



A new nurse frog from Southwestern Amazonian highlands, with notes on the phylogenetic affinities of *Allobates alessandroi* Aromobatidae)

Paulo R. Melo-Sampaio ^a, Ivan Prates ^{b,c}, Pedro L. V. Peloso ^d, Renato Recoder ^e,
Francisco Dal Vechio ^e, Sergio Marques-Souza ^e and Miguel T. Rodrigues ^e

^aDepartamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; ^bDepartment of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ^cDepartment of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA; ^dInstituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Brazil; ^eDepartamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

BSTRACT

Few studies have focused on the diverse fauna of southwestern Brazilian Amazonia. This region, spared from large-scale human occupation until the second half of the twentieth century, has been threatened by expanding agriculture, logging, and mining. Here, we describe a new nurse frog (*Allobates*, Aromobatidae) from the open highland habitats of Serra dos Pacaás Novos, one of the few large remnants of relatively intact native vegetation in the Brazilian state of Rondônia. The new species is diagnosed by its metallic orange dorsal colouration, weakly expanded discs on hands, and presence of two subarticular tubercles on Finger IV. A phylogenetic analysis based on DNA sequence data recovered the new species as more closely related to nurse frogs from the Madeira and Tapajós River basins; this pattern may reflect a history of dispersal uphill from a lowland ancestor. Our results also indicate that the generic assignment of *Allobates alessandroi* (Grant and Rodriguez, 2001), a high-elevation species from the Andes of Peru, needs to be reassessed; we discuss the phylogenetic affinities of this species based on morphological attributes.

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Introduction

The onset of European exploration in Amazonia dates back to the 15th century, when explorers entered major rivers to map the region or in search of the so-called natural productions (Levy 2011). Within a century, Iberian powers started to occupy the Amazon Basin to secure territories; in 1616, the Portuguese built the Forte do Presépio at the mouth of Amazon River, where now lies the metropolis of Belém, and in 1669 the Forte de São José da Barra do Rio Negro, presently Manaus (Costa 2014). However, European occupation took longer to reach southwestern Brazilian Amazonia. It was only in 1783 that the area along the Guaporé River, a tributary of the Madeira, acquired geopolitical significance due to the discovery of gold,

CONTACT Paulo R. Melo-Sampaio  prmelosampaio@gmail.com

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which led to the foundation of the Forte Príncipe da Beira to prevent Spanish incursions. Nevertheless, southwestern Brazilian Amazonia was spared from large-scale human occupation until the second half of the 20th century. As a result, this remote region was visited by few natural historians and scientists until recently, and regional biotas remain poorly known.

In the 1960–80s, government-sponsored infrastructure and immigration initiatives in southwestern Amazonia triggered the destruction of vast rainforest areas (Fearnside 1987). In the Brazilian state of Rondônia, large-scale ‘slash-and-burn’ has been widely employed to expand land for pastures and crops. Today, most of Rondônia’s remaining forests lie within conservation units and indigenous reserves, which are constantly threatened by illegal logging, hunting, mining, and volatile environmental policies by local and federal governments. Recent systematic biodiversity inventories have revealed that these imperilled forests host one of the most diverse biotas on Earth (e.g. Marçal et al. 2011), including many undescribed species. Biological surveys are instrumental to improve the documentation and description of these biotas and allow an assessment of species that may have been extirpated from the surrounding areas (Carleial and Bigio 2014; Ohara et al. 2015).

In 2013 a research team, including many of us, performed a three-week-long herpetological survey in the Parque Nacional de Pacaás Novos (Pacaás Novos National Park), which is entirely inserted within the largest section of remaining native vegetation in Rondônia. There, we collected specimens of at least 51 amphibian and 52 reptile species. Among them is an undescribed nurse-frog of the genus *Allobates* Zimmermann and Zimmermann, 1988 a group of small anurans where most species are cryptically coloured and inhabit the leaf litter of Neotropical wet forests. Species diversity in *Allobates* is underestimated, with more than 60 of the recognised species having been named in the 21st century (Melo-Sampaio et al. 2018; Simões et al. 2018, 2019; Frost 2019). Here, we name and describe this new species based on external morphology and discuss its relationships oriented by a phylogenetic analysis inferred from DNA sequence data – analyses based on a combination of new sequences of our specimens and data from previous investigations. By examining the type series of other *Allobates* species associated with mountains, we also found compelling evidence that the generic allocation of *Allobates alessandroi* (Grant and Rodríguez 2001) needs to be reassessed.

Material and methods

Specimen sampling

Specimens of the new species were collected at the Pico do Tracoá (Tracoá Peak) in the Parque Nacional dos Pacaás Novos in April 2013. Individuals were killed with a peritoneal injection of lidocaine hydrochloride, subsequently fixed in 10% formalin for 24 hours and transferred to 70% ethanol for permanent storage. Liver samples were taken prior to fixation and stored in absolute ethanol for posterior genetic analyses. Specimens were deposited at Coleção Herpetológica Osvaldo Rodrigues da Cunha, Museu Paraense Emílio Goeldi (MPEG); Universidade Federal do Acre at Rio Branco (UFAC-RB); Museu Nacional do Rio de Janeiro (MNRJ); and Museu de Zoologia, Universidade de São Paulo (MZUSP).

Genetic sampling and analyses

We generated DNA sequence data for three specimens of the new species and combined these data with published sequences of 59 specimens representing all named *Allobates* species with DNA sequences available on Genbank – sequences were available for 35 of 54 named species. As outgroups, we included one representative of each of the other four aromobatid genera, namely *Anomaloglossus stepheni* (Martins, 1989), *Aromobates nocturnus* Myers, Paolillo and Daly, 1991, *Mannophryne collaris* (Boulenger, 1912), and *Rheobates palmatus* (Werner, 1899). Following the revisionary work of Grant et al. (2017), we rooted our tree by considering *Allobates* as the sister of a clade composed of the remaining Aromobatidae.

We generated sequences of the mitochondrial gene 16S subunit ribosomal RNA (16S) following Palumbi (1996). Sequences were edited using Geneious Pro 6 (Biomatters, Auckland) and deposited in Genbank (Benson et al. 2013) (accession numbers MT076998–MT077000). To improve phylogenetic inference in *Allobates*, we included in our data matrix sequences of nine additional genes (totalling 10 genes and 6,721 base pairs) available on Genbank, as follows: the nuclear genes 28S ribosomal RNA (28S), histone H3 (HH3), recombination activating gene 1 (RAG1), rhodopsin (RHO), seventh in absentia (SIA), and tyrosinase (TYR); and the mitochondrial genes 12S subunit ribosomal RNA (12S), cytochrome oxidase subunit I (COI), and cytochrome B (CYTB). An alignment was built for each gene in MAFFT 7.309 (Katoh and Standley 2013) as implemented in Geneious using default settings. We refrained from manually modifying alignments to avoid subjective changes; however, visual inspection suggested no alignment anomalies. A concatenated alignment file was generated using SequenceMatrix (Vaidya et al. 2011).

For phylogenetic inference, models of nucleotide evolution and best-fit partition schemes were determined using PartitionFinder 2.1.1 (Lanfear et al. 2016) with PhyML for likelihood estimation (Guindon et al. 2010) and the Bayesian Information Criterion for model selection (Sullivan and Joyce 2005). Based on PartitionFinder results, codon partitioning was used for the protein-coding genes. We performed phylogenetic inference under a Bayesian framework using MrBayes 3.2.6 (Ronquist et al. 2012), implementing three independent runs of four Markov chains of 10 million generations each and sampling every 1,000 generations. We used Tracer 1.6 (Rambaut et al. 2018) to assess whether Markov chain mixing was adequate (effective sample sizes > 200) and to visually assess model parameter stationarity and convergence between runs. We then combined runs using LogCombiner 1.8.4 (with 20 discarded as burn-in) and summarised a maximum clade credibility tree in TreeAnnotator 1.8.4 (Drummond et al. 2012). Resulting topologies were visualised in FigTree 1.4.3 (Drummond et al. 2012).

Morphological data and analyses

We analysed and measured preserved specimens under a stereo-microscope. We determined sex and life stage (i.e. juvenile or adult) from direct observation of sexual characteristics; namely, presence of vocal slits in males (if two vocal slits were present, the specimen was considered as an adult) or presence of eggs in the abdomen in females.

Morphological characters were scored in all specimens following definitions and terminology suggested in Barrio-Amorós and Santos (2009). We scored most morphological data, including colour in preservative, from direct observation of specimens, with the exception of colour in life, which were obtained from digital photographs taken in the field. For

comparisons with other *Allobates* species, we examined specimens deposited at MNRJ, MPEG, MZUSP, UFAC-RB, Museo de la Universidad San Marcos (MUSM), American Museum of Natural History (AMNH), Instituto Nacional de Pesquisas da Amazônia (INPA), and Smithsonian National Museum of Natural History (USNM) (see [Appendix A](#)).

A digital caliper was used to take morphometric measurements to the nearest 0.1 mm. The following measurements were taken: snout-vent length (SVL); head length, from jaw articulation to the tip of the snout (HL); head width, at the level of the jaw articulation (HW); interorbital distance (IOD); eye–snout distance, from the anterior corner of the eye to the tip of snout (ET); internarial distance (IND); eye diameter (ED); diameter of tympanum (TD); forearm length (FAL); upper arm length (UAL); hand length, from the proximal edge of palmar tubercle to the tip of Finger III (HandIII); width of the disc of Finger III (WDFIII); tibia length (TL); foot length, from the proximal edge of the metatarsal tubercle to the tip of toe IV (FL); width of the disc of toe IV (WDTIV).

Results

Phylogenetic relationships

Our phylogenetic results ([Figure 1](#)) are largely consistent with those of previous analyses of *Allobates* (Grant et al. 2006, 2017; Melo-Sampaio et al. 2018). *Allobates offersioides* (Lutz, 1925) from the Brazilian Atlantic Forest was inferred as the sister of all remaining *Allobates*, followed by *Allobates undulatus* (Myers and Donnelly, 2001) from *tepui* highlands. A clade composed of the trans-Andean taxa *Allobates talamancae* (Cope, 1875) and *Allobates niputidea* Grant, Acosta-Galvis, and Rada, 2007 is the sister of a clade formed by the remaining cis-Andean species.

This cis-Andean clade encompasses three major lineages ([Figure 1](#)): a highly supported clade (posterior probability (PP) = 1) composed of brightly coloured species thought to mimic poison frogs; a poorly supported clade (PP = 0.43) composed of small-bodied and cryptically coloured species from the Andean foothills, *tepui* highlands, and Amazonian lowlands; and a moderately supported clade (PP = 0.80) composed of large-bodied species that occur south of the Amazon River.

The last of those three clades includes the new species from Parque Nacional dos Pacaás Novos, along with *Allobates aviventris* Melo-Sampaio, Souza and Peloso, 2013, *Allobates magnussoni* (Lima, Simões and Kaefer, 2014), *Allobates masniger* (Morales, 2002), *Allobates nidicola* (Caldwell and Lima, 2003), and *Allobates nunciatus* (Moraes, Pavan and Lima, 2019). The new species was inferred to be the sister of, and yet highly divergent from, the most recent common ancestor all of the other five species in this clade ([Figure 1](#)).

Allobates pacaas sp. nov.

Figures 2–4.

Holotype MZUSP 158934 (field number MTR 25831 [Figures 2–4](#)), an adult female from Pico do Tracoá, Parque Nacional dos Pacaás Novos, Campo Novo de Rondônia municipality, state of Rondônia, Brazil (10.84892°S, 63.63312°W; 911 m above sea level), collected by I. Prates, R. Recoder, F. Dal Vechio, S. M. de Souza, M. Teixeira Jr., and M. T. Rodrigues on 21 April 2013.

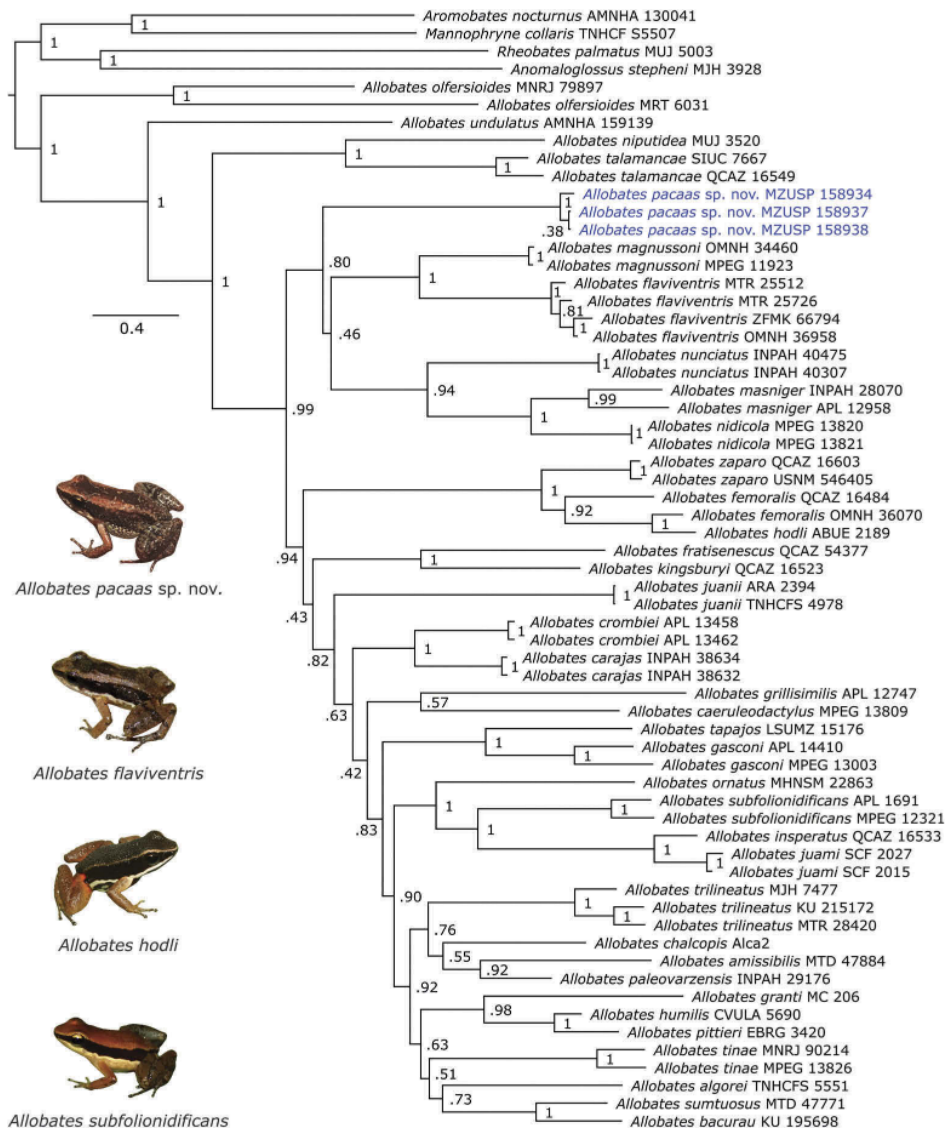


Figure 1 Phylogenetic relationships of *Allobates* inferred under a Bayesian framework based on 10 molecular markers.

Paratopotypes Eight males MNRJ 93128 (field number MTR 25829), MNRJ 93129 (MTR 25839), MZUSP 158935 (MTR 25867), MZUSP 158936 (MTR 25843), MZUSP 158937 (MTR 25868), MZUSP 158938 (MTR 25840), MPEG 42818 (MTR 25837), MPEG 42819 (MTR 25838), and one female UFAC-RB 9470 (MTR 25830), all adults and with the same collection data as the holotype.

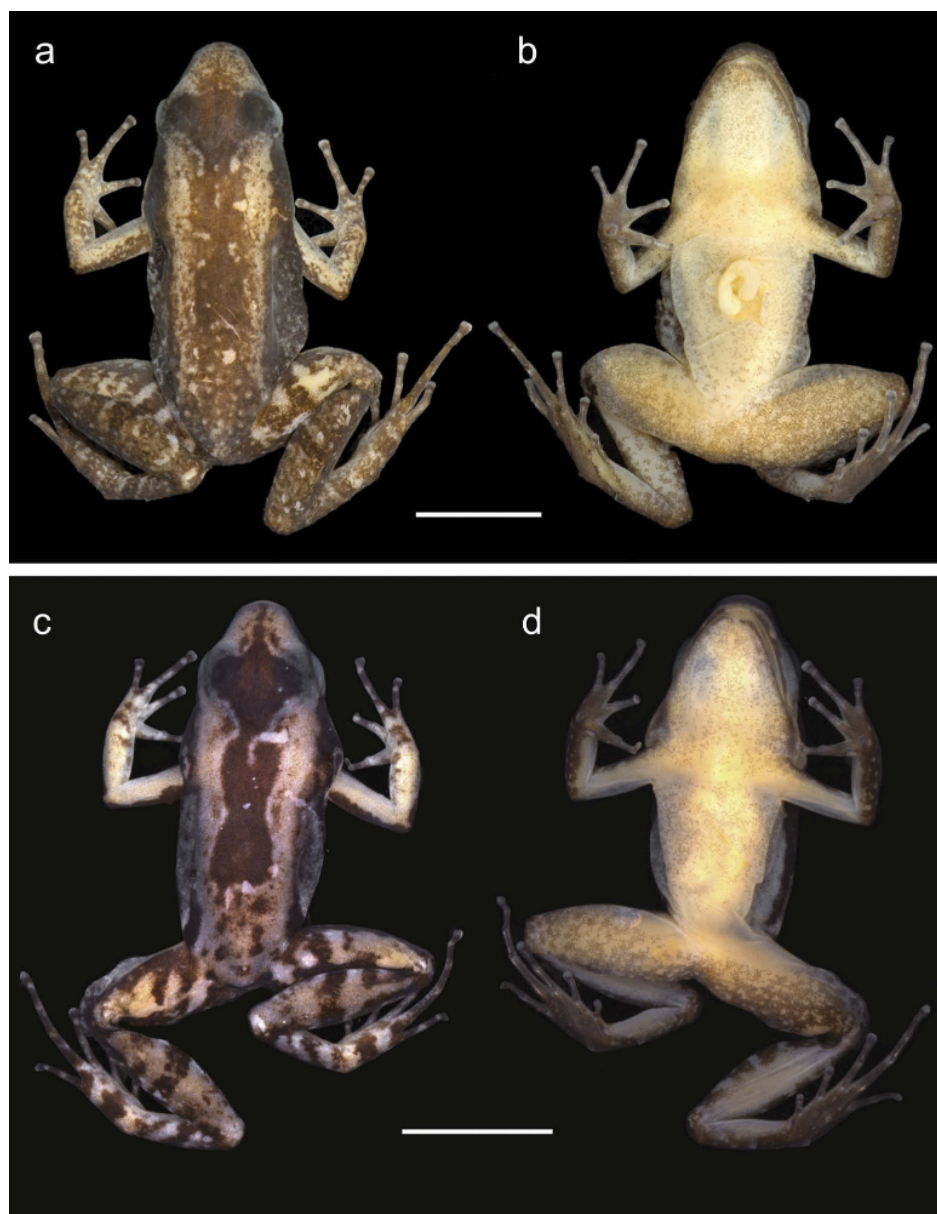


Figure 2 Dorsal (a) and ventral (b) views of the holotype of *Allobates pacaas* sp. nov. (MZUSP 158934, female). Dorsal (c) and ventral (d) views of paratype (MZUSP 158937, male). Scale bar = 5 mm.

Referred specimen UFAC-RB 6798, from Parque Nacional Pacaás Novos, Base Candeias (10.79111°S, 63.63030°W; 410 m above sea level), collected by K. D. Zaqueo on 17 July 2014.

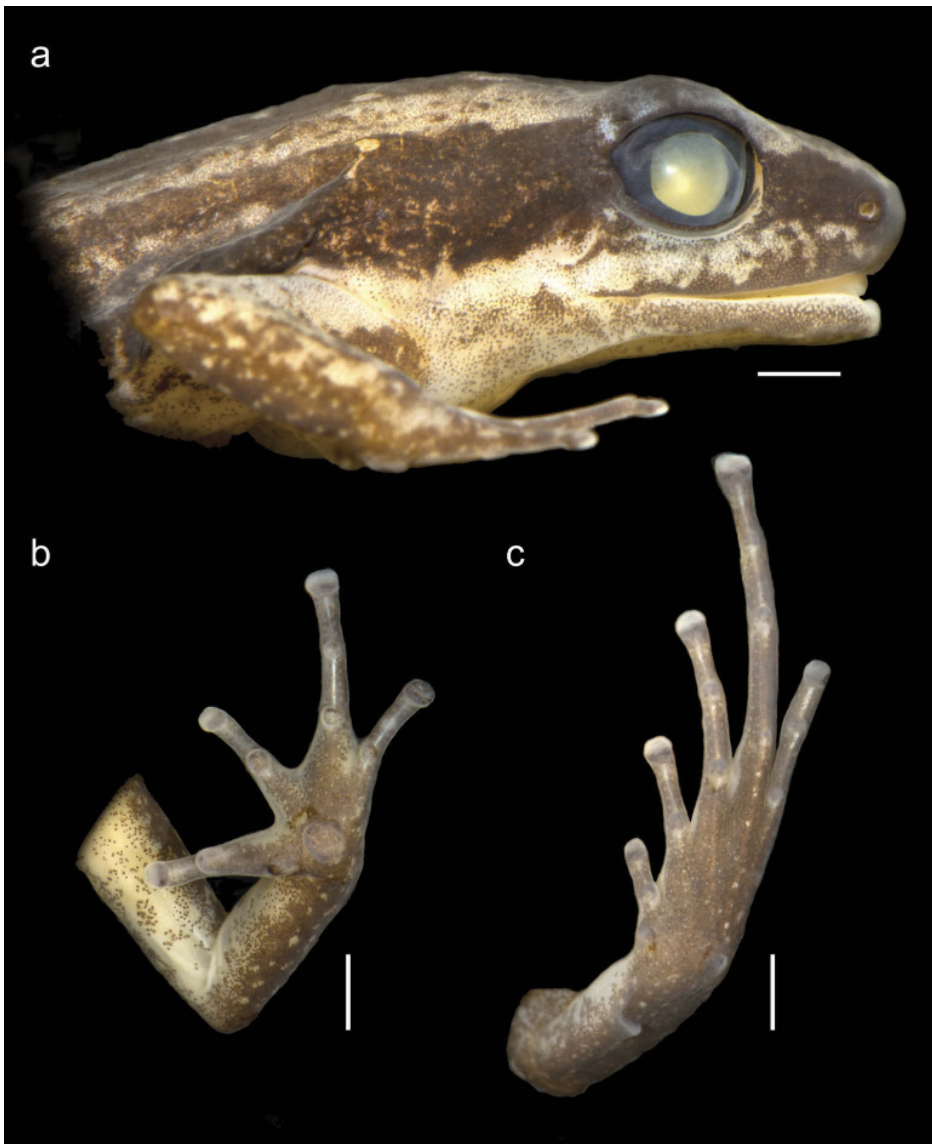


Figure 3 Head (a), left hand (b), and left foot (c) of the holotype of *Allobates pacaas* sp. nov. (MZUSP 158934). Scale bars = 1 mm.

Diagnosis The new species is assigned to *Allobates* based on morphological similarity and on results of a phylogenetic analysis of DNA sequence data. The species is diagnosed from its congeners by the following combination of characteristics: (i) snout rounded in lateral and dorsal views; (ii) a small-sized species, mean SVL of males 13.9 mm (range 12.8–15.2 mm), mean SVL of females 15.6 mm (range 14.7–16.6 mm); (iii) dorsal surface of snout light brown, same colour extending posteriorly until eye level; dorsum tan brown in life with an hourglass pattern and scattered golden flecks; (iv) skin texture smooth

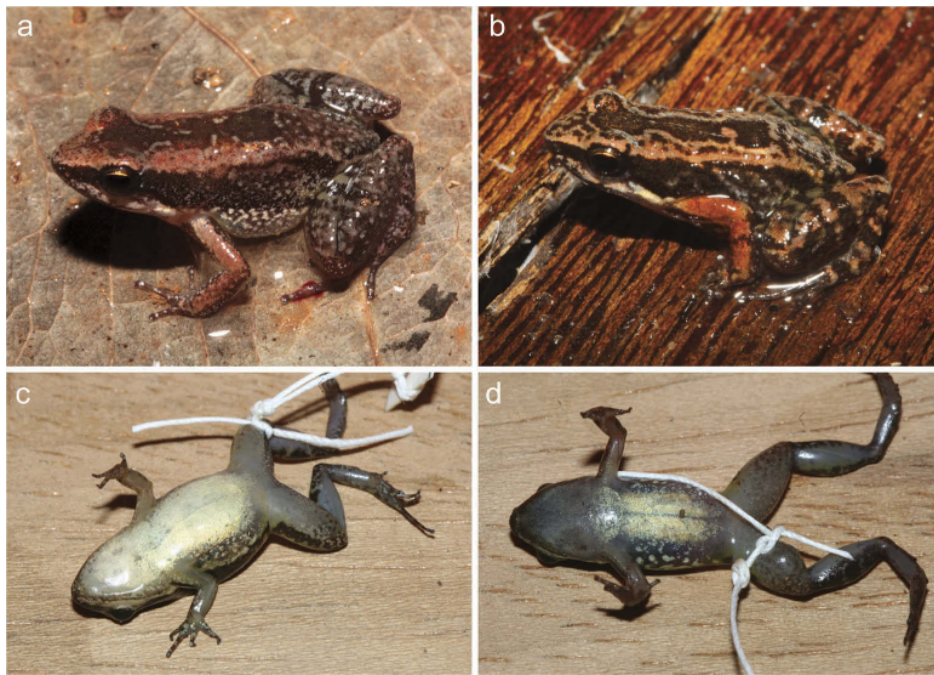


Figure 4 Dorsal (a) and ventral (c) views of the female holotype of *Allobates pacaas* sp. nov. (MZUSP 158934) in life. Dorsal (b) and ventral (d) views of male paratype MZUSP 158938 in life.

throughout the body; (v) lateral dark brown band with well-delimited upper edge and ill-defined lower edges; (vi) abdominal surface cream with scattered melanophores; melanophores more concentrated around lips and ventral surface of thigh; (vii) dorsal surface of legs with conspicuous dark brown transversal stripes; (viii) dark throat-collar absent; (ix) iris metallic gold to coppery brown without vermiculation; (x) vocal sac distinct, single, subgular; (xi) maxillary teeth present; (xii) Finger III not swollen in males; (xiii) presence of two subarticular tubercles in Finger IV; (xiv) rudimentary basal webbing present between Toes III and IV; (xv) slightly expanded discs on fingers and toes; (xvi) fringes absent on finger and toes.

Description of the holotype An adult female with SVL 16.6 mm, HL 4.9 mm, HW 4.2 mm, IOD 3.0 mm, ET 2.2 mm, IND 1.5 mm, ED 1.7 mm, TD 0.8 mm, FAL 3.4 mm, UAL 4.0 mm, HANDIII 4.1 mm, WDFIII 0.5 mm, TL 7.6 mm, FL 7.4 mm, WDTIV 0.5 mm. Head slightly longer than wide, HL 115.6% of HW, HW 25.5% of SVL, HL 29.4% of SVL. Snout rounded to nearly truncate in dorsal and lateral views (Figures 2–4). ETD 44.9% of HL, IND 36.2% of HW, ETD 79.5% of ED. Nares opening laterally. Tympanum behind and lower than the eye, close to the angle of jaws, covered superiorly by a supratympanic fold; lower part of tympanic annulus visible; TD 44.8% of ED. Tongue nearly twice as long as wide, attached anteriorly, rounded posteriorly; median lingual process absent. Skin texture smooth throughout the body. Cloacal tubercles absent.

Forearm and upper arm nearly identical in thickness; FAL 117.6 of UAL. Finger III length 24.8 of SVL; tip of Finger IV reaching distal subarticular tubercle of Finger III when fingers are adpressed; Finger II reaching the distal subarticular tubercle of Finger III; relative length of fingers III>I> II>IV; Finger III not swollen; webbing and lateral fringes absent on fingers. Palmar tubercle nearly ovoid; thenar tubercle present, ovoid; a single subarticular tubercle present on Finger I, and II, two subarticular tubercles on Finger III and IV. Discs of Fingers I, II, III and IV slightly expanded, all with distinct dorsal paired scutes; width of the disc on Finger III 0.47 mm.

Tibia length 45.9 of SVL. Relative length of toes IV>III>V> II>I; rudimentary basal webbing present between Toes III–IV; webbing absent between Toes II–III; fringes absent on all toes. Tip of Toe I barely reaching proximal subarticular tubercle of Toe II when toes are appressed; tip of Toe III reaching medial tubercle of Toe IV. Inner metatarsal tubercle ovoid and outer metatarsal tubercle rounded. Metatarsal fold present, weakly developed; tarsal keel present, conspicuous and curved, tubercle-like. A single subarticular tubercle present on Toe I; two subarticular tubercles present on Toes II, III and V; three subarticular tubercles present on Toe IV.

Colour of the holotype in preservative Dorsal surface of body generally dark brown, except on the snout region (anterior corner of the eyes to the tip of snout) and for the two dorsolateral stripes, where colour is paler (light brown; golden) delimiting an hourglass dorsal pattern (Figure 2(a)). Scattered golden flecks are present on the posterior surface of the dorsum. Dorsolateral stripes are present from the posterior corner of the eye to the groin, becoming faint posteriorly; weakly defined on the inner margins (view under magnification). Lateral surface of the body characterised by a solid, wide, dark brown stripe, extending from the tip of the snout to the groin; ventral margin ill-defined.

Arms and hands pale brown in dorsal view; tip of the fingers light brown; paired scutes on finger discs brown. Dorsal surface of thighs with intercalating light and dark brown stripes of similar width; dorsal surface of tibias with intercalating brown and dark brown stripes of similar width; dorsal surface of feet brown.

Throat, gular, and anterior pectoral regions pale, with a conspicuous presence of melanophores on the marginal areas of the throat, evenly spaced elsewhere. Pectoral region, abdomen and ventral surface of thigh insertion pale cream; scattered and evenly spaced melanophores on pectoral and abdominal region; heavily pigmented (high concentration of melanophores) on ventral surface of thigh and knees; ventral surface of upper arm similar to pectoral and abdominal region, forearm and hand completely pigmented (brown). Ventral surface of legs and feet completely pigmented (brown). Tips of fingers and toes light brown.

Arms cream to pale brown in dorsal view, melanophores concentrated in small and regular light brown blotches on posterior surfaces of upper arm, forearm and hand. Tip of fingers light brown. Paired scutes on finger discs cream. Upper arm cream in ventral view. Forearm and hand tan brown in ventral view.

Colour variation in preserved specimens Trunk dorsal colour pattern varies considerably in the type series. UFAC-RB 9470 and MZUSP 158935 are most similar to holotype. In MPEG 42818, MZUSP 158936, and MZUSP 158937, the inner margins of the dorsolateral

stripes are rugged, forming an hourglass-shaped pattern. On MPEG 42819 and MNRJ 93129, the inner margins of the dorsolateral stripes are irregular with projections inwards, forming a reticulated pattern. The lateral stripes are uniformly brown in the MZUSP 158935, MZUSP 158938 and UFAC-RB 9470. Oblique lateral stripes are present in MPEG 42818, MZUSP 158936 and MZUSP 158937; golden mottling over the stripes is present in MPEG 42819 and MNRJ 93129.

Colour of adults in life Head copper to pinkish with small dark brown spots (Figure 5). Copper to tan-brown dorsum with a dark brown hourglass surrounded by narrow golden line (incomplete in darker specimens). Lateral bands dark brown. Pale ventrolateral stripes interrupted at the level of arm insertion, running faded from axilla to groin. Forelimbs

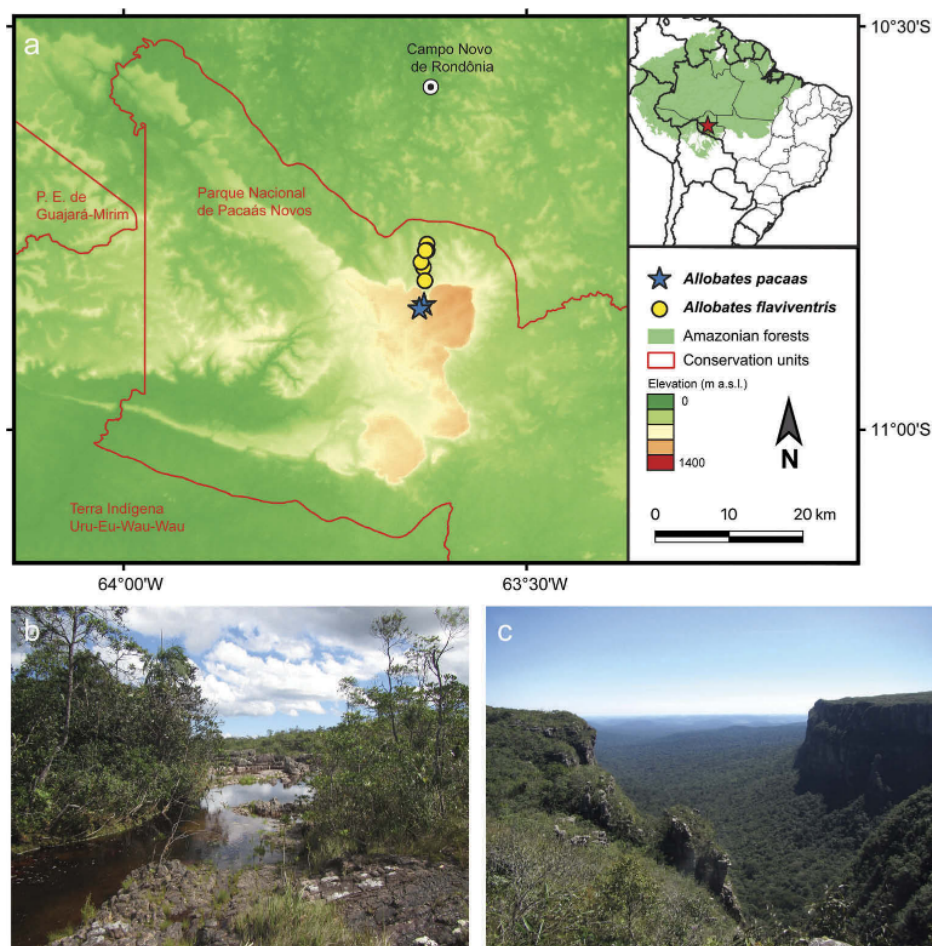


Figure 5 (a) Altitudinal map depicting the location of the Parque Nacional dos Pacaás Novos in southwestern Brazilian Amazonia and conservation units in the area (red lines). Collection sites of *Allobates pacaas* sp. nov. in the highlands of the park (blue stars) and of *Allobates flaviventris* in lowland forests (yellow circles) are indicated. (b) Habitat where *A. pacaas* was found in the Serra dos Pacaás National Park. (c) View of highland savannahs and lowland rainforests from the top of the Pico do Tracoá.

orange in dorsal surface. Hindlimbs copper to tan brown with dark brown transversal stripes. Ventral surface of female metallic cream; males with violet throat and metallic cream chest and belly.

Comparisons We compare the new species to the other species inferred as members of the same major clade within *Allobates* (Figure 1) and with sympatric or geographically close species from southwestern Amazonia.

The presence of two subarticular tubercles on Finger IV distinguishes *A. pacaas* from all other Amazonian *Allobates* species except *Allobates hodli* (Simões, Lima and Farias, 2010).

Regarding its closest relatives, *A. pacaas* is distinguished from *A. masniger*, *A. nidicola*, and *A. nunciatus* in having an hourglass-shaped, dark brown, pattern on dorsum and orange arms (uniform brown dorsum and pale brown arms in *A. masniger*, *A. nidicola*, and *A. nunciatus*). *Allobates pacaas* further differs from *A. masniger* and *A. nidicola* in having a light white belly (dark belly in *A. masniger* and *A. nidicola*). *Allobates pacaas* differs from *A. aviventris* and *A. magnussoni* by the absence of a golden-yellow belly (present in *A. aviventris* and *A. magnussoni*).

Relative to other southwestern Amazonian species, *Allobates pacaas* differs from *Allobates brunneus* (Cope, 1887) by having a smaller size (maximum SVL < 16.6 mm in females), dorsal skin smooth, and metallic white venter (maximum SVL 19.8 in females, dorsal skin granular, and lemon-yellow venter in *A. brunneus*). *Allobates pacaas* differs from *Allobates gasconi* (Morales, 2002) by having the Finger III not swollen and dark throat in males in life (swollen Finger III and white throat in life in *A. gasconi*). *Allobates pacaas* differs from *Allobates conspicuus* (Morales, 2002), *A. grillisimilis* (Simões, Sturaro, Peloso and Lima, 2013b), and *A. subfolionidificans* (Lima, Sanchez and Souza, 2007) by having an hourglass pattern on the dorsum (uniform dorsum with dorsolateral and ventrolateral stripes in *A. conspicuus* and *A. grillisimilis*; without ventrolateral stripes in *A. subfolionidificans*). *Allobates pacaas* further differs from *A. conspicuus* and *A. subfolionidificans* by having a dark throat in live male specimens (throat white to translucent in males of *A. conspicuus* and *A. subfolionidificans*). *Allobates pacaas* differs from *A. tapajos* by having a violet throat in live male specimens (golden yellow in *A. tapajos*). *Allobates pacaas* differs from *A. tinae* Melo-Sampaio, Oliveira, and Prates, 2018 by having dark brown transversal stripes on thighs and dark throat in males (absent transversal stripes on thighs and golden-yellow throat in males of *A. tinae*). *Allobates pacaas* differs from *Allobates fuscillus* (Morales, 2002) and *A. trilineatus* (Boulenger, 1883) by the absence of swelling on Finger III and presence of a dorsal hourglass pattern (swollen Finger III and dorsum uniform with dorsolateral stripes in *A. fuscillus* and *A. trilineatus*). Lastly, *A. pacaas* differs from *Allobates femoralis* (Boulenger, 1883) and *A. hodli* by the absence of red, orange or yellow marks on the dorsal surface of thighs and axilla (red, orange or yellow ash marks on thighs and axilla in *A. femoralis* and *A. hodli*).

Etymology *Allobates pacaas* is named in recognition of an important natural refuge in the Brazilian state of Rondônia. The region has experienced intense deforestation since the 1960s with large-scale loss of biodiversity (Fearnside 1987). The specific epithet is a noun in apposition to the genus.

Distribution and natural history The Parque Nacional de Pacaás Novos is situated in the western part of the state of Rondônia and covers an area of about 765,000 ha. The

national park widely overlaps with the Terra Indígena (indigenous territory) Uru-Eu-Wau-Wau (Figure 5(a)). Major rivers draining the area are the Madeira, Mamoré, Guaporé, Machado and Candeias. Elevation varies from about 200 up to 1,230 m on the Pico do Tracoá, in a rare instance of mountains in the western part of the Amazon Basin east of the Andes. There is an altitudinal gradient of habitat types in the park; most of the lower areas are covered by tropical rainforest, while above 700 m, forests progressively dwindle and give space to open Cerrado-like savannahs with rocky fields and scattered vegetation.

The type series of *A. pacaas* was collected at two nearby sites on top of the Pico do Tracoá, at an approximate elevation of 900 m (Figure 5(b-c)) (IBGE 2012). The first site (10.84892°S, 63.63312°W) is characterised by rocky outcrops and low vegetation adjacent to a stream (the Rio Pacaás Novos) with clear waters and waterfalls, where some specimens were found hiding in clusters of tall grasses on river banks. The second site (10.84361°S, 63.62726°W) is a dense and short gallery forest adjacent to the same stream, where the species was found on the leaf litter. All specimens were found active during the day. *Allobates pacaas* was not detected in two well-sampled lowland rainforest sites, where *A. aviventris* was found (Figure 5(a)). We did not witness any reproductive or call activity, potentially as a result of the lack of rains during the sampling period.

Discussion

Over the last two decades, the taxonomy of *Allobates* has tremendously benefitted from improved diagnoses based on morphological attributes (Lima and Caldwell 2001; Grant and Rodríguez 2001; Caldwell et al. 2002; Lima et al. 2009; Lima et al. 2012; Simões and Lima 2012) and from integrative taxonomic approaches that incorporated molecular phylogenetics, vocalisation, reproduction behaviour, and colour in life data (Lima et al. 2007, 2010, 2014, 2015; Simões et al. 2010, 2013a, 2013b, 2014, 2019; Melo-Sampaio et al. 2013, 2018; Simões 2016; Moraes et al. 2019). Improved knowledge on species boundaries has supported the discovery and description of several new taxa and provided a picture of both wide-ranged and narrowly distributed species in Amazonian *Allobates*. Nevertheless, the discovery of *A. pacaas* suggests that the diversity of nurse frogs remains underestimated. As illustrated by this investigation, comprehensive biodiversity inventories are instrumental to refine species distributions and reveal distinct evolutionary lineages in Amazonia (Peloso 2010; Carneiro et al. 2016; Magnusson et al. 2016; Melo-Sampaio et al. 2018; Sturaro et al., forthcoming).

Allobates pacaas is one of the several nurse frog species that occur in Amazonian highlands. Other examples include *Allobates fratisenescus* (Morales, 2002), *Allobates ornatus* (Morales, 2002), *Allobates algorei* Barrio-Amorós and Santos, 2009, and *Allobates humilis* (Rivero, 1980) from the Andean foothills; *Allobates amissibilis* Kok Hölting, and Ernst, 2013, *Allobates granti* (Kok, MacCulloch, Gaucher, Poelman, Bourne, Lathrop, and Lenglet, 2006), and *A. undulatus* from the tepui highlands; and *Allobates carajas* Simões, Rojas, and Lima, 2019 from the Serra dos Carajás in eastern Brazilian Amazonia. A history of several independent dispersals into montane settings may have contributed to nurse frog diversification and endemism in highlands. Accordingly, studies in other Amazonian frog clades have found that species dispersal between lowlands and highlands played an important role in shaping current distribution patterns (Santos et al. 2009; Kok et al. 2012; Salerno et al. 2012). Our phylogenetic analyses clustered *A. pacaas* with five species from the Madeira and Tapajós river basins, which may

reflect a lowland origin of the former. One of those species, *A. aviventris*, was sampled in the lowland forests adjacent to the Pico do Tracoá within the Parque Nacional dos Pacaás Novos. Moreover, our finding of a deep divergence between *A. pacaas* and closely related taxa poses the hypothesis of prolonged population persistence on the highlands, as seen in other montane endemics (Prates et al. 2017, 2020). However, the clade grouping *A. pacaas* with lowland species received only moderate support (posterior probability = 0.80). An improved sampling of genetic variation will be necessary to further test the hypotheses of a lowland-to-highland colonisation and of long-term population persistence in highland habitats.

Serra dos Pacaás is a massive rocky plateau isolated in southwestern Amazonia. Few scientists have visited this unique landscape, and the local biota is poorly known. The discovery of *A. pacaas*, likely endemic to the region, suggests that the Serra dos Pacaás hosts species found nowhere else. Besides the new nurse frog, we sampled at least five additional unnamed species of the frog genus *Pristimantis* Jiménez de la Espada, 1870 one unnamed *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991, and one possibly unnamed *Cercosaura*; Wagler 1830 in the park. Given its high biodiversity, species new to science, and unique combination of habitats, the Parque Nacional dos Pacaás Novos emerges as a key region for the study and conservation of biodiversity in southwestern Amazonia. However, conservation units in this region are now threatened by illegal logging and mining and by rapidly expanding farmlands. Therefore, we urge that the Brazilian government and society commit to the integral preservation of this biodiversity sanctuary.

Notes on *Allobates alessandroi*

After examination of the morphology of other *Allobates* species known to occur in montane areas, we concluded that *Allobates alessandroi* (Grant and Rodríguez, 2001) is likely taxonomically misplaced.

Allobates alessandroi (Figure 6) was described based on five specimens collected in the Peruvian Andes (Cusco and Puno provinces). The species has a large body size in males when compared to most *Allobates* taxa (>20 mm SVL). It is also the only species of *Allobates* with well-developed fringes on both edges of all toes (Figure 6(a)), keel-like lateral fringes on the fingers (most prominent distally), and two subarticular tubercles on Finger IV (Figure 6(b)) (Grant and Rodríguez 2001). The presence of webbed or fringed toes, unknown in other *Allobates*, is a diagnostic character of *Hyloxalus* (Noble 1931; Dunn 1933; Grant et al. 2006). Furthermore, the following additional characters of *A. alessandroi* are also diagnostic for *Hyloxalus* Jiménez de la Espada, 1870 (as proposed by Grant et al. 2006; emendation by, Grant et al. 2017 in bold): (1) dorsal colouration generally cryptic, brown, grey, or black; (2) pale oblique lateral stripe present; (3) pale dorsolateral stripe absent in most (but not all) species; (4) pale ventrolateral stripe usually absent; (5) dorsal skin texture posteriorly granular; (6) toe webbing varying from absent in most species to basal or extensive in some species; (7) Finger III of adult males (**often**) not swollen; (8) Finger I (**often**) shorter than Finger II; (9) finger discs narrow to moderately expanded; (10) median lingual process absent; and (11) dark throat collar absent. Recently, Grant et al. (2017) found a swollen Finger III in species of *Hyloxalus*, a trait that is also present in *A. alessandroi*.

To our knowledge, *A. alessandroi* is known only from the type series and has no DNA sequence data available; the current phylogenetic placement of this species can thus be considered tentative. In the absence of molecular data and based on the morphological

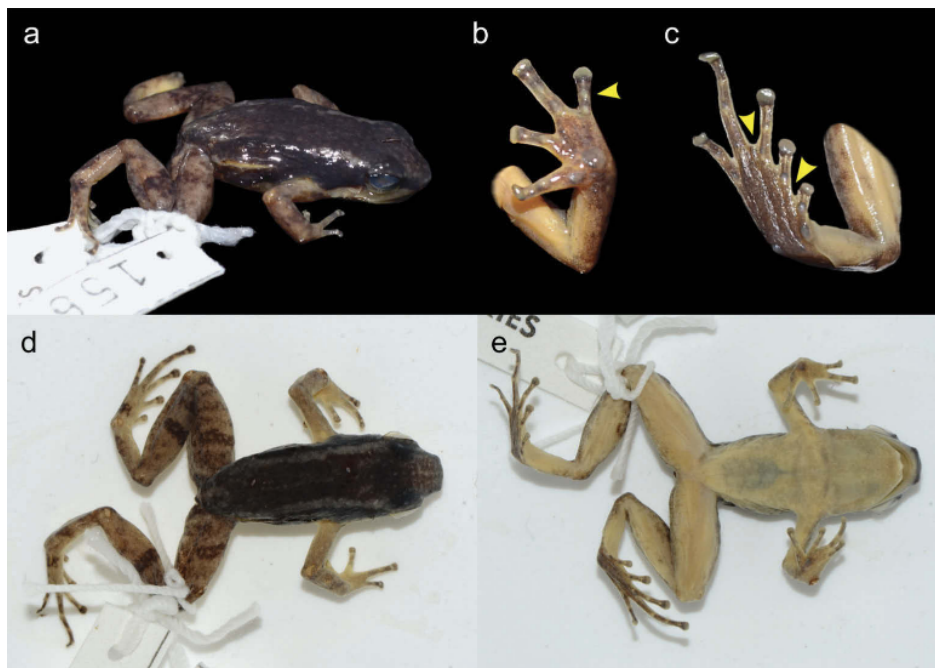


Figure 6 Holotype of *Allobates alessandroi* (MUSM 15609) in dorsolateral view (a). Hand of holotype (b) showing two subarticular tubercles in Finger IV (arrow). Foot of holotype (c) showing lateral fringes and basal webs (arrow). Dorsal (d) and ventral (e) view of paratype AMNH 157004.

attributes presented above, we assign *A. alessandroi* to the genus *Hyloxalus* under *Hyloxalus alessandroi* **new combination**. If this species was indeed a member of *Allobates*, its unique morphology would represent a remarkable case of convergence with *Hyloxalus*, potentially as an adaptation to life in rocky streams. Such traits, however, are not observed in *A. pacaas* and other *Allobates* species known to inhabit similar habitats.

Other *Allobates* species have unclear taxonomic affinities and may need to be reassessed. Recently, *Allobates picachos* (Ardila-Robayo, Acosta-Galvis, and Coloma, 2000) was allocated to *Hyloxalus* by Acosta-Galvis and Vargas-Ramírez (2018) based on molecular data. Other species that may deserve consideration are *Allobates mandelorum* (Schmidt, 1932) (as noted by Barrio-Amorós et al. 2019), *Allobates sanmartini* (Rivero, Langone, and Prigioni, 1986), and *Allobates wayuu* (Acosta-Galvis, Cuentas, and Coloma, 1999), three species tentatively allocated to *Allobates* by Grant et al. (2006). Similar to *A. alessandroi*, the last two species have attributes typical of *Hyloxalus*, such as Finger I smaller than Finger II, two tubercles on Finger IV, well-developed interdigital membranes on toes, and presence of an oblique lateral line. To reassess the generic status of these and other poorly known species, future studies will benefit from the re-examination of morphological attributes and incorporation of additional data types.

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ORCID

Paulo R. Melo-Sampaio  <http://orcid.org/0000-0003-1858-1643>

Pedro L. V. Peloso  <http://orcid.org/0000-0003-0127-8293>

Miguel T. Rodrigues  <http://orcid.org/0000-0003-3958-9919>

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Appendices

Appendix A Examined specimens

Allobates alessandroi (n = 4): PERU: Cusco: Paucartambo: MUSM 15609 (holotype), MUSM 17737 (paratype), AMNH 157004 (paratype, through photographs); Puno: MUSM 15608 (paratype).

Allobates brunneus (n = 18): BRAZIL: Mato Grosso: Chapada dos Guimarães: INPA-H 10116–18, 10120–21, 10123, 10127, 10129–31, 10131, 10133, 10136, 10140–42, 10148, UFAC-RB 2910.

Allobates caeruleodactylus (n = 9): BRAZIL: Amazonas: Autazes: INPA-H 7238 (holotype), 7229–32, 7234–37 (paratypes).

Allobates conspicuus (n = 2): PERU: Madre de Dios: Manu: MUSM 24238. MUSM 31601.

Allobates femoralis (n = 15): BRAZIL: Acre: Cruzeiro do Sul: UFAC-RB 2775; Sena Madureira: UFAC-RB 4714; Amazonas: Careiro da Várzea: MPEG 13806–17. Rondônia: Porto Velho: INPA-H 16728.

Allobates aviventris (n = 88): BRAZIL: Acre: Rio Branco: UFAC-RB 5536; Senador Guimard: UFAC-RB 4650 (holotype), 4599–4603, 4631–4635, 4640–4641, 4649, 4657–4667, 4669–4671, 4675–4678 (paratypes), MNRJ 91945, UFAC-RB 0159, 0161, 0165, 0166, 0349, 0401, 0520, 0521, 0524, 0525, 0535, 0567, 4639, 4718, 4953. Amazonas: Boca do Acre: UFAC-RB 4627. Rondônia: Campo Novo de Rondônia: MZUSP 158939–49; Nova Mamoré: MPEG 13348–75.

Allobates fuscus (n = 6): BRAZIL: Amazonas: Ipixuna: INPA 2532 (holotype), 2531; Itamarati: INPA 3114, 3250, 3270, 3514 (paratypes).

Allobates gasconi (n = 84): BRAZIL: Acre: Bujari: UFAC-RB 4252–54; Feijó: MCP 13630; Nova Vida: INPA 4726 (paratype); Plácido de Castro: UFAC-RB 1163, 1164, 1166, 1238, 1273. Porto Acre: UFAC-RB 1055, 1122; Rio Branco: MNRJ 91679, UFAC-RB 3471–73, 3478–81, 3484–85 3494–3495, 3551; Sena Madureira: UFAC-RB 4616–17, 4719–25. Amazonas: Boca do Acre: MNRJ 90229–40, UFAC-RB 4668; Itamarati: INPA 3082 (holotype), 3079, 3085, 3090, 3150–51, 3172, 3249, 3406, 3415, 3483–84, 3491, 3494, 3496, 3512–13 (paratypes), 3073 (as a paratype of *A. vanzolinus*); Lábrea: MPEG 12992–13010.

Allobates goianus (n = 1): BRAZIL: Goiás: Alto Paraíso: MZUSP 76652 (holotype; former WCAB 47779).

Allobates grillisimilis (n = 8): BRAZIL: Amazonas: Nova Olinda do Norte: INPA-H 30809–30810 (paratypes); Borba: INPA-H 30784, 30794, 30799–30801, 30804 (paratopotypes).

Allobates hodli (n = 35): BRAZIL: Acre: Plácido de Castro: UFAC-RB 1242; Senador Guimard: UFAC-RB 4654–56, 4674, 4949. Amazonas: Boca do Acre:

Rondônia: Porto Velho: INPA-H 16555 (holotype), INPA-H 16541, 16553–68 (paratopotypes); INPA-H 16622, INPA-H 16818, INPA-H 16788, 16767, 16756, 16805, 16758, 16788, 16739, 16771, 16777, 16730 (paratypes).

Allobates magnussoni (n = 30): BRAZIL: Pará: Santarém: MPEG 11921–50.

Allobates marchesianus (n = 9): BRAZIL: Amazonas: Barcelos: INPA-H 7970–72, 7976–77, 7988, 10212, UFAC-RB 4181 (topotypes); São Gabriel da Cachoeira: UFAC-RB 4812.

Allobates masniger (n = 5): BRAZIL: Pará: Itaituba: Parque Nacional da Amazônia: USNM 303585 (holotype), USNM 303584, 303587, 303589–90.

Allobates mcdiarmidi (n = 3): BOLÍVIA: Cochabamba: Chapare: San Onofre: USNM 257805 (holotype), USNM 257804, USNM 257806.

Allobates melanolaemus (n = 4): PERU: Loreto: MUSM 17741 (holotype), MUSM 16507 (paratype), AMNH FS 11920, 11922 (paratypes).

Allobates nidicola (n = 4): BRAZIL: Amazonas: Boca do Acre: UFAC-RB 4626; Castanho: INPA 8093 (holotype), MPEG 13820–21.

Allobates olfersioides (n = 12): BRAZIL: Alagoas: Mangabeiras: MZUSP 73707 (former WCAB 2801; holotype of *Colostethus alagoanus*); Bahia: Ibirapitanga: MNRJ 28952; Espírito Santo: Linhares: MNRJ 40420–21; Linhares: MZUSP 73752 (former WCAB 1952; holotype of *Colostethus capixaba*); Santa Teresa: MNRJ 32425; Rio de Janeiro: Angra dos Reis: MNRJ 8094 (topotype), USNM 96539–96540 (syntypes); Nictheroy: USNM 96412 (syntype); Teresópolis: MNRJ 23722–23.

Allobates paleovarzensis (n = 1): BRAZIL: Amazonas: Castanho: INPA-H 20904 (holotype).

Allobates subfolionidificans (n = 74): BRAZIL: Acre: Brasília: UFAC-RB 5869; Cruzeiro do Sul: UFAC-RB 1838, 184, 1952, UFAC-RB 3810; Mâncio Lima: UFAC-RB 2785; Manoel Urbano: UFAC-RB 4540, 4542; Marechal Thaumaturgo: UFAC-RB 828, 855, 857, 2020, 2022, 2155, 2164; UFAC-RB 2663; UFAC-RB 5618; UFAC-RB 2316–2317; UFAC-RB 2429, 2433, 2479; UFAC-RB 2613, 2615; Porto Acre: UFAC-RB 982, 1093; Porto Walter: MPEG 12318–12326; Rio Branco: UFAC-RB 4874; INPA-H 13760 (holotype), UFAC-RB 4616–4617, 4682–4683, 4700–4701; Tarauacá: UFAC-RB 5647; UFAC-RB 5668; Santa Rosa do Purus: UFAC-RB 5362. PERU: Loreto: Genaro Herrera: MUSM 15612.

Allobates sumtuosus (n = 12): BRAZIL: Amazonas: Manaus: INPA-H 31949–31951; Pará: Reserva Biológica Trombetas: USNM 303591 (holotype), USNM 303592–93, INPA-H 31954–55, 31959–60; Roraima: São João da Baliza: INPA-H 31956–57, (former AMNH 20210–11).

Allobates tapajos (n = 7): BRAZIL: Amazonas: INPA-H 36509–10, 36512, 36518, 36548, 36563, 36581.

Allobates tinae (n = 21): BRAZIL: Acre: Senador Guimard: UFAC-RB 4604, 4636–37 (paratypes), UFAC-RB 4638. Amazonas: Boca do Acre: MNRJ 90214 (holotype), MNRJ 90215–28 (paratypes), UFAC-RB 4625 (paratype), MNRJ 90213. *Allobates trilineatus* (n = 56): BRAZIL: Acre: Cruzeiro do Sul: UFAC-RB 2776; Marechal Thaumaturgo: UFAC-RB 2508; Mâncio Lima: MCP 13618, 13639; Senador Guimard: UFAC-RB 0160, 0162–64; Porto Walter: MPEG 12328–12360; Rio Branco: UFAC-RB 3550, 4605–4615, 5203; Sena Madureira: UFAC-RB 4703–04, 5557; Xapuri: UFAC-RB 0089. Amazonas: Envira: MCP 13628. PERU: Madre de Dios: Cocha Cashu: MUSM 15618. San Martín: Yurimaguas: MUSM 15611.

Allobates vanzolinus (n = 7): BRAZIL: Amazonas: Ipixuna: INPA 3381, 3413 (paratypes); Jurua: INPA 4896 (holotype), 4903, 4904, 4905, 4912 (paratypes).