls (3–15 notes; nine males, 72 calls and 216 notes); iii) advertisement calls (17–61 notes; nine males, 61 calls and 549 notes); and iv) singular multipulsed notes (16–33 pulses; voucher CHUFPB30256 [AAGARDA12595], six calls). For single note calls,~~s~~ we measured the following temporal and spectral parameters: call duration, silent interval between calls and dominant, minimum, and maximum frequencies of the call. Because warming-up and advertisement calls presented a multi-note structure, we sampled a subset of notes to describe the ~~micro~~temporal and spectral parameters of ~~the~~ calls. For warming-up calls, we measured the following parameters of the whole call

Comentado [r3]: It seems that the fieldwork was almost continuous along this 4 years? If not, please make more explicit how many field campaigns

Comentado [r4]: Note that throughout my correction I avoided using the term lineage, which can be a bit confusing in a taxonomic study.

Comentado [r5]: or 14? You cited 24 specimens

Formatado: Fonte: Itálico

Formatado: Fonte: Itálico

and of the first, most central, and last notes of each call (i.e. n = 3 notes per call): Temporal parameters – call duration, silent interval between advertisement calls, number of notes per call, duration of the first three notes, silent interval between the first three notes, duration of the most central three notes, silent interval between the three most central notes, duration of last three notes, silent interval between the last three notes, and note repetition rate. Spectral parameters – dominant, minimum and maximum frequencies of the whole call, first three notes, most central three notes, and last three notes.

For advertisement calls, we measured the following parameters of the whole call and of the first three, three most central, and last three notes of each call (i.e. n = 9 notes per call). Temporal parameters – call duration, silent interval between the advertisement calls, number of notes per call, duration of first three notes, silent interval between the first three notes, duration of the most central three notes, silent interval between the three most central notes, duration of last three notes, silent interval between the three last notes, and note repetition rate. Spectral parameters - dominant, minimum and maximum frequencies of the whole call, first three notes, most central three notes, and last three notes. For calls composed of multipulsed singular notes, we measured the call duration, silent interval between the multipulsed singular notes, number of pulses per call, and dominant, minimum and maximum frequencies of each call. For calls consisting in single notes, we measured call duration, silent interval between calls, and dominant, minimum and maximum frequencies of the call.

We adapted the methodology proposed by Lima et al., (2015) for the call description of ~~*Allobates*~~ A. aff. *tapajos*. From each recording, we selected a section with uninterrupted calls around the middle length of the recording. In this section we analyzed 20–24 calls and 34–52 notes per recording, from which we measured both temporal and spectral parameters: duration, dominant, minimum and maximum frequencies, silent interval between the calls, and silent interval between notes in all recordings. We also estimated the rate of note emission (number of notes/seconds) from these sections in all recordings.

Comentado [r6]: adopted?

Morphology

We measured specimens using a digital caliper to the nearest 0.1 mm. We followed Fabrezi & Alberch, (1996), Grant et al., (2006), Lima et al., (2007), and Barrio-Amorós & Santos~~,~~ (2009) for morphometric measurements and terminology: snout-to-vent length, head length from tip of snout to posterior edge of maxilla articulation, head width at the level of maxilla articulation, snout length from tip of snout to the center of nostril, eye-to-nostril distance from anterior corner of the eye to the center of nostril, internarial distance, eye diameter from anterior to posterior corner, interorbital distance, maximum diameter of tympanum, forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow, upper arm length from anterior corner of arm insertion to the outer edge of flexed elbow, lengths from proximal edge of palmar tubercle to tips of fingers II, III, IV and V; width of disc on Finger III, width of Finger III's third phalanx, diameter of palmar tubercle, diameter of thenar tubercle, leg length from the posterior extremity of the urostyle region to the outer edge of flexed knee, tibia length from outer edge of flexed knee to heel, foot length from proximal edge of outer metatarsal tubercle to tip of Toe IV, and width of disc on Toe IV (WTD).

Molecular Analysis

We extracted DNA from ~~muscle~~ tissue samples using the sodium-chloride salt precipitation method (Bruford et al., 1992). For the polymerase chain reaction (PCR) amplification, we used 7.5µl of Taq DNA Polymerase Master Mix (Ampliqon S/A, Denmark), 0.4 µl of either primer (forward/backward), and 1–2µl of DNA, then we complemented with Mili Q water for a final volume reaction of 15µl. Then, we amplified a fragment of the mitochondrial DNA (mtDNA) ~~16S fragments~~ gene 16S using primers 16Sar and 16Sbr of Palumbi~~,~~ (1996). The PCR protocol was configured with one initial cycle of 94 °C for 3 min, followed by 35 cycles of 94 °C for 20 s, 48 °C for 20 s, 68 °C for 40 s, and a final extension cycle of 68 °C for 5 min. The purification of PCR products and sequencing were performed by Macrogen Inc. (Seoul, South Korea).

To assist our diagnosis evaluation and enlighten the phylogenetic ~~position~~ relationships of the two sympatric species of *Allobates* ~~lineages~~ herein addressed, we compared their newly generated 16S ~~fragment~~ sequences with homologous sequences ~~with~~ of ~~all~~ other XX *Allobates* species ~~species with compatible fragment~~ deposited in the online repository GenBank. Sequences of the closely related *Anomaloglossus stepheni* (Martins, 1989), also obtained from GenBank, were included as outgroup. We aligned this 16S mtDNA ~~gene fragments~~ dataset using MAFFT algorithm (Katoh et al., 2002) in Geneious v 9.0.5 with default settings. ~~We aligned our 16S sequences with 16S sequences of other species of *Allobates* and with the outgroup *Anomaloglossus stepheni* (Martins, 1989), which were available in GenBank (Supplemental Figure S1).~~ Due to the immense ~~quantity~~ amount of 16S sequences available (> 850 sequences), many of them from the same species, we chose, when available, up to three sequences from each species and candidate/non-described species identified in previous works (e.g. Simões, Lima & Farias, 2010; Grant et al., 2017; Fouquet, Vidal & Dewynter, 2019; Lima, Ferrão & Silva, 2020). The final dataset ~~used in all analyses~~ comprised 187 sequences of a 387 base pairs (bp) fragment of the 16S (Supplemental Document S1). All GenBank accession numbers and genetic vouchers used here are listed in the Supplemental Table S1.

To infer a phylogenetic tree, ~~W~~we used the Bayesian Information Criterion in jModelTest (Darriba et al., 2012) to select the best model of nucleotide substitution (GTR+I+G) for our dataset.

The tree was inferred ~~We~~under a Bayesian framework us~~ed~~ing BEAST v.2.6.3 (Bouckaert et al., 2019) with 50 million generations, sampling every 5,000 steps using a Yule Process tree prior ~~to conduct a bayesian phylogenetic analysis~~. We checked for stationarity of parameters by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.7.1 (Rambaut et al., 2018). The first 10% of the genealogies sampled were discarded as burn-in, and a maximum clade credibility tree with median node ages was constructed in TreeAnnotator v.2.6.3

Comentado [r7]: how many spp.?

Formatado: Cor da fonte: Vermelho

Formatado: Cor da fonte: Vermelho

(Bouckaert et al., 2019). We also calculated sequence divergences (uncorrected p-distances) among species/individuals using MEGA v10.1.1 (Kumar et al., 2018).

Interspecific comparisons

The new species described here is only known to occur in ombrophilous forests from southern Brazilian Amazonia, in the boundary of the southern Pará ~~s~~State with northern Mato Grosso state, Brazil~~, in the ombrophilous forests of the medium Teles Pires River~~. ~~Herein~~Based on this restricted distribution and molecular phylogenetic affinities, here~~,~~ we phenotypically compare ~~it~~ the new species with all 31 Brazilian congeners (XX% of the genus diversity) ~~distributed throughout Brazil~~ (Fig. 1): *Allobates bacurau* Simões, 2016, *A. brunneus*, *Allobates* ~~*A.*~~ *caeruleodactylus* (Lima & Caldwell, 2001), *Allobates* ~~*A.*~~ *caldwellae* Lima, Ferrão & Silva, 2020, *A. carajas*, *Allobates* ~~*A.*~~ *conspicuus* (Morales, 2002), *A. crombiei*, *A. femoralis*, *Allobates* ~~*A.*~~ *flaviventris* Melo-Sampaio, Souza & Peloso, 2013, *Allobates* ~~*A.*~~ *fuscellus* (Morales, 2002), *Allobates* ~~*A.*~~ *gasconi* (Morales, 2002), *Allobates* ~~*A.*~~ *goianus* (Bokermann, 1975), *Allobates* ~~*A.*~~ *grillicantus* (Moraes & Lima, 2021), *A. grillisimilis*, *A. hodli*, *Allobates* ~~*A.*~~ *magnussoni* Lima, Simões & Kaefer, 2014, *Allobates* ~~*A.*~~ *marchesianus* (Melin, 1941), *Allobates* ~~*A.*~~ *masniger* (Morales, 2002), *A. myersi*, *Allobates* ~~*A.*~~ *nidicola* (Caldwell & Lima, 2003), *Allobates* ~~*A.*~~ *nunciatus* Moraes, Pavan & Lima, 2019, *A. olfersioides*, *Allobates* ~~*A.*~~ *pacaas* Melo-Sampaio et al., 2020, *Allobates* ~~*A.*~~ *paleovarzensis* Lima et al., 2010, *Allobates* ~~*A.*~~ *subfolionidificans* (Lima, Sanchez & Souza, 2007), *Allobates* ~~*A.*~~ *sumtuosus* (Morales, 2002), *A. tapajos*, *Allobates* ~~*A.*~~ *tinae* Melo-Sampaio, Oliveira & Prates, 2018, *Allobates* ~~*A.*~~ *trilineatus* (Boulenger, 1884), *Allobates* ~~*A.*~~ *vanzolinius* (Morales, 2002), and *Allobates* ~~*A.*~~ *velocicantus* Souza, Ferrão, Hanken & Lima, 2020.

Formatado: Cor da fonte: Vermelho

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:6B0FDB3B-30B2-471E-9E06-8B7F296F454F. The electronic edition of this work was published in a journal with an ISSN, has been archived, and is available from the following digital repository: www.peerj.com/.

Results

***Allobates paleci* sp. nov.**

Figure 2, Figure 3, Figure 4, Figure 7, Table 1.

Holotype. CHUFPB30253. Adult male collected by L.A. Silva and H. Folly, on 17 February 2019, on the right bank of the Teles Pires River, Jacareacanga municipality, Pará state, Brazil (-9.258367º, -56.805723º; datum = WGS84).

Comentado [r8]: please check for standardization in "State"

Paratopotypes. Eight adult males: CHUFPB30244–45, CHUFPB30248, CHUFPB30251–52, CHUFPB30256, CHUFPB30281, CHUFPB30306; one adult female CHUFPB30242; all collected by ~~.~~L.A. Silva and H. Folly ~~collected all specimens~~ between 16–17 February 2019~~ at the same locality of the holotype~~.

Etymology. Indigenous populations of the Apiaká ethnic group historically inhabited areas along the major tributaries of the Tapajós, Juruena, Teles Pires, and Arinos ~~Rivers~~rivers. Different familiar groups of the Apiaká ~~who live spread throughout this area can~~are ~~be named~~called according to their residence ~~regions.~~regions, and ~~The~~ families inhabiting the middle~~edium~~ Teles Pires River are ~~called~~ known as Paleci. The specific epithet “paleci” is a noun in apposition referring to these families~~ of the Apiaká ethnic group who inhabit the medium Teles Pires River~~, who live ~~-~~on the vicinities of the new species’ type locality. We also suggest the following Portuguese vernacular names for the new species: “sapinho-foguete-dos-paleci” or “rãzinha-dos-paleci”.

Generic placement. The new species is assigned to the genus *Allobates* based on molecular evidence (mtDNA 16S, Figure 5) and by presence of the following morphological characteristics: Finger V length not reaching the distal subarticular tubercle of Finger IV, basal webbing with lateral fringe on the preaxial side of Toe IV, presence of pale paracloacal marks, presence of a pale ventrolateral stripe, and the presence of a diffuse oblique lateral stripe (Grant et al., 2017).

Diagnosis. *Allobates paleci* sp. nov. can be distinguished from the other species of the genus ~~occurring in the Brazil~~ by the following combination of ~~set of~~ character~~s~~s: (1) dorsum light brown, with a dark brown hourglass mark ranging from the interorbital level to the urostyle region~~dorsal surface of thigh light brown, abdomen immaculate yellowish in life; (2~~; (2) abdomen immaculate yellowish in life~~) dorsum light brown with dark brown hourglass mark ranging from the interorbital level to the urostyle region~~; (3) gular region of males yellowish in life, lacking obvious melanophores; (4) dorsal surface of thighs light brown lacking dark brown transversal bars; (5) in life, presence of a light golden interrupted ventrolateral stripe; (6) advertisement calls formed by trills with a duration of 0.97–3.57 s (2.29 ± 0.65), 17–61 notes (39.93 ± 11.18) emitted at a rate of 16.32–19.10 notes per second (17.49 ± 0.68), and dominant frequency ranging between 5,168–6,202 Hz (5,717 ± 220.81)~~; (7) molecular data~~.

Morphological comparisons. ~~The new species is known only for two sites in southern Amazonia, in the Tapajós center of endemism. Therefore, we compared the morphology of the new species with all other valid *Allobates* species ranging along Brazil (i.e. 31 spp., Segalla et al., 2021; Moraes, Pavan & Lima, 2019; Lima, Ferrão & Silva, 2020; Melo-Sampaio et al., 2020; Souza et al., 2020).~~ The character states of the compared species are given in parentheses.

Allobates paleci sp. nov. can be easily distinguished from *A. femoralis*, *A. hodli*, and *A. myersi* by having dorsal surface of the thigh light brown, abdomen immaculate and yellowish in life (red or yellow flash mark on dorsal surface of thigh, and black and white marbling on the abdomen) (Boulenger, 1884; Pyburn, 1981; Simões, Lima & Farias, 2010).

From *A. bacurau*, *A. caeruleodactylus*, *A. caldwellae*, *A. conspicuus*, *A. fuscellus*, *A. grillicantus*, *A. grillisimilis*, *A. juami*, *A. marchesianus*, *A. masniger*, *A. nidicola*, *A. nunciatus*, *A. paleovarzensis*, *A. subfolionidificans*, *A. sumtuosus*, *A. tinae*, *A. vanzolinius*, and *A. velocicantus*, *A. paleci* sp. nov. can be distinguished by have a light

Comentado [r9]: A striking character of this species, which from what I have noticed to occur in all specimens, is that its dark lateral stripe, generally well-marked and continuous in other Allobates, is here quite interrupted. Note that this is even different from A. tapajos, whose dark stripe seems longer and fades after approximately the middle of the body. The only species that have such a condition of a widely interrupted lateral stripe would be A. magnussoni, A. flaviventris, A. brunneus and A. goianus, but it still looks different from the one found here. The condition in this species is as if the “diffuse clear oblique line” that cross the dark stripe of the Allobates has extended until there is almost no space for the dark band. I don't know if I was able to explain it well, but I think that this character is very unique of this species and it must be mentioned and used as a diagnosis. Or at least cite this as an “absence of a continuous and well-marked dark lateral stripe from the snout to groin” or something similar

Comentado [r10R9]: Also remember to cite the absence of a dorsolateral light stripe, a character often used in Allobates taxonomy

Comentado [r11]: Other recommendation is that you include here in the diagnosis data about the male and female SVL (range and mean). These may not be very strong diagnostic characters for this species, but it is important to be comparable with the literature.

Comentado [r12]: I think you can drop it. If it is not going to detail every character of the molecular data that diagnoses the species, better omit.

Comentado [r13]: According to what reference? Consider removing that. These centers of endemism are very much focused for birds and primates. In fact, this entire paragraph has already been said in the methodology brown dorsum ~~light brown~~ with dark brown hourglass mark ranging from the interorbital level to the urostyle region (dorsum without ~~large dark~~ contrasting~~patches or~~ marks) (Melin, 1941; Lima & Caldwell, 2001; Morales, 2002; Caldwell & Lima, 2003; Lima, Sanchez & Souza, 2007; Lima et al., 2010; Simões et al., 2013a; Simões et al., 2013b; Simões, 2016; Melo-Sampaio, Oliveira & Prates, 2018; Simões et al., 2018; Moraes, Pavan & Lima, 2019; Lima, Ferrão & Silva, 2020; Souza et al., 2020). *Allobates paleci* sp. nov. is distinguished from *A. flaviventris*, *A. gasconi*, *A. magnussoni*, *A. pacaas*, and *A. trilineatus* by presenting yellowish throat in live males, free of obvious melanophores (throat light gray to dark grey) (Boulenger, 1884; Morales, 2002; Melo-Sampaio, Souza & Peloso, 2013; Lima, Simões & Kaefer, 2014; Melo-Sampaio et al., 2020). *Allobates paleci* sp. nov. presents dorsal surface of thighs lacking dark brown transversal bars and thus is distinguished from *A. brunneus*, *A. carajas*, *A. crombiei*, *A. goianus*, and *A. olfersioides* (dark brown transverse bands/blotches on thigh) (Cope, 1887; Lutz, 1925; Bokermann, 1975; Morales, 2002; Lima, Caldwell & Strüssmann, 2009; Simões, Rojas & Lima, 2019). *Allobates tapajos* is the most morphologically/chromatically similar species, but the new species presents a light golden ventrolateral stripe interrupted in life (ventrolateral stripe absent) (Lima, Simões & Kaefer, 2015). Furthermore, these two species are acoustically and molecularly very distinct (see ~~sessions~~ below).

Call description. Advertisement calls (n = 61, nine males) of *Allobates paleci* sp. nov. (Figure 6A, Table 2) are characterized by relatively long trills of 17–61 notes (mean 39.9 notes ± 11.9) ~~notes~~ emitted at a rate of 16.3–19.1 pulses per second (mean 17.49 pulses per second ± 0.68), and call duration raging between 0.97–3.57 s (mean 2.29 s ± 0.649). The call ~~was~~ is irregularly emitted ~~at~~ between intervals ~~between~~ lasting 8.06–55.83 s (mean 16.36 s ± 9.88). The durations of the first, central and last notes ~~were~~ are similar: 13.00–41.10 ms (mean 27.79 ms ± 6.86), 14.50–48.20 ms (mean 29.08 ms ± 7.58), and 15.20–50.20 ms (mean 30.23 ms ± 8.56), respectively. The intervals of pulses from begin, middle and final portion of the call ~~were~~ are also similar: 12.60–37.40 ms (mean 24.28 ms ± 6.01), 14.60–48.40 ms (mean 29.02 ms ± 7.77), and 15.10–56.10 ms (mean 33.35 ms ± 8.41), respectively. Furthermore, each note was composed of two or three visible pulses. The amplitude modulation along the call ~~was~~ is homogeneous. The dominant frequency of the call range~~d~~ between 5,168–6,202 Hz (mean 5,717 Hz ± 221). As observed for the call amplitude modulation, the frequency ~~was~~ is also maintained through the entire call, with the first, central and last notes similar in dominant frequencies: 4,996–6,202 Hz (mean 5,669 Hz ± 239), 5,168–6,202 Hz (mean 5,715 Hz ± 215), and 4,996–6,202 Hz (mean 5,700 Hz ± 223), respectively.

Warming-up calls (n = 72, nine males) of *Allobates paleci* sp. nov. (Figure 6B and Table 3) are composed of ~~small~~ short trills of 3–15 notes (mean 6.65 notes ± 2.35) ~~notes~~ emitted at a rate of 17.35–23.00 pulses per second (mean 20.06 pulses per second ± 1.29), and call duration raging between 0.14–0.79 s (mean 0.34 s ± 0.14). The call ~~was~~ is irregularly emitted between intervals lasting ~~at intervals between~~ 0.21–22.73 s (mean 4.72 s ± 5.26). The durations of the first, central and last notes ~~were~~ are similar, 23.40–48.50 ms (mean 33.15 ms ± 5.98), 15.20–55.10 ms (mean 30.18 ms ± 7.20), and 17.60–51.20 ms (mean 30.48 ms ± 9.08), respectively. The interval of pulses from begin,

Comentado [r14]: A. marchesianus have hourglass marks, no?

Comentado [r15]: A. trilineatus could it be part of the first compared group? This species has a uniform dorsum. Please check on the redescription

Jaramillo-Martinez, A. F., L. A. G. Gagliardi-Urrutia, P. I. Simões, and S. Castroviejo-Fisher. 2021. Redescription and phylogenetics of *Allobates trilineatus* (Boulenger 1884 "1883") (Anura: Aromobatidae) based on topotypic specimens. Zootaxa 4951: 201–235 (https://doi.org/10.11646/zootaxa.4951.2.1).

middle and final portion of the call were also similar, 10.70–33.50 ms (mean 22.84 ms ± 5.08), 11.40–36.60 ms (mean 25.05 ms ± 5.30), and 21.10–44.70 ms (mean 32.79 ms ± 4.96), respectively. Each note ~~was~~ is composed of two or three ~~visible~~ pulses. The amplitude modulation along the call ~~was~~is homogeneous. The dominant frequency of warming-up calls range~~d~~ between 4,996–6,029 Hz (mean 5,663 Hz ± 243.51). As observed for the call amplitude modulation, the frequency is~~was~~ also maintained through the entire call, with the first, central and last notes ~~showing~~ having similar dominant frequencies: 4,996–6,029 Hz (mean 5,641 Hz ± 256.57), 4,996–6,202 Hz (mean 5,669 Hz ± 240.74), and 4,996–6,029 (mean 5,638 Hz ± 235.75), respectively.

Additionally, we recorded the male CHUFPB30245 (field number AAGARDA12596) emitting three consecutive calls composed of a single unpulsed note before the emission of a warming-up call (Figure 6C and Table 4). These calls ~~presented a duration of~~last for 15.30–26.10 ms (mean 21.97 ms ± 5.83), are emitted at a rate of 196.90–215.30 calls per ms (mean 206.10 calls per ms ± 9.20) and have a dominant frequency of 4,996 Hz. We also recorded the male CHUFPB30256 (field number AAGARDA12595) emitting six calls composed of single multipulsed notes at the end of an advertisement call emission series (Figure 6D and Table 5). ~~Each t~~These calls ~~presented a duration of~~last for 0.18–0.34 s (mean 0.24 s ± 0.06), are emitted at a rate of 0.36–2.56 calls per second (mean 0.93 calls per second ± 0.93), hav~~e~~ing between 16–33 pulses (mean 22.00 pulses ± 6.26) and a dominant frequency of 5,513–6,029 Hz (mean 5,828 Hz ± 169.37). ~~During field sampling, w~~We observed two other males (both unvouchered) emitting this last call type just before moving through the leaflitter (one of them can be ~~watched~~ seen ~~here~~in the following footage: https://youtu.be/WvngJ1tEMYI). Although no other individual ~~(male or female)~~ of *Allobates paleci* sp. nov. ~~the same species w~~was observed around the calling males ~~who emitted~~emitting these ~~solitary~~ multipulsed notes, we suggest that this call type were emitted in a courtship or territorial context.~~this call type may correspond to a courtship or territorial call.~~ In fact, ~~T~~the acoustic envelope of th~~is~~e call type ~~described herein~~ resembles both the courtship call described for *A. hodli* (Simões, Lima & Farias, 2010) ~~as~~ and the aggressive call described for ~~of~~ *A. olfersioides* (Forti, Silva & Toledo, 2017). Further field observations are needed to clarify the social context of this call type.

Bioacoustic comparison. *Allobates paleci* sp. nov. has a unique combination of the following acoustic parameters: advertisement calls formed by trills lasting 0.97–3.57 s (mean 2.27 s ± 0.65) with 17–61 notes (mean 39.93 notes ± 11.18) emitted at 16.32–19.10 notes/s (mean 17.49 notes/s ± 0.68), and dominant frequency ranging between 5,168–6,202 Hz (mean 5,717 Hz ± 221). ~~Five of the 31 species compared here with *A. paleci* sp. nov. have no information regarding their advertisement calls: *A. conspicuus*, *A. fuscellus*, *A. gasconi*, *A. pacaas*, and *A. vanzolinius* (Morales, 2002; Melo-Sampaio et al., 2020). Nevertheless, as mentioned above, the new species is easily distinguished from them based on morphology.~~

~~While the advertisement call of *Allobates paleci* sp. nov. is always arranged in trills of 17–61 notes, with a duration of 0.970–3.574 s,~~ This call structure differs from the calls of six compared species, which ~~compared here have calls~~ consist~~ing~~ of single notes emitted continuously: *A. caeruleodactylus*, *A. magnussoni*, *A. masniger*, *A. nidicola*, *A.*

Formatado: Fonte: Itálico

Formatado: Fonte: Itálico

Comentado [r16]:

Formatado: Recuo: Primeira linha: 0 cm *olfersioides*, and *A. subfolionidificans* (Lima & Caldwell, 2001; Caldwell & Lima, 2003; Lima, Sanchez & Souza, 2007; Tsuji-Nishikido et al., 2012; Lima, Simões & Kaefer, 2014; Simões, 2016; Forti, Silva & Toledo, 2017). Three other species also have a~~, in addition to a~~ call type consisting of single notes emitted continuously, but may ~~may also~~ emit~~produce~~ trills~~ of notes~~: *A. marchesianus*: trills of 21–24 notes and duration of 3.39–4.40 s (Caldwell, Lima & Keller, 2002); *A. sumtuosus*: trills of 23–35 notes and duration of 3.949–5.878 s (Simões et al., 2013b); and *A. brunneus*: trills of 6–11 notes and duration of 1.68–4.18 s (Lima, Caldwell & Strüssmann, 2009). Two species, *A. carajas* and *A. tapajos*, have at least four different temporal call arrangements, also including a trilled call type~~ with a trilled structure~~: *A. carajas* – continuous emission of notes separated by regular silent intervals, continuous emission of notes separated by irregular silent intervals, emission of discrete note trills, and sporadic emission of single notes (Simões, Rojas & Lima, 2019); when emitting trills, they reach longer durations (up to 7.05 s) but with a lower note number (up to 22 notes) when compared to *A. paleci* sp. nov.; *A. tapajos* possess note pairs (most common arrangement), single notes emitted between note pairs, and note trios (rarest arrangement); just one male emitted trills similar in duration to the calls of *A. paleci* sp. nov. (2.46–3.37 s), but with fewer notes per trill (10–14) (Lima, Simões & Kaefer, 2015).

The 15 remaining species ~~produce~~ emit only trills~~ed in~~ advertisement calls, with a general temporal pattern of note emission shared with *Allobates paleci* sp. nov. Nine of these species emit~~presented~~ shorter trills~~ durations~~ and fewer notes/call when compared to *A. paleci* (call duration 0.970–3.574 s and 17–61 notes): *A. caldwellae* has calls ranging between 0.259–1.255 s and 3–7 notes (Lima, Ferrão & Silva, 2020); *A. femoralis* has calls composed of groups of one, three or four notes, with 2–4 notes, lasting between 0.183–0.528 s (Amézquita et al., 2009; Simões, Lima & Farias, 2010); *A. flaviventris* has 2–10 notes within each trill and presumably a shorter call duration (not informed in the original description, Melo-Sampaio, Souza & Peloso, 2013); *A. grillicantus* has trills lasting 0.151–0.507 s with 3–15 notes (Moraes & Lima, 2021); *A. grillisimilis* has trills ranging 0.122–0.305 s with 3–15 notes (Simões et al., 2013a; Simões, 2016); *A. hodli* has short trills ranging 0.140–0.198 s with two whistle-like notes (Simões, Lima & Farias, 2010); *A. myersi* has three different temporal arrangements: short trills (mean 0.35 ± 0.02 s) of two, three, or four notes (Simões & Lima, 2011); *A. nunciatus* has trills of up to 0.357 s and of four notes (Moraes, Pavan & Lima, 2019); and *A. trilineatus* has trills of 0.97–1.55 s with 9–13 notes (Grant & Rodríguez, 2001).

Four species presented trills with similar durations and number of notes, but with smaller note repetition rates (note repetition rate of 16.32–19.10, mean of 17.49 ± 0.68 in *Allobates paleci* sp. nov.): *A. goianus* calls have trills with a mean duration of 3.9 s and 2–41 notes emitted at 3.1–3.9 notes per second (Carvalho, Martins & Giaretta, 2016); *A. paleovarzensis* trills of 0.72–3.02 s with 3–21 notes emitted at 6.97 notes/s (Lima et al., 2010); *A. tinae* trills last 0.285–2.27 s (1.50 ± 0.45) with 2–9 notes (mode = 8; n = 45) emitted at 5.34 notes/s (Melo-Sampaio, Oliveira & Prates, 2018). Finally, *A. velocicantus* presented a similar trill duration of 1.87–2.89 s (2.49 ± 0.22), with 66–

Comentado [r17]: It is not clear how the call of the new species differs from trills of these species.

Comentado [r18]: This paragraph is confusing. I don't know if all of these species can be considered as having a trilled call. A. femoralis, A. nunciatus, A. hodli, A. myersi emit some regular group of notes, but they are not strictly sequential. I now that there's a bit of subjectivism here, but I suggest rephrase this paragraph considering these species as another acoustic group, followed by a final paragraph comparing A. paleci with only with species with strictly-trilled calls.

Comentado [r19R18]: In fact, this section seemed to me the most confusing so far in the text. I think it's worth trying to improve her fluidity in general. Maybe better separating these acoustic groups is enough

Comentado [r20]: I don't consider this structure as a trill.

138 notes emitted at 51.2 ± 5.8 (38.4–56.8) notes/s (Souza et al., 2020). Two species presented longer trills with more notes: *A. bacurau* has trills of 7–11 s with 60–81 notes (Simões, 2016); and *A. juami* has trills of 2.5–5.09 s (4.51 ± 0.37) with 60–73 notes (65 ± 4) (Simões et al., 2018).

Allobates crombiei shows the most similar trill regarding the calls emitted by *A. paleci* sp. nov. (Lima, Erdtmann & Amézquita, 2012). *Allobates crombiei* presents trills with mean duration of 3.52 ± 0.49 s (1.91–4.53) with 43 ± 6.38 notes (25–59), and, being the main distinction compared to the advertisement call of *A. paleci* sp. nov., showed a lower note repetition rate of about 12.21 notes per second (call duration of 0.970–3.574 s [2.286 ± 0.649], 17–61 notes [39.934 ± 11.183], and note repetition rate of 16.32–19.10 [17.49 ± 0.68] in *A. paleci* sp. nov.). In respect to spectral parameters, *A. crombiei* has an ascendant but quick frequency modulation, while *A. paleci* sp. nov. lacks frequency modulation.

The remaining five of the 31 compared species have no information regarding their advertisement calls: *A. conspicuus*, *A. fuscellus*, *A. gasconi*, *A. pacaas*, and *A. vanzolinius* (Morales, 2002; Melo-Sampaio et al., 2020). Nevertheless, the new species is easily distinguished from them based on morphological variation (see above).

Description of the holotype. Adult male, CHUFPB 30253, SVL = 13.4 mm (Figure 2 and Figure 3), other~~. All holotype~~ measurements ~~are~~ detailed in Table 1. Skin texture slightly granular on dorsum and limbs, smooth on venter. Head wider than longer; head length 77% of head width; head width and head length 40% and 27.7% of SVL, respectively. Interorbital distance 71% of head width. Eye diameter 1.2 times longer than eye-nostril distance; eye diameter 49% of head length. Tympanum round with smooth margins, barely visible to the naked eye. Snout slightly rounded in dorsal view, nearly truncate; snout rounded in lateral view; snout length (eye–~~-~~nostril distance + nostril–snout distance) 49% of head length. Nostrils located laterally at the tip of the snout; internostril distance 44% of head width. *Canthus rostralis* from the tip of the snout to the anterior corner of the eye, barely defined. Loreal region vertical. Vocal sac single and subgular. Vomerine teeth absent; maxillary teeth visible under 50X magnification. Choanae located laterally, anterior to eye bulge. Vocal slits conspicuous, laterally located~~ laterally~~. Tongue longer than wider, attached in the anterior portion of the jaw. Cloacal tubercles absent.

Upper arm length 22% of SVL~~ size~~; forearm length 21% of SVL~~ size~~; upper arm slightly thicker than forearm. Hand without fringes or webbing. Palmar tubercle conspicuous, round to slightly elliptical. Thenar tubercle present, elliptical, less conspicuous than palmar tubercle and half of its size. Subarticular tubercles of fingers III and IV smaller than the width of the finger; subarticular tubercles of fingers II and III round and protuberant. Distal subarticular tubercle present on finger V, small and round. Supernumerary tubercles and accessory palmar tubercles absent. Metacarpal fold absent. Fingers III and V do not reach the distal subarticular tubercle of finger IV when fingers are a~~p~~dpressed; relative fingers length: IV > II > III > V. Finger IV not swollen. Discs of fingers II–V moderately expanded; width of finger IV disc 75% the size of finger IV third phalanx.

Thigh length and tibia length of similar size, 51% of SVL size each. Tarsal keel present, tubercle-like, softly curved. Inner metatarsal tubercle present, elliptical and conspicuous. Subarticular tubercle of toe I slightly smaller than inner metatarsal tubercle, similar in size to the width of toe I; subarticular tubercles of toes II–IV round and protuberant. Tip of toe I not reaching mid-level of subarticular tubercle of toe II when toes are ~~appressed~~adpressed; tip of toe III reaching past the proximal subarticular tubercle of toe IV; tip of toe V reaching past one third of the third phalange of toe IV. Metatarsal fold absent. Basal webbing present between toes II–III and III–IV. Basal webbing absent between other toes. Relative toe length: IV > III > V > II > I. Discs of toes II–V moderately expanded.

~~The specimen i~~In preservative, ~~shows the~~ dorsum ~~of the body~~ cream~~,~~ with small dark brown granules from the tip of the snout to the vent region, with~~; presence of~~ a dark brown hourglass mark ~~in an hourglass shape~~ at the center.~~ of dorsum.~~ Dorsolateral light stripe absent. Dark brown lateral stripe present but discontinuous, strongly pigmented at snout and behind eyes, fading ~~towards~~ towards the lateral of the body, and becoming pigmented again in the inguinal region. Ventrolateral stripe indistinct. Arms and legs cream, as pigmented as the dorsum background; legs more pigmented than arms. In dorsal view, ~~inguinal region,~~ anterior and posterior region of thigh with dark brown ~~patches~~markings. Gular region, chest, belly, upper arms, and thigh cream in ventral view, with ~~;~~ only few melanophores in~~around~~ the jaw; forearm, tibia and tarsal region cream with melanophores~~ present~~ in ventral view, except at anterior and posterior margins. Palmar and thenar surfaces dark brown.

~~Type series v~~Variation. The single collected female (SVL = 16.2 mm) is larger than 90% of all males of the type series, suggesting the existence of sexual dimorphism in body proportions~~ SVL~~, but larger samples are needed to explicitly test this hypothesis. ~~M~~Complete morphometric variation of~~in~~ the type series is presented in Table 1. Regarding coloration, variation is more evident~~Most of the variation was~~ in ~~on coloration in~~ preserved specimens~~ative~~ (Figure 4). The concentration of small dark brown granules ~~varied~~vary, as only the holotype and three other males showed cream coloration on dorsum, while the remaining specimens show more concentration of these granules, ~~resembling~~ having a brownish coloration; the female ~~has~~ have the dark dorsum typical of other specimens in the type series. Beside the holotype, five other males ~~showed~~have a well-defined pigmented hourglass-shaped mark~~spot~~ at the center of dorsum. In contrast, ~~O~~other three males and the female ~~showed~~have ~~-~~this dark hourglass ~~shape~~ mark ~~not so~~indistinct~~-defined~~, as~~because~~ their ~~remaining~~ dorsum background ~~was~~ are also darker. Three specimens have~~showed~~ thighs cream with sparse melanophores in ventral view (immaculate cream in the remaining specimens)~~; ventral portion of thighs of all other specimens show immaculate cream coloration~~. Tibia coloration in ventral view is cream at the center and the margins range from almost no pigmentation to highly pigmented with melanophores. The dark brown lateral stripe is well defined at snout and from behind eyes until the arm line, then fades in different degrees toward the inguinal region in each specimen.

Arms are less pigmented in dorsal view in all specimens, with irregular blotches on the forearm near the elbow and near the hand. Hands in dorsal view are as pigmented as the arms. All specimens presented dark brown patches at the anterior and posterior region of thigh. Patches in thigh are of irregular shape, either restricted as a stripe on the anterior portion of the thigh or extending towards the posterior portion of the thigh. Small blotches of dark coloration are observed in tibia, tarsus, and foot in dorsal view.

The only specimen from the type series photographed in life was the male CHUFPB 30252 (Figure 7). Nevertheless, the remaining type series was generally concordant with the coloration pattern observed for this individual. In life, the specimen CHUFPB 30252 (SVL = 14.3 mm) had the dorsum of the body light brown, with a dark brown hourglass mark at the center. Dorsolateral stripe absent. Dark brown lateral stripe present, strongly pigmented at snout and behind eyes, fading towards the inguinal region. Light golden ventrolateral stripe interrupted, more evident at the medial portion of the body towards the inguinal region. Arms and legs light brown as pigmented on the dorsum. Anterior and posterior region of thigh with longitudinal dark brown patches. Gular region yellow without obvious melanophores, chest yellowish, belly yellowish with a white subjacent peritoneum. Upper arms in ventral view yellowish. Thigh in ventral view yellowish at distal portion becoming whitish towards the insertion of legs; few iridophores along the lower margin of the thigh's ventral surfaces; forearm, tibia and tarsal regions light yellowish in ventral view with melanophores, except at anterior and posterior margins. Ventral surface of foot dark brown; ventral surface of hand with a dark brown pigmentation on the palmar region and around tubercles, fingers light brown scattered with few melanophores.

Geographic distribution and ~~Natural~~ natural history ~~and geographic distribution~~. *Allobates paleci* sp. nov. is known ~~to occur~~ only ~~at~~from the type locality, in the right bank of the Teles Pires River, Jacareacanga municipality, southern Pará state, Brazil. In this locality, ~~T~~the new species was only ~~found~~ recorded ~~only~~ inside dense ombrophilous forests. During the rainy season (specially between November and February), several males of the new species were found calling from the moist leaf litter. Despite being recorded in very close sites and after a considerable sampling effort, *Allobates paleci* sp. nov. and *A.* aff. *tapajos* (see more details below) were never~~ot~~ found calling ~~at the same sites~~intopically ~~in any of the field expeditions~~. Furthermore, *A. paleci* sp. nov. was less abundant at the type locality~~, whereas~~ when compared to the sympatric *A.* aff. *tapajos* ~~was commonly observed during diurnal surveys~~. ~~*Allobates paleci* sp. nov. is known to occur only at the type locality, in the right bank of the Teles Pires River, Jacareacanga municipality, southern Pará state, Brazil.~~

~~Remarks~~Molecular relationships. ~~Our~~ The inferred gene tree from a fragment of the mtDNA gene 16S ~~tree~~ (Figure 5) confidently recovered *Allobates paleci* sp. nov. ~~nested~~ as part~~within~~ of the genus *Allobates*. However, the molecular affinities of the new species in relation to the compared congeners are more uncertain ~~some nodes presented lower posterior probabilities~~ (Supplemental Figure S1). ~~In our analysis~~Considering the tree topology, *A. paleci* sp. nov. was recovered as the sister taxon of *A. caeruleodactylus*, ~~with a low~~although with a low branch support ~~posterior probability~~. Considering genetic distances, the sequence from *A. paleci* sp. nov. was recovered as more similar to the ones pertaining to *A. carajas*. Average sequence divergence between

Comentado [r21]: Please mention the activity period if you have, maybe also calling period. Any data about the spawning clutches, tadpoles?

the new species and ~~its~~ the ~~described~~ compared congeners was 14.3%, ranging from 11.8% (*A. carajas*~~*A. carajas*~~) to 18.3% (*A. niputidea*) (Supplemental Table S1).

Remarks on the sympatric *Allobates*. The coloration pattern and ~~morphology~~ measurements (mean SVL 14.4 ± 1.7, range 12.2–16.8 mm; n = 15 males) of the putative new species of *Allobates* related to *A. tapajos* ~~lineage~~ found in sympatry with *A. paleci* sp. nov. ~~agrees with~~fitted in the variation of~~original description of~~ ~~*A. tapajos*~~the nominal species (Lima, Simões & Kaefer, 2015) (Figure 8). Acoustic parameters of this ~~species~~ population also ~~often~~ overlapped with those ~~of the original description of *A. tapajos*~~described for the nominal *A. tapajos*, except for number of notes, which range from 1–4 but most commonly presents three notes (calls ranging from 1–3 notes for *A. tapajos*, most common pattern described includes calls with two notes, Lima, Simões & Kaefer, 2015) (Figure 8, Table 6).~~;~~ A footage of this species calling can be seen in https://www.youtube.com/watch?v=0fu2JJLRhHo&ab~~)~~. Recently, Réjaud et al., (2020) reported three undescribed lineages ~~attributed~~ related to *A. tapajos*. Our gene tree recovered the population from Teles Pires River as the~~a~~ sister taxon of *A.* aff. *tapajos* 3 ~~from~~ sensu Réjaud et al., (2020). ~~In fact, s~~Sequence divergence between the Teles Pires River population reach up to 4.8% when compared to~~and t he~~ *A.* aff. *tapajos* 3 ~~recovered by~~sensu Réjaud et al., (2020), and ~~was 4.8% and nearly~~ up to 6.5% ~~to~~ when compared to *A. tapajos* from its type locality (Supplemental Table S1). ~~For these reasons we refer to this population from the Teles Pires River as *A.* aff. *tapajos*.~~

Discussion

The type and number of molecular markers, as well as different sources of morphological evidence used to infer phylogenetic relationships among organisms have direct impacts on the topology recovered, despite th~~ee phylogenetic~~ method employed~~.~~ (e.g. Mott & Vieites, 2009; de Sá et al., 2014). This is also the case for phylogenetic relationships within the genus~~For~~ *Allobates*~~, this is not different~~ (Grant et al., 2017), which slightly differ in the different studies focused on this theme. Nevertheless, the use of molecular evidence derived from single-locus comparisons within an integrative taxonomy framework has proven useful to help identify, diagnose and describe morphologically similar species of *Allobates* (e.g. Simões et al., 2018; Lima, Ferrão & Silva, 2020; Jaramillo et al., 2021). It is noteworthy that, a~~A~~lthough the ~~cryptic~~ new species~~lineage~~ cryptic to *A. tapajos* reported here ~~related to *A. tapajos* recorded~~ was already previously reported in the literature ~~elsewhere~~ (Réjaud et al., 2020), *A. paleci* sp. nov. remained ~~was never~~ un~~recovered~~ sampled in all of the previous phylogenetic/taxonomic appraisals of the genus. This case reinforce ~~. This underscores~~ the tremendous underestimated and unknown diversity~~number of yet to be described species of *Allobates*~~ of this genus in Amazonia (Reference).

We also provide, for the first time, morphological and acoustic data on a previously recognized cryptic lineage related to *A. tapajos* (Réjaud et al., 2020). The high genetic distance between the Teles Pires River population and *A. tapajos* suggests ~~first~~ that this population may in fact represent~~be~~ an undescribed species. However, our morphological and acoustic data does not unequivocally separate it from the nominal *A. tapajos*, suggesting either a case of cryptic speciation or ~~hidden~~ a higher genetic

Formatado: Fonte: Itálico

Formatado: Fonte: Itálico

Formatado: Fonte: Não Itálico

Formatado: Fonte: Itálico

Formatado: Fonte: Itálico

Formatado: Não Realce

diversity across a larger geographic range than previously thought. In cases of cryptic species, ~~the real challenge faced by the~~ taxonomists are frequently challenged ~~sometimes is~~ to accurately ~~establish~~ identify the species limits (not necessarily their validity) between the involved taxa (e.g. Silva et al., 2020). To solve ~~this~~ the taxonomic issue of *A. tapajos* and its molecularly divergent lineages, further studies should focus on compare these ~~different pp~~populations ~~currently assigned to *A. tapajos*~~ in a more comprehensive framework, ~~exploring~~ with a greater amount and comprehensiveness of ~~different~~ molecular ~~markers,~~ and morphological data~~evidence of adults and tadpoles, and acoustic data~~.

We further stress the need of an objective standardization for advertisement call description for *Allobates*, especially for when trilled calls with high rates of note repetition are present. For example, based on the oscillograms, the same acoustic structure periodically emitted (repetition unity) by cryptic species is considered either as notes (*A. grillisimilis*, Simões et al., 2013a) or pulses (*A. grillicantus*, Moraes & Lima, 2021). The current lack of an objective framework for advertisement call descriptions hampers comparisons and may even cause noise in the taxonomy of the genus. Because *A. paleci* sp. nov. shows an advertisement call with complete amplitude modulation, including a clear but short silent interval between consecutive repetition units, we refer to them as notes (Köhler et al., 2017). Finally, recent studies have proposed such standardization for other anuran groups, as the review of acoustic traits for *Physalaemus* (Hepp & Pombal Jr., 2020), which may represent a useful example of what could be proposed for *Allobates*.

In addition to *Ameerega munduruku* Neves, Silva, Akieda, Cabrera, Koroiva & Santana 2017, *Pristimantis pictus* Oliveira, Silva, Guimarães, Penhacek, Martínez, Rodrigues, Santana, & Hernández-Ruz, 2020 and *P. pluvian* Oliveira, Silva, Guimarães, Penhacek, Martínez, Rodrigues, Santana, & Hernández-Ruz, 2020, *Allobates paleci* sp. nov. represents the fourth species of amphibian described for the Teles Pires River region in the last five years. These findings reinforce the high levels of hidden diversity ~~of~~ in th~~ise study area~~ river basin. Unfortunately, this river basin has also~~, which in turn has~~ been severely impacted by anthropic pressure in the last two decades, mainly by the establishment of large hydroelectric power plants and livestock farms (Fearnside, 2005; Fearnside & Pueyo, 2012). The maintenance of long-term studies and the consideration of this high species richness concentration of type localities in the elaboration of public policies is crucial to preserve the ~~its~~ remarkable regional ~~bio~~diversity.

Formatado: Fonte: Itálico

Comentado [r22]: I agree with you, call descriptions need for standardization, but it's easier to define this structure as composed of notes in long trills. In the case of supershort trills, the intervals between call elements are so short that even measurements become imprecise, and so these elements are better referred to as 'pulses' (the case of the grillisimilis-grillicantus species pair). By seeing these animals calling you also notice that the vocal sac extends several times in the long trill of A. paleci, indicating breathing between notes...while the A. grillicantus seems to breathe just to initiate the call.

Acknowledgments

We are in debt with Adrian A. Garda for the critical review of the manuscript, improvement of the English, and provide his lab so we could do extraction, amplification, and sequencing of DNA used in the present study.

References

Amézquita A, Lima AP, Jehle R, Castellanos L, Ramos O, Crawford AJ, Gasser H, Hödl W. 2009. Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. Biological Journal of the Linnean Society 98(4):826–838 https://doi.org/10.1111/j.1095-8312.2009.01324.x

Barrio-Amorós CL, Santos JC. 2009. Description of a new *Allobates* (Anura, Dendrobatidae) from the eastern Andean piedmont, Venezuela. Phyllomedusa Journal of Herpetology 8(2):89–104 https://doi.org/10.11606/issn.2316-9079.v8i2p89-104

Bokermann WCA. 1975. Uma nova espécie de *Colostethus* do Brasil Central (Anura, Dendrobatidae). Iheringia. Série Zoologia 46:13–19.

Boulenger GA. 1884 "1883". On a collection of frogs from Yurimaguas, Huallaga River, Northern Peru. Proceedings of the Zoological Society of London 51(1):635–638 https://doi.org/10.1111/j.1469-7998.1883.tb06669.x

Bruford MW, Hanotte O, Brookfield JFY, Burke T. 1992. Single-locus and multilocus DNA fingerprinting. In: Hoelzel AR, ed. Molecular Genetic Analysis of Populations: A Practical Approach. Oxford 225–269.

Caldwell JP, Lima AP. 2003. A new Amazonian species of *Colostethus* (Anura: Dendrobatidae) with a nidicolous tadpole. Herpetologica 59(2):219–234 https://doi.org/10.1655/0018-0831(2003)059[0219:ANASOC]2.0.CO;2

Caldwell JP, Lima AP, Keller C. 2002. Redescription of *Colostethus marchesianus* from its type locality. Copeia 2002(1):157–165 https://doi.org/10.1643/0045-8511(2002)002[0157:ROCMMF]2.0.CO;2

Carvalho T, Martins LB, Giaretta AA. 2016. A new account for the endangered Cerrado Rocket Frog *Allobates goianus* (Bokermann, 1975) (Anura: Aromobatidae), with comments on taxonomy and conservation. Acta Herpetologica 11(1):21–30 https://doi.org/10.13128/Acta_Herpetol-17491

Cope ED. 1875 "1876". On the Batrachia and Reptilia of Costa Rica. Journal of the Academy of Natural Sciences of Philadelphia 2(8):93–154 https://doi.org/10.5962/bhl.title.5028

Cope ED. 1887. Synopsis of the Batrachia and Reptilia obtained by H. H. Smith, in the Province of Mato Grosso, Brazil. Proceedings of the American Philosophical Society 24(125):44–60.

de Sá RO, Grant T, Camargo A, Heyer WR, Ponssa ML, Stanley E. 2014. Systematics of the neotropical genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): phylogeny, the relevance of non-molecular evidence, and species accounts. South American Journal of Herpetology 9:S1–S128 https://doi.org/10.2994/SAJH-D-13-00022.1

Fabrezi M, Alberch P. 1996. The carpal elements of anurans. Herpetologica 52(2):188–204.

Fearnside PM. 2005. Deforestation in Brazilian Amazonia: history, rates, and consequences. Conservation Biology 19(3):680–688.

Formatado: Inglês (Estados Unidos)

Fearnside PM, Pueyo S. 2012. Greenhouse-gas emissions from tropical dams. Nature Climate Change 2(6):382–384 https://doi.org/10.1038/nclimate1540.

Forti LR, Silva TRA, Toledo LF. 2017. The acoustic repertoire of the Atlantic Forest Rocket Frog and its consequences for taxonomy and conservation (*Allobates*, Aromobatidae). ZooKeys 692:141–153 https://doi.org/10.3897/zookeys.692.12187

Fouquet A, Vidal N, Dewynter M. 2019. The Amphibians of the Mitaraka massif, French Guiana. Zoosystema 41(sp1):359–374 https://doi.org/10.5252/zoosystema2019v41a19

Grant T, Rodríguez LO. 2001. Two new species of frogs of the genus *Colostethus* (Dendrobatidae) from Peru and a redescription of *C. trilineatus* (Boulenger, 1883). American Museum Novitates 2001(3355):1–24.

Grant T, Acosta A, Rada M. 2007. A name for the species of *Allobates* (Anura: Dendrobatoidea: Aromobatidae) from the Magdalena Valley of Colombia. Copeia 2007(4):844–854 https://doi.org/10.1643/0045-8511(2007)7[844:ANFTSO]2.0.CO;2

Grant T, Frost DR, Caldwell JP, Gagliardo RON, Haddad CF, Kok PJ, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). Bulletin of the American Museum of Natural History 2006(299):1–262 https://doi.org/10.1206/0003-0090(2006)299[1:PSODFA]2.0.CO;2

Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV. 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). South American Journal of Herpetology 12(1):1–90 https://doi.org/10.2994/SAJH-D-17-00017.1

Hepp F, Pombal Jr JP. 2020. Review of bioacoustical traits in the genus *Physalaemus* Fitzinger, 1826 (Anura: Leptodactylidae: Leiuperinae). Zootaxa 4725(1):1–106 https://doi.org/10.11646/zootaxa.4725.1.1

Jaramillo AF, Gagliardi-Urrutia G, Simões PI, Castroviejo-Fisher S. 2021. Redescription and phylogenetics of *Allobates trilineatus* (Boulenger 1884 “1883”) (Anura: Aromobatidae) based on topotypic specimens. Zootaxa 4951(2):201–235. https://doi.org/10.11646/zootaxa.4951.2.1

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14):3059–3066 https://doi.org/10.1093/nar/gkf436

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution 35(6):1547–1549 https://doi.org/10.1093/molbev/msy096

Lima AP, Caldwell JP, Biavati G, Montanarin A. 2010. A new species of *Allobates* (Anura: Aromobatidae) from paleovárzea forest in Amazonas, Brazil. Zootaxa 2337(1):1–17 https://doi.org/10.11646/zootaxa.2337.1.1

Lima AP, Caldwell JP. 2001. A new Amazonian species of *Colostethus* with sky blue digits. Herpetologica 52(2):180-189.

Lima AP, Caldwell JP, Strüssmann C. 2009. Redescription of *Allobates brunneus* (Cope) 1887 (Anura: Aromobatidae: Allobatinae), with a description of the tadpole, call, and reproductive behavior. Zootaxa 1988(1):1–16 https://doi.org/10.11646/zootaxa.1988.1.1

Lima AP, Erdtmann LK, Amézquita A. 2012. Advertisement call and colour in life of *Allobates crombiei* (Morales) "2000" [2002] (Anura: Aromobatidae) from the type Locality (Cachoeira do Espelho), Xingu River, Brazil. Zootaxa 3475(1):86–88 https://doi.org/10.11646/zootaxa.3475.1.8

Lima AP, Ferrão M, Silva DL. 2020. Not as widespread as thought: Integrative taxonomy reveals cryptic diversity in the Amazonian nurse frog *Allobates tinae* Melo-Sampaio, Oliveira and Prates, 2018 and description of a new species. Journal of Zoological Systematics and Evolutionary Research 58(4):1173–1194 https://doi.org/10.1111/jzs.12406

Lima AP, Sanchez DE, Souza JR. 2007. A new Amazonian species of the frog genus *Colostethus* (Dendrobatidae) that lays its eggs on undersides of leaves. Copeia 2007(1):114–122 https://doi.org/10.1643/0045-8511(2007)7[114:ANASOT]2.0.CO;2

Lima AP, Simões PI, Kaefer IL. 2014. A new species of *Allobates* (Anura: Aromobatidae) from the Tapajós River basin, Pará State, Brazil. Zootaxa 3889(3):355–387 https://doi.org/10.11646/zootaxa.3889.3.2

Lima AP, Simões PI, Kaefer IL. 2015. A new species of *Allobates* (Anura: Aromobatidae) from Parque Nacional da Amazônia, Pará State, Brazil. Zootaxa 3980(4):501–525 https://doi.org/10.11646/zootaxa.3980.4.3

Lutz A. 1925. Batraciens du Brésil. Comptes Rendus et Mémoires Hebdomadaires des Séances de la Société de Biologie et des ses Filiales. Paris 93(2):137–139.

Maia GF, Lima AP, Kaefer IL. 2017. Not just the river: genes, shapes, and sounds reveal population-structured diversification in the Amazonian frog *Allobates tapajos* (Dendrobatoidea). Biological Journal of the Linnean Society 121(1):95–108 https://doi.org/10.1093/biolinnean/blw017

Martins M. 1989. Nova espécie de *Colostethus* da Amazônia central (Amphibia: Dendrobatidae). Revista Brasileira de Biologia 49(4):1009–1012.

Melin DE. 1941. Contributions to the knowledge of the Amphibia of South America. Elanders boktr.

Melo-Sampaio PR, Souza MB, Peloso PLV. 2013. A new, riparian, species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura: Aromobatidae) from southwestern Amazonia. Zootaxa 3716(3):336–348 https://doi.org/10.11646/zootaxa.3716.3.2

Melo-Sampaio PR, Oliveira RM, Prates I. 2018. A new nurse frog from Brazil (Aromobatidae: *Allobates*), with data on the distribution and phenotypic variation of western Amazonian species. South American Journal of Herpetology 13(2):131–149 https://doi.org/10.2994/SAJH-D-17-00098.1

Melo-Sampaio PR, Prates I, Peloso PL, Recoder R, Dal Vechio F, Marques-Souza S, Rodrigues MT. 2020. A new nurse frog from Southwestern Amazonian highlands, with notes on the phylogenetic affinities of *Allobates alessandroi* (Aromobatidae).

Formatado: Inglês (Estados Unidos)

Journal of Natural History 54(1–4):43–62 https://doi.org/10.1080/00222933.2020.1727972

Moraes LJCL, Pavan D, Lima AP. 2019. A new nurse frog of *Allobates masniger-nidicola* complex (Anura, Aromobatidae) from the east bank of Tapajós River, eastern Amazonia. Zootaxa 4648(3):401–434 https://doi.org/10.11646/zootaxa.4648.3.1

Moraes LJ, Lima AP. 2021. A new nurse frog (*Allobates*, Aromobatidae) with a cricket-like advertisement call from eastern Amazonia. Herpetologica 77(2):146–163 https://doi.org/10.1655/Herpetologica-D-20-00010.1

Morales VR. 2002 "2000". Sistematica y biogeografía del grupo *trilineatus* (Amphibia, Anura, Dendrobatidae, *Colostethus*), con descripción de once nuevas especies. Publicaciones de la Asociación de Amigos de Doñana 13:1–59.

Mott T, Vieites DR. 2009. Molecular phylogenetics reveals extreme morphological homoplasy in Brazilian worm lizards challenging current taxonomy. Molecular Phylogenetics and Evolution 51(2):190–200 https://doi.org/10.1016/j.ympev.2009.01.014

Oliveira EA, Silva LA, Silva EAP, Guimarães KLA, Penhacek M, Martínez JG, Rodrigues LRR, Santana DJ, Hernández-Ruz E J. 2020. Four new species of *Pristimantis* Jiménez de la Espada, 1870 (Anura: Craugastoridae) in the eastern Amazon. PLoS One 15(3):1–28 https://doi.org/10.1371/journal.pone.0229971

Palumbi SR. 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK. ed. Molecular Systematics. Sinauer, Sunderland 205–247.

Pyburn WF. 1981. A new poison-dart frog (Anura: Dendrobatidae) from the forest of southeastern Colombia. Proceedings of the Biological Society of Washington 94: 67–75.

Réjaud A, Rodrigues MT, Crawford AJ, Castroviejo-Fisher S, Jaramillo-Martinez AF, Chaparro JC, Glaw F, Gagliardi-Urrutia LAG, Moravec J, De la Riva I, Pérez-Peña PE, Lima AP, Werneck FP, Hrbek T, Ron SR, Ernst R, Kok PJR, Driskell AM, Chave J, Fouquet A. 2020. Historical biogeography identifies a possible role of Miocene wetlands in the diversification of the Amazonian rocket frogs (Aromobatidae: *Allobates*). Journal of Biogeography 47(11):2472–2482 https://doi.org/10.1111/jbi.13937

Segalla MV, Berneck B, Canedo C, Caramaschi U, Cruz CAG, Garcia PCA, Grant T, Haddad CFB, Lourenço ACC, Mângia S, Mott T, Nascimento LB, Toledo LF, Werneck FP, Langone JA. 2021. List of Brazilian Amphibians. Herpetologia Brasileira 8(1):65–96.

Silva LA, Magalhães FM, Thomassen H, Leite FS, Garda AA, Brandão RA, Haddad CFB, Giaretta AA, Carvalho TR. 2020. Unraveling the species diversity and relationships in the *Leptodactylus mystaceus* complex (Anura: Leptodactylidae), with the description of three new Brazilian species. Zootaxa 4779(2):151–189 https://doi.org/10.11646/zootaxa.4779.2.1

Silverstone PA. 1976. A revision of the poison-arrow frogs of the genus *Phyllobates* Bibron in Sagra (family Dendrobatidae). Science Bulletin. Natural History Museum of Los Angeles County 27:1–53.

Simões PI. 2016. A new species of nurse-frog (Aromobatidae, *Allobates*) from the Madeira River basin with a small geographic range. Zootaxa 4083(4):501–525 https://doi.org/10.11646/zootaxa.4083.4.3

Simões PI, Lima AP. 2011. The complex advertisement calls of *Allobates myersi* (Pyburn, 1981) (Anura: Aromobatidae) from São Gabriel da Cachoeira, Brazil. Zootaxa 2988(1):66–68 https://doi.org/10.11646/zootaxa.2988.1.5

Simões PI, Lima AP, Farias IP. 2010. The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger, 1883) (Anura: Aromobatidae). Zootaxa 2406:1–28 https://doi.org/10.11646/zootaxa.2406.1.1

Simões PI, Gagliardi-Urrutia LAG, Rojas-Runjaic FJ, Castroviejo-Fisher S. 2018. A new species of nurse-frog (Aromobatidae, *Allobates*) from the Juami River basin, northwestern Brazilian Amazonia. Zootaxa 4387(1):109–133 https://doi.org/10.11646/zootaxa.4387.1.5

Simões PI, Kaefer IL, Farias IP, Lima AP. 2013b. An integrative appraisal of the diagnosis and distribution of *Allobates sumtuosus* (Morales, 2002) (Anura, Aromobatidae). Zootaxa 3746(3):401–421 https://doi.org/10.11646/zootaxa.3746.3.1

Simões PI, Rojas D, Lima AP. 2019. A name for the nurse-frog (*Allobates*, Aromobatidae) of Floresta Nacional de Carajás, Eastern Brazilian Amazonia. Zootaxa 4550(1):71–100 https://doi.org/10.11646/zootaxa.4550.1.3

Simões PI, Sturaro MJ, Peloso PLV, Lima AP. 2013a. A new diminutive species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura, Aromobatidae) from the northwestern Rio Madeira-Rio Tapajós interfluve, Amazonas, Brazil. Zootaxa 3609(3):251–273 10.11646/zootaxa.3609.3.1

Souza JR, Ferrão M, Hanken J, Lima AP. 2020. A new nurse frog (Anura: *Allobates*) from Brazilian Amazonia with a remarkably fast multi-noted advertisement call. PeerJ 8:e9979 https://doi.org/10.7717/peerj.9979

Tsuji-Nishikido BM, Kaefer IL, Freitas FC, Menin M, Lima AP. 2012. Significant but not diagnostic: differentiation through morphology and calls in the Amazonian frogs *Allobates nidicola* and *A. masniger*. The Herpetological Journal 22(2):105–114.