

Genetic and Phenotypic Evidence Reveals a Complex Evolutionary History within the Amazonian *Allobates juami/insperatus* Clade (Anura, Aromobatidae), with the Description of a New Species

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GENETIC AND PHENOTYPIC EVIDENCE REVEALS A COMPLEX
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ALLOBATES JUAMI/INSPERATUS CLADE (ANURA,
AROMOBATIDAE), WITH THE DESCRIPTION OF A NEW SPECIES

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CONTENTS

Abstract.....	3
Introduction.....	3
Materials and Methods.....	5
Field Work.....	5
Morphology.....	5
Osteology.....	6
Bioacoustics.....	6
Molecular Data.....	6
Phylogenetic Analyses.....	8
Genetic Distances.....	8
Species Delimitation and Genetic Structure.....	9
Phenotypic Differentiation.....	9
Results.....	11
Phylogenetics and Molecular Species Delimitation.....	11
Morphometric and Acoustic Differentiation.....	17
Data Integration.....	20
Taxonomy.....	20
<i>Allobates insperatus</i> (Morales, 2002).....	20
<i>Allobates juami</i> Simões et al., 2018.....	38
<i>Allobates liniaureum</i> , new species.....	43
Discussion.....	60
Conclusion.....	66
Acknowledgments.....	66
References.....	67
Appendix 1. Examined Specimens.....	73
Appendix 2. Outgroup Genbank Accession Numbers.....	74
Appendix 3. Locality Data and Genbank Accession Numbers of Ingroup Employed in This Study.....	76
Appendix 4. Partition Scheme.....	80
Appendix 5. Morphometric Principal Components Analysis Loadings.....	81
Appendix 6. Acoustic Principal Components Analysis Loadings.....	82
Appendix 7. Uncorrected-Pairwise Genetic Distances.....	83

ABSTRACT

Amazonia is a great natural laboratory, allowing the study of complex evolutionary mechanisms that promote diversification associated with dynamic geomorphological, climatic, and ecological processes, which often generate fuzzy species boundaries. We investigated the evolutionary history of two sister species of nurse frogs distributed in northwestern Amazonia, *Allobates insperatus* and *A. juami*, which have been considered as different entities based on molecular evidence, despite the absence of substantial phenotypic differences. We obtained morphological, osteological, and acoustic data, and DNA sequences of one mitochondrial and six nuclear gene fragments across the species distribution. The mitochondrial tree and species delimitation methods suggest the existence of at least four main evolutionary lineages; however, the signal of the nuclear markers is discordant among some groups, showing the admixture of those lineages that may reflect introgression and/or incomplete lineage sorting. Considering all sources of evidence, we confirm *A. insperatus* and *A. juami* as valid species and redescribe the former. In addition, we recognize and describe a new species supported by all data and analyses. Our findings suggest that speciation of *Allobates*, and perhaps of other sympatric anurans, in western Amazonia may have been influenced by the Pebas megawetland, with subsequent dispersion through the Andean foothills before the formation of the main Amazonian rivers, followed by colonization of the emerging northwestern Amazonian lowlands and secondary contact of newly diverged sister lineages.

INTRODUCTION

Amazonia undoubtedly harbors an exceptional diversity of amphibians (Jenkins et al., 2013; Manion et al., 2014; Ochoa-Ochoa et al., 2020). During the past 25 years or so, several studies on different amphibian groups or regions have suggested that species richness is 21%–400% higher than currently recognized (Fouquet et al., 2007a, 2007b, 2014; Padial and De la Riva, 2009, Angulo and Icochea, 2010; Funk et al., 2012; Jungfer et al., 2013; Caminer and Ron, 2014; Gehara et al., 2014; Lourenço et al., 2015; Jaramillo et al., 2020; Réjaud et al., 2020). Several of the hypotheses that explain the outstanding Amazonian diversity are based on allopatric speciation involving physical or ecological barriers, such as rivers, orogenic formations, and ecological gradients (Antonelli et al., 2010, and references therein). However, recent studies show that the impact of these physical barriers to promote speciation varies across taxa (Naka et al., 2012; Oliveira et al., 2017; Pirani et al., 2019). Concomitantly, it becomes apparent that introgression, gene flow, and secondary contact between species may have been more common than previously thought (Pereyra et al., 2016, 2021; French et al., 2019). This suggests more complex and dynamic

scenarios for species formation, which often require the evaluation of factors such as demographic histories, ecological factors, and behavioral traits (Noonan and Gaucher, 2005, 2006; Santos et al., 2014; Dias-Terceiro et al., 2015). More complex speciation scenarios may also imply that species limits are not as clear as those found when species are allopatric, often resulting in unstable taxonomies (Padial and De la Riva, 2021; French et al., 2019; Fouquet et al., 2019).

Nurse frogs, *Allobates* Zimmermann and Zimmermann, 1988, are a monophyletic group restricted to the Neotropics (Grant et al., 2006, 2017; Santos et al., 2009; Pyron and Wiens, 2011; Réjaud et al., 2020). There are currently 63 species of *Allobates* recognized and the vast majority are restricted to Amazonia (Frost, 2023). When studying the historical biogeography of the group, Réjaud et al. (2020) reported a large proportion of undescribed species, with an estimate of 21% more species than currently recognized. Furthermore, the external morphology of many *Allobates* species is highly similar, making their morphological identification challenging, a situation that is exacerbated when comparing only museum specimens (Melo-Sampaio et al., 2018; Jaramillo et al., 2021; Gagliardi-Urrutia et al., 2021). In this

context, it is not a surprise that *Allobates* species-level taxonomy is in flux, with a fast increase of species descriptions and reevaluations in the past two decades (Jaramillo et al., 2021). Additionally, evidence suggests that evolutionary scenarios favoring geographic isolation could explain phenotypic and genetic divergence within *Allobates*. Mechanisms such as river barriers (Kaefer et al., 2012; Maia et al., 2017), isolation by distance (Fernandes et al., 2021), geomorphological barriers (Simões et al., 2008), and mimicry (Amezquita et al., 2009) have been suggested as complementary explanations. Also, the potentially homogenizing processes of hybridization and introgression have been already detected in Amazonian nurse frogs (Simões et al., 2012). These challenges make nurse frogs an exciting group in which to apply theoretical and methodological advances in evolutionary biology to evaluate species limits in Amazonia, the hottest hotspot of amphibian diversity.

In this study, we focus on *Allobates insperatus* (Morales, 2002) and *A. juami* Simões et al., 2018, two species that are distributed in northwestern Amazonia, between Brazil, Colombia, Ecuador, and Peru (Frost, 2023). We chose these species because, as explained below, they encapsulate many of the difficulties associated with species delimitation in the Neotropics. Morales (2002) proposed *A. insperatus* based on a cursory and brief description of morphological characters of preserved specimens from six localities between the rivers Aguarico, Coca, and Napo in Ecuador, without mentioning any geographic variation among populations. This led to considerable uncertainty regarding the identification of specimens as *A. insperatus* in subsequent studies, which was often explicitly acknowledged (Darst et al., 2005; Grant et al., 2006; Santos et al., 2009). Simões et al. (2018) described *A. juami* using genetic, morphological, and behavioral data from four adult males and three adult females from a single locality close to the Japurá and Juami rivers in northwestern Brazil. Although Simões et al. (2018) provided a phenotypic diagnosis of *A. juami* and *A. insperatus*, they also highlighted their high similarity, and that the

variation observed among available acoustic data assigned to *A. insperatus* may correspond to more than one species. Subsequent studies consistently inferred *A. insperatus* and *A. juami* as sister species when including one or few specimens per species and multiple mitochondrial and nuclear markers (Melo-Sampaio et al., 2020; Souza et al., 2020; Moraes and Lima, 2021). When Réjaud et al. (2020) analyzed a large dataset of *Allobates* 16S sequences, they found a clade of 21 specimens associated with these two species from different localities from Brazil, Colombia, Ecuador, and Peru. Their results show that some purported individuals of *A. insperatus* were more closely related to those of *A. juami* type series than to other *A. insperatus* individuals (a result confirmed by Jaramillo et al., 2021). Their genetic distances and branch lengths within this clade were small when compared to other pairs of well-delimited sister species of *Allobates*. These results could be explained by a scenario of a single species with a large distribution and associated intraspecific variation or, alternatively, by a conflict in character optimization resulting from biological processes such as rapid speciation, incomplete sorting of ancestral polymorphism or genetic introgression (Glor, 2010; Alexander et al., 2017; Beckman et al., 2018).

Unfortunately, genetic or phenotypic data from specimens unambiguously identified as *A. insperatus*, especially collected at the species type locality, have not been previously reported. The type locality of *A. insperatus* is Santa Cecilia, Sucumbíos province, Ecuador (0.066640° N, 76.991347° W, 319 m elev.) and, to date, the geographically closest analyzed data are DNA sequences from ten specimens collected in Estación Científica de la Universidad Católica, Sucumbíos, Ecuador (0.0886° S, 76.1419° W, 221 m), ~96 km southeast of the type locality (Grant et al., 2006; Réjaud et al., 2020). This means that one could not discard that at least part of the conflicting results found so far (Réjaud et al., 2020; Jaramillo et al., 2021) were due to misidentified specimens.

In this study, we evaluate the conflicting results within the *A. juami/insperatus* clade and propose

a new species delimitation framework. For this, we compare mitochondrial and nuclear DNA sequences as well as phenotypic data (external morphology, osteology, and bioacoustics). Based on our results, we redescribe *A. insperatus* using newly collected topotypic specimens and associated data, report on mito-nuclear discordance within the *A. juami/insperatus* clade, and describe a new species sister of these nominal species. In addition, we discuss the observed patterns of geographic distribution of genetic and phenotypic diversity within this group and its potential relationships with historical geological and hydrological dynamics of northwestern Amazonia.

MATERIALS AND METHODS

FIELD WORK

We conducted field surveys in Puerto Nuevo Yurimaguas, Yurimaguas, Alto Amazonas, Loreto, Peru (5.85724° S, 76.16201° W, 202 m) and in Santa Cecilia, Lago Agrio, Sucumbíos province, Ecuador (0.07329° S, 76.98927° W, 323 m) on February 11–12 and March 2–5, 2019, respectively. We performed active searches during the day (~6:00–11:00 and 16:00–19:00). We georeferenced localities in situ using a portable GPS with datum WGS 84. We manually collected all individuals and kept them in plastics bags with leaf litter and abundant humidity. In improvised laboratories, we photographed live specimens, which were then euthanized with an overdose of topical 5% lidocaine solution. From each specimen, we collected a piece of muscle from the left hind limb and preserved it in 96% ethanol for molecular analyses. We subsequently fixed all specimens in a 10% formalin solution for three days and then preserved them in 70% ethanol. All collected specimens were deposited at the Coleção de Anfíbios of the Museu de Ciências e Tecnologia of Pontifícia Universidade Católica do Rio Grande do Sul (MCP) in Brazil, Museo de Zoología of the Universidad San Francisco de Quito (ZSFQ) in Ecuador, and Colección Referencial de Biodiversidad del Instituto de Investigaciones de la Amazonía Peruana (CRBIAP) in Peru.

MORPHOLOGY

We identified individuals as adult males using secondary sexual characters (i.e., direct observation of vocalization behavior, swollen fingers, presence of vocal sacs or vocal slits) and as adult females those that lacked these characters and that had a larger body size (snout-to-vent length) than the smallest adult male. We followed Grant et al. (2006), Lima et al. (2007), and Simões et al. (2018, 2019) for the description of qualitative and quantitative external morphological characters. Fingers were named I–IV as in Grant et al. (2006). Using a digital caliper or a stereomicroscope with graduated lenses (precision 0.1 mm), we measured the following 40 morphometric variables (Gagliardi-Urrutia et al., 2021): snout-to-vent length (SVL); head length (HL), from tip of snout to commissure; head width (HW), at the level of commissure; snout length (SL), from tip of snout to center of nostril; eye-to-nostril distance (EN), from anterior corner of the eye to the center of nostril; internarial distance (IN), between the posterior edges of nares; eye diameter (ED), from anterior to posterior corner; eyelid width (EW), greatest transverse width of upper eyelid; interorbital distance (IO); maximum tympanum diameter (TYM); forearm length (FAL), from proximal edge of palmar tubercle to outer edge of flexed elbow; upper arm length (UAL), from anterior corner of arm insertion to the outer edge of flexed elbow; finger lengths, from proximal edge of palmar tubercle to tips of fingers I (HAND I), II (HAND II), III (HAND III), and IV (HAND IV); disc width of finger I (WFID), II (WFIID), III (WFIID), IV (WFIVD); distal phalanx width of finger I (WFIP), II (WFIIP), III (WFIIP), IV (WFIVP); palmar tubercle diameter (DPT); thetar tubercle diameter (DTT); thigh length (TL), from the posterior extremity of the urostyle region to the outer edge of flexed knee; shank length (SAL), from outer edge of flexed knee to heel; tarsus length (TAL), from the heel to the proximal edge of inner metatarsal tubercle; foot length (FL), from proximal edge of inner metatarsal tubercle to tip of toe IV; disc width of toe I

(WTID), II (WTIID), III (WTIIID), IV (WTIVD), V (WTVTD); and distal phalanx width of toe I (WTIP), II (WTIIP), III (WTIIIP), IV (WTIVP), V (WTVIP). We list all examined specimens in appendix 1. Species comparisons for which we could not access individuals are based on original descriptions and other relevant literature cited in the Diagnosis sections.

OSTEOLOGY

We produced osteological data using high resolution X-ray microcomputed tomography (μ CT) of an adult female paratype of *Allobates juami* (MCP 13288) and an adult male from the Huallaga-Marañón interfluvio (MCP 14295). Scanning was performed at Instituto do Petróleo e dos Recursos Naturais (IPR) – PUCRS, using a BRUKER skyscan 1173 with voltage and current set to 40 kV and 55 μ A, respectively. We processed raw X-ray data using NRecon software v. 1.7.1.6 to reconstruct cross-section slices to generate volumes, with a final voxel resolution of 5.99 μ m. We imported the resulting μ CT volume files into Avisoft 2019.1 to generate an osteological model. Computed tomography data (bmp stacks and mesh files) have been deposited in MorphoSource (<https://www.morphosource.org>) under the identification codes 000703432 and 000705152. For osteological terminology we followed Lynch (1971), Trueb (1973), Fabrezi and Alberch (1996), and Kaplan (2004).

BIOACOUSTICS

We recorded vocalizing males in the field using Tascam DR-05X and DR-07X digital recorders with Sennheiser Me-80 and K6 directional microphones, respectively. We set recorders at a sampling rate of 44.1 kHz and a 16-bit depth, and recordings were stored as .WAV files. We positioned microphones approximately 0.5 to 1.5 m from calling males and measured air temperature at time of recording with a digital thermometer at the ground level. We used 10 notes from the middle section of the call for analysis of

acoustic parameters. The sampled notes were distributed in intervals of two or three notes, never sampling consecutive notes. We did not sample the first three or the last two notes of any call to avoid “warm up” notes or variation in acoustic parameters caused by fatigue.

We measured the duration of calls, notes, and silent intervals between notes from oscillograms (Koehler et al., 2017; Gagliardi-Urrutia et al., 2021). We quantified the number of notes per call from spectrograms. We measured lower, upper, and peak (i.e., dominant) note frequencies from power spectrum graphs (Simões et al., 2010). We conducted all spectral analyses using a Blackman window type, with a frequency resolution of 82 Hz and DFT size of 2048 samples. We measured lower and upper frequencies of notes 20 dB below the peak frequency intensity to avoid overlap with background noise. We used the software Raven Pro interactive sound analysis software, version 1.6.1 (Center for Conservation Bioacoustics, Cornell Lab of Ornithology, Ithaca, NY, 2019: <http://www.birds.cornell.edu/raven>) to measure all acoustic variables. We report the values of acoustic parameters of call and note respectively as the arithmetic means, standard deviations, and ranges for each recorded species. We deposited all recordings in the acoustic repository of Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (MNCN-CSIC) in Madrid, Spain (FonoZoo: 14940–14949).

MOLECULAR DATA

We extracted genomic DNA from preserved tissue samples using a standard phenol-chloroform protocol with ethanol precipitation or a Wizard Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacturer's protocols. First, we amplified a fragment of the mitochondrial gene 16S rRNA of ~570 base pairs (bp) using the polymerase chain reaction (PCR). Each reaction sample contained a total volume of 25.0 μ L, comprising 2.0 μ L of genomic DNA, 12.5 μ L (1X) of Hot Star Taq Master Mix-Qiagen, 0.75 μ L (0.3 μ M) for each primer, 0.75 μ L (1.5 mM) of $MgCl_2$

TABLE 1

List of primers for nuclear markers designed in this study, their amplicon size, and annealing temperature
F = forward; R = reverse.

Marker	Primer Pair		Product Size (bp)	Annealing T (° C)
	F5'-3'	R5'-3'		
DOLK	TCTTCTTGCCGTGGCTVCAG	WAAGGAGGAKGCACAGAGTC	~437	50.0
LIG4	TYAAAGTTCTTGGTTTGCCC	TCGCTAAGRCACACACTGGG	~390	50.0
LRRC8D	ACCATCTTAGACAAAAAGGATGGG	CAGATAAGAAGACACCAAAGCG	~452	49.3
MED13	GCAACATGAACATTAAAGGCG	CTTGTTCTTCCACACGAGCC	~340	48.4
PPL	GAAGAGCARAAGAAGCGCC	CGGTCTCAAGAAATTTGCCTC	~443	50.0
SALL1	TTGACCCCATTTATTAAGACCG	GGGTCTTTCTCCTGTGTGT	~455	48.1

and 8.25 µL with MiliQ H₂O to complete the total volume. We used the universal primers 16Sar-F (5'-CGCCTGTTTATCAAAAACAT-3'), 16Sc-F (GTRGGCCTAAAAGCAGCCAC), and 16Sbr-R (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi, 1991), with the following PCR conditions: initial denaturation 95° C for 15 min, followed by 35 cycles of 94° C for 0.5 min, 50° C for 0.5 min and 64° C for 1 min, and final extension 72° C for 10 min. We sequenced PCR products in both directions using Sanger sequencing at Centro de Biotecnologia (CBiot) of Universidade Federal do Rio Grande do Sul (UFRGS) in Brazil.

For nuclear markers, we amplified six fragments (DOLK, LIG4, LRRC8D, MED13, PPL, SALL1) from Shen et al. (2013) and Feng et al. (2017) using newly designed primers (table 1) for MiSeq sequencing, using as templates the sequences of three species of dendrobatoids (*Allobates femoralis* [Boulenger, 1884], *Hyloxalus jacobuspetersi* [Rivero, 1991], and *Ranitomeya imitator* [Schulte, 1986]) from Feng et al. (2017). We used Multiplex PCR (Thermo Scientific Phusion U Green Multiplex PCR Master Mix) protocols, where we pooled all primers together at differential concentration varying from 2.14–3.50 µM. We used the following thermocycler conditions: initial denaturation 98° C for 5 min; followed by a first round of 25 cycles of 98° C for 10 s, 53° C (reducing 0.2° C per round) for 30 s, and 72° C for 45 s; a second round of 25 cycles of 98° C

for 10 s, 53° C for 30 s, and 72° C for 45 s; and a final extension of 72° C for 5 min. The amplicons were purified with SPRI beads and were ligated to individual indexes in a second PCR using Kapa Hot Star Ready Mix: 98° C for 3 min; followed by 18 cycles of 98° C for 20 s, 60° C for 15 s, and 72° C for 15 s; final extension 72° C for 5 min. We quantified the ligated products on an agarose gel with a reference standard in the software Image Lab (Bio-Rad Laboratories), pooled the libraries at equal concentrations, and purified and sequenced the products on an Illumina MiSeq.2500 sequencer platforms at the Genetic Resources Core Facility (GRCF) at Johns Hopkins University (Baltimore, MD). For some samples, we generated the amplicons for LRRC8D, PPL, and SALL1 through individual PCR reactions, using these conditions: initial denaturation 94° C for 5 min; followed by a first set of 25 cycles of 94° C for 30 s, 60° C (-0.2° C per round) for LRRC8D and SALL1 or 53° C (-0.2° C) for PPL 30 s, and 72° C for 40 s; second set of 25 cycles of 94° C for 30 s, 55° C for 30 s for LRRC8D and SALL1 or 52° C for 30 s for PPL, and 72° C for 40 s; final extension 72° C for 3 min. We sequenced these products in both directions using Sanger sequencing.

For Sanger sequencing, we assembled and edited contigs using the original chromatograms in Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, MI). We trimmed the Illumina MiSeq raw reads in Trimmomatic v. 0.36 (Bolger

et al., 2014) with sliding window 4:10, retaining only reads of >100 bp, and leading and trailing to 5 bp. We removed the Illumina universal adaptors using Cutadapt v. 2.8 (Martin, 2011). We mapped these edited reads against *Ranitomeya imitator* reference (accession codes DOLK: KX201459, LIG4: KX205195, LRRC8D: KX205782, MED13: KX206477, PPL: KX208501, SALL1: KX209641) with BWA-MEM algorithm v. 0.7.17 (Li, 2013). We converted the outputs into BAM files, and then sorted, merged, and removed PCR duplicates with SAMTOOLS v. 1.9 (Li et al., 2009). We obtained the final sequences with SAMTOOLS and iVar v. 1.2 (Grubaugh et al., 2019), considering a value of 10 in base quality minimum, and minimum frequency threshold to count base of 0.6.

PHYLOGENETIC ANALYSES

To delimit the *Allobates juami/insperatus* clade, we evaluated the phylogenetic position within *Allobates* of our new and previously available samples using DNA sequences of 16S, the best represented DNA marker available in public databases. For this, we downloaded from GenBank homologous sequences of 16S for 43 *Allobates* nominal species and 12 potentially new species (here referred as sp., aff., or cf.) according to Grant et al. (2017), Melo-Sampaio et al. (2018, 2020), Réjaud et al. (2020), and Lima et al. (2020). We combined this dataset with our newly generated 16S sequences. As outgroups, we used sequences of the closely related aromobatids *Anomaloglossus beebei* (Noble, 1923) and *Rheobates palmatus* (Werner, 1899) (Grant et al., 2017). We list all samples used in this study and their respective GenBank accession codes in appendixes 2 and 3.

Based on the results of the previous analysis, we selected all samples more closely related to topotypic *A. insperatus* and *A. juami* specimens than to any other nominal species. We then built four types of datasets restricted to the *A. juami/insperatus* clade: (1) only mitochondrial 16S data; (2) only nuclear data; (3) combined nuclear and mitochondrial data but excluding terminals

with just 16S; (4) total evidence. We used *A. femoralis* as the single outgroup for these datasets because it is the only species with available homologous sequences for the six analyzed nuclear markers in public databases.

Each fragment was individually aligned using the E-INS-i strategy of MAFFT (Katoh et al., 2005, 2019). The best partition scheme and model of nucleotide evolution for each dataset was selected using PartitionFinder 2 (Lanfear et al., 2017), considering values of the corrected Akaike Information Criteria (AICc), “linked” branch lengths estimation for partitions, and “all” models of nucleotide evolution. We performed maximum-likelihood tree searches using Garli (Zwickl, 2006) through the cluster Amazonia, from Laboratório de Alto Desempenho (LAD) – PUCRS high-performance computing. We ran 500 independent searches using a random tree (“streefname = stepwise”), 100,000 generations without topology improvement required for termination (gentreshfortopoterm), tree rejection threshold at 50 (treerejectionthreshold), and the maximum number of branches away from original location that a branch may be reattached during a limited SPR move at 6 (limsprange). The best tree was selected according to the highest value of log likelihood score.

We calculated 1000 bootstrap (BS) pseudoreplicates using the same tree-search strategy outlined above. We summarized the frequency of clades found through bootstrapping on the most optimal tree using SumTrees 4.3.0 of DendroPy 4.3.0 (Sukumaran and Holder, 2010). The best partition schemes, their related models of nucleotide evolution, and alignment information for each dataset are in appendix 4, while the different DNA datasets are available from the open research repository Zenodo (<https://zenodo.org/records/14503577>).

GENETIC DISTANCES

We estimated the genetic variation by calculating uncorrected-pairwise genetic distances (i.e., p-distances) among closely individuals of the *A. juami/insperatus* clade in MEGA 7.0.14 (Kumar et al., 2018), using the option “Site Cov-

erage Cutoff (%) = 5%” to remove highly ambiguous sites from pairwise comparisons.

SPECIES DELIMITATION AND GENETIC STRUCTURE

We used three approaches based on molecular data to estimate species diversity in the *A. juami/insperatus* clade. The automatic barcode-gap discovery (ABGD) searches for the gap that divides the distribution between intra- and interspecific genetic distances, and recursively assigns the individuals to candidate groups. We used the ABGD command line (Puillandre et al., 2012) entering as input the 16S genetic distance matrix resulting from MEGA, considering the variation of intraspecific distances (P values) between 0.001 to 0.03, gap width 0.01, and 50 bins. We considered only intermediate P values because extremes can lead to trivial clustering, where each terminal is considered as an individual cluster (low P values) or all terminals are clumped into one (high P values) (Puillandre et al., 2012; Pardo et al., 2014).

We also used Bayesian phylogenetics and phylogeography (BPP v4.0; Flouri et al., 2018) for nuclear markers only. This approach performs multilocus coalescent species delimitation analyses to evaluate different models of species assignments in a Bayesian framework. As BPP requires a user-specified guide tree, we used the nuclear-only tree. However, the prior distribution for the ancestral population size and root age can influence the posterior probabilities of models (Yang and Rannala, 2010). Hence, we used the strategy of Leaché and Fujita (2010) and tested three different prior configurations that rely on the adjustment of effective population size (N_e ; inverse-gamma distribution $\theta = a, b$: where “a” and “b” are shape and rate parameters, respectively) and divergence time (inverse-gamma distribution $\tau = a, b$: where “a” and “b” are shape and rate parameters, respectively) priors: (1) we assumed large ancestral N_e ($\theta = 3, 0.02$) with deep divergence time ($\tau = 3, 0.3$); (2) medium ancestral

N_e ($\theta = 3, 0.002$) with medium divergence time ($\tau = 3, 0.03$); (3) small ancestral N_e ($\theta = 3, 0.0002$) with shallow divergence time ($\tau = 3, 0.003$). We ran each Markov chain Monte Carlo (MCMC) for 5×10^5 generations, sampling every 100, and with a 10% burn-in. We used the option “algorithm1” with the fine-tuning parameter (a m).

We used STRUCTURE to group samples into K clusters, trying to minimize departures from Hardy-Weinberg equilibrium and linkage disequilibrium (Pritchard et al., 2000). We ran STRUCTURE with nuclear datasets. We transformed the alignments into Variant Call files (VCF) using “SNP-sites” (Page et al., 2016). Then, this file was filtered and transformed into a STRUCTURE file with the program *population* of Stacks software applying default parameters (Catchen, 2013). In STRUCTURE v. 2.3.4 (Pritchard et al., 2000), we ran 10 replicates for $K = 1-10$ using admixture model and retaining 2×10^6 generations of MCMC and discarding 1×10^3 generations as burn-in. We chose the best K to explain the data following Evanno et al. (2005) and the best log probability of the K parameter ($\text{Prob}[K=k]$) and summarized the results with CLUMPAK online version (Kopelman et al., 2015).

PHENOTYPIC DIFFERENTIATION

To evaluate whether the variation on morphometric and acoustic data is concordant with the genetic results, we conducted principal component analyses (PCA) retaining the variation of the first two PCs independently for the morphometric and acoustic datasets. For the morphometric dataset, we conducted two PCAs: one of the raw measurements and one removing the effect of body size by using the residuals of a linear regression of each morphological variable against SVL. For the acoustic dataset, we also performed two PCAs: one considering variables from the complete advertisement call and the other using notes as the comparative unit. We proceeded this way because some calls were arranged in trills of very short notes, while other calls were composed of single notes.

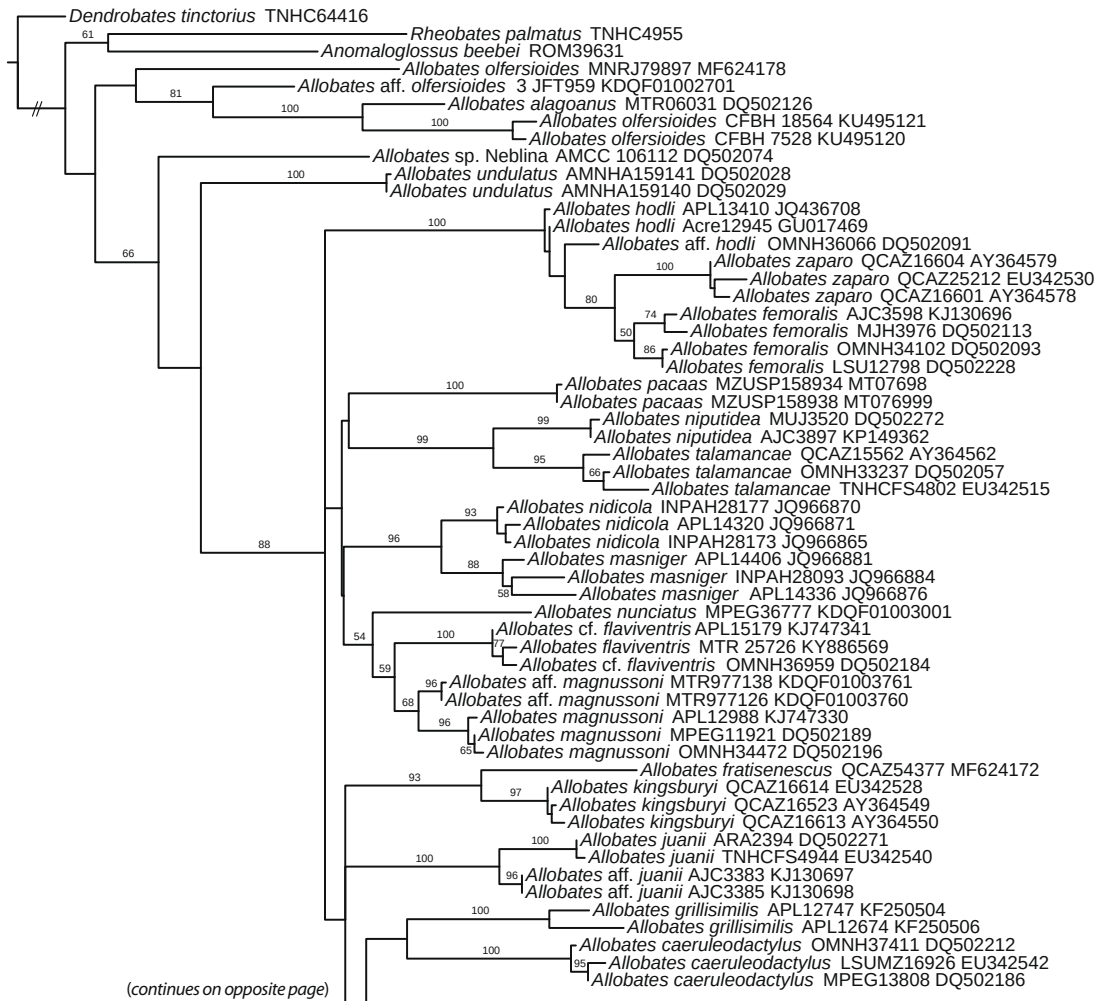
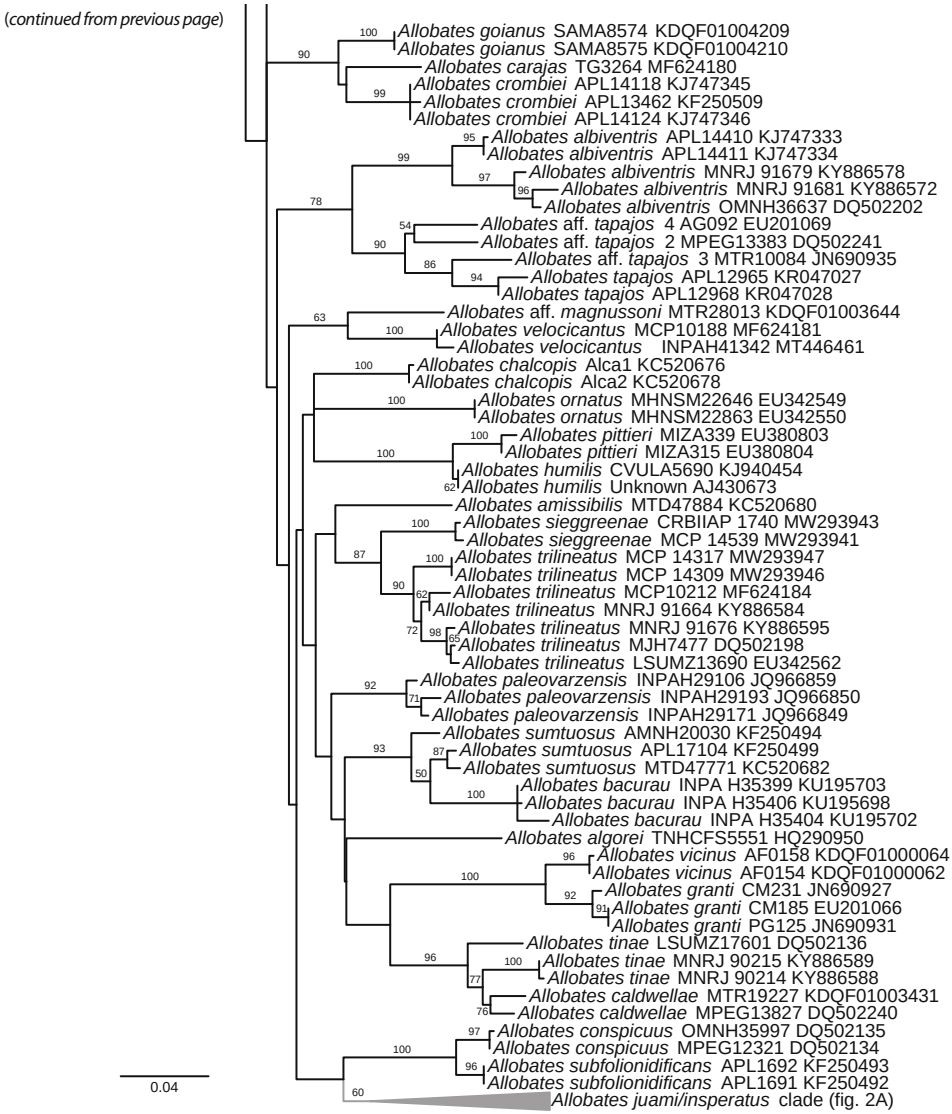


FIGURE 1. (above and following page) Phylogenetic relationships of *Allobates* inferred by maximum likelihood (log likelihood score = -9071.1) from a fragment of 16S (601 bp). Numbers on branches are bootstrap frequencies (percentage) of 1000 pseudoreplicates >50%. For ease of representation, the *A. juami/insperatus* clade is collapsed into a gray triangle.

To test for statistical significance between overlapping variables, we performed a nonparametric pairwise Wilcoxon test (data violated the normality and homogeneity assumptions of parametric tests) with the Benjamini and Hochberg (1995) multiple-comparisons correction for p-value in the function *pairwise.wilcox.test()* of stats package in R. However, for all statistical analyses we considered males and females together, because although preliminary results for raw datasets show differences between sexes (nonparametric

MANOVA: $F = 10.27$, $df = 1$, $p = 0.002$) and species (nonparametric MANOVA: $F = 24.02$, $df = 1$, $p = 0.001$), it does not for their interaction (nonparametric MANOVA: $F = 1.50$, $df = 1$, $p = 0.217$). A similar pattern was found for the body size corrected dataset, indicating differences among species (nonparametric MANOVA: $F = 6.67$, $df = 1$, $p = 0.001$), but not for sexes (nonparametric MANOVA: $F = 1.04$, $df = 1$, $p = 0.334$) or their interaction (nonparametric MANOVA: $F = 1.08$, $df = 1$, $p = 0.389$).



RESULTS

PHYLOGENETICS AND MOLECULAR SPECIES
DELIMITATION

The phylogenetic relationships among *Allobates* inferred from our 16S dataset (fig. 1) was generally consistent with other hypotheses based on multilocus datasets with similar taxon sampling (Réjaud et al., 2020; Moraes and Lima, 2021). Differences were mostly restricted to deeper relationships, probably caused by our smaller character sampling. The *Allo-*

bates juami/insperatus clade (bootstrap support, BS = 60) was recovered as sister of a clade formed by *A. conspicuus* (Morales, 2002) and *A. subfolionidificans* (Lima et al., 2007) (BS = 100). Our target clade contained 44 specimens (fig. 2A) from different localities of Brazil, Colombia, Ecuador, and Peru (fig. 3), of which 14 were sequenced for the first time, including topotypical *A. insperatus*. Within the *A. juami/insperatus* clade, we identified four mitochondrial clades (fig. 2A). The first was represented by specimens sampled in terra-firme forests of the

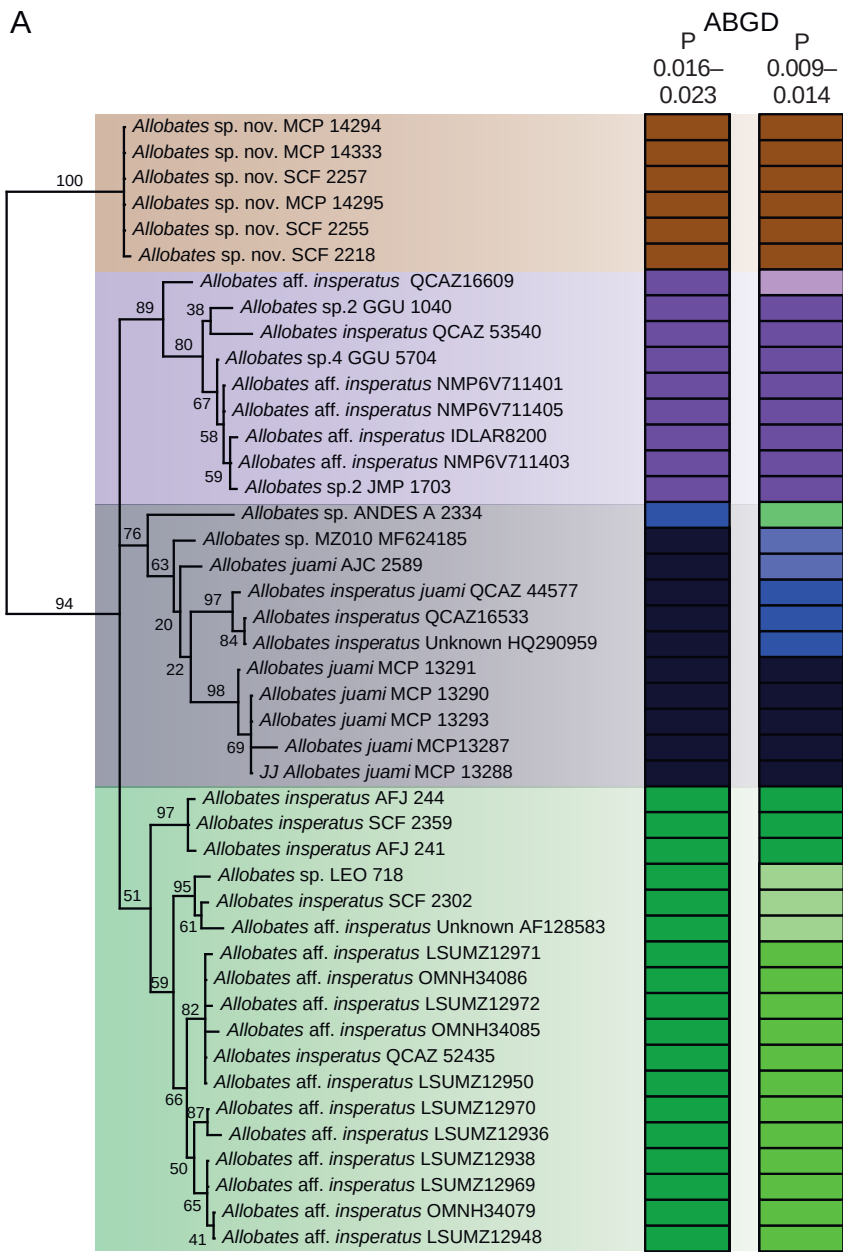
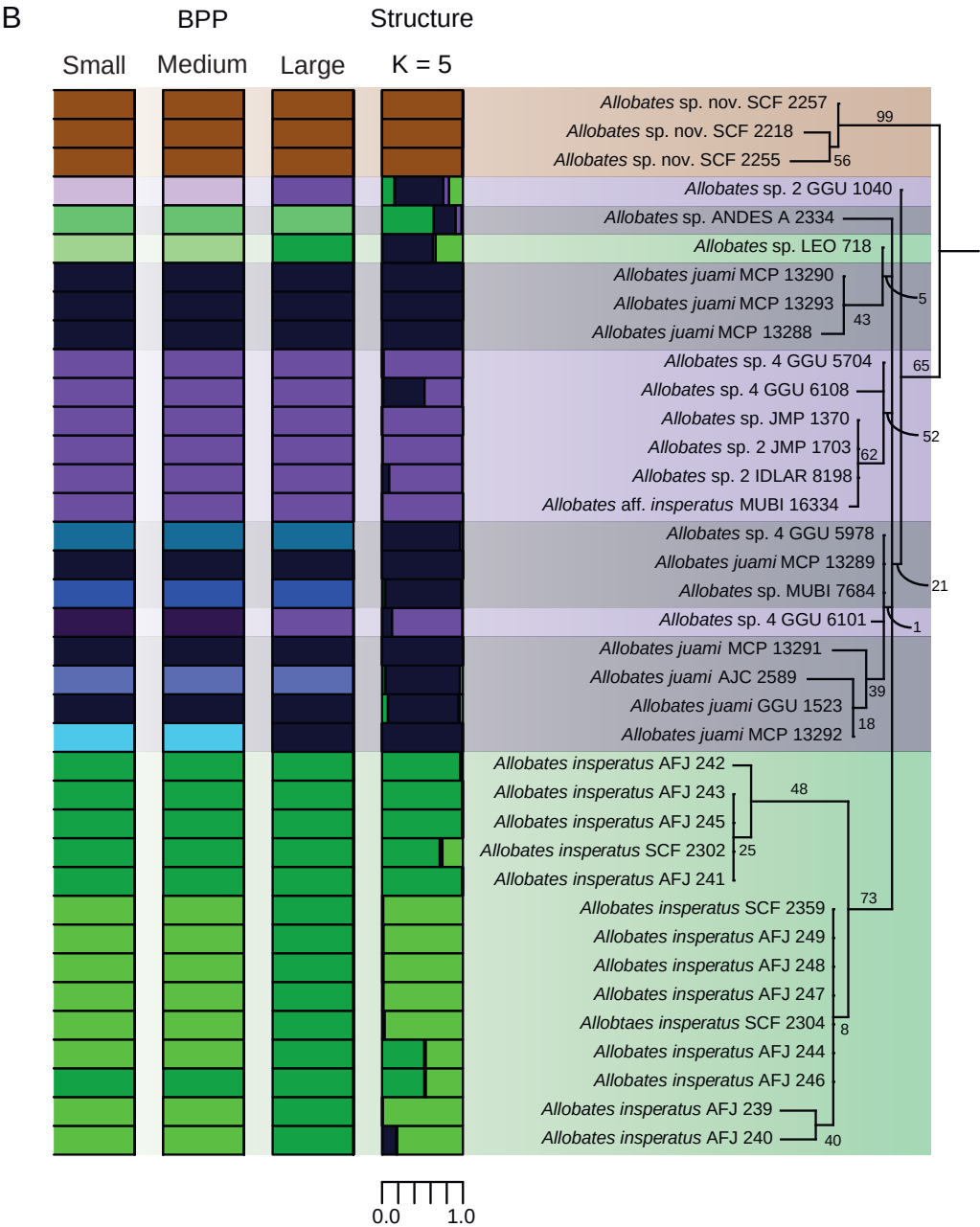
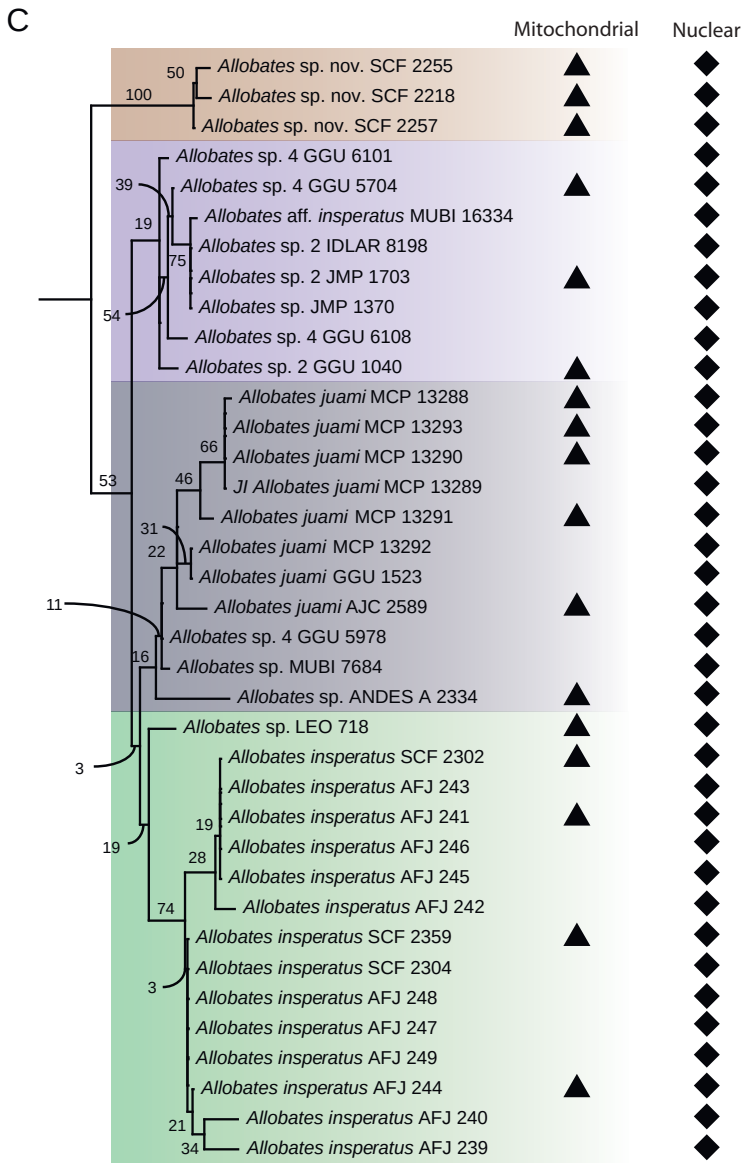


FIGURE 2. (above and following three pages) Maximum-likelihood phylogenetic relationships of the *Allobates juami/insperatus* clade inferred from four datasets. Background colors highlight the four mitochondrial clades discussed in the text: *A. sp.* Huallaga-Marañón (brown), *A. aff. juami* (purple), *A. juami* (blue), *A. insperatus* (green). Numbers on branches are bootstrap values. A. 16S dataset [log likelihood score: -9071.1], with detailed relationship of figure 1 and the ABGD delimitation with two P range values (continues next page).



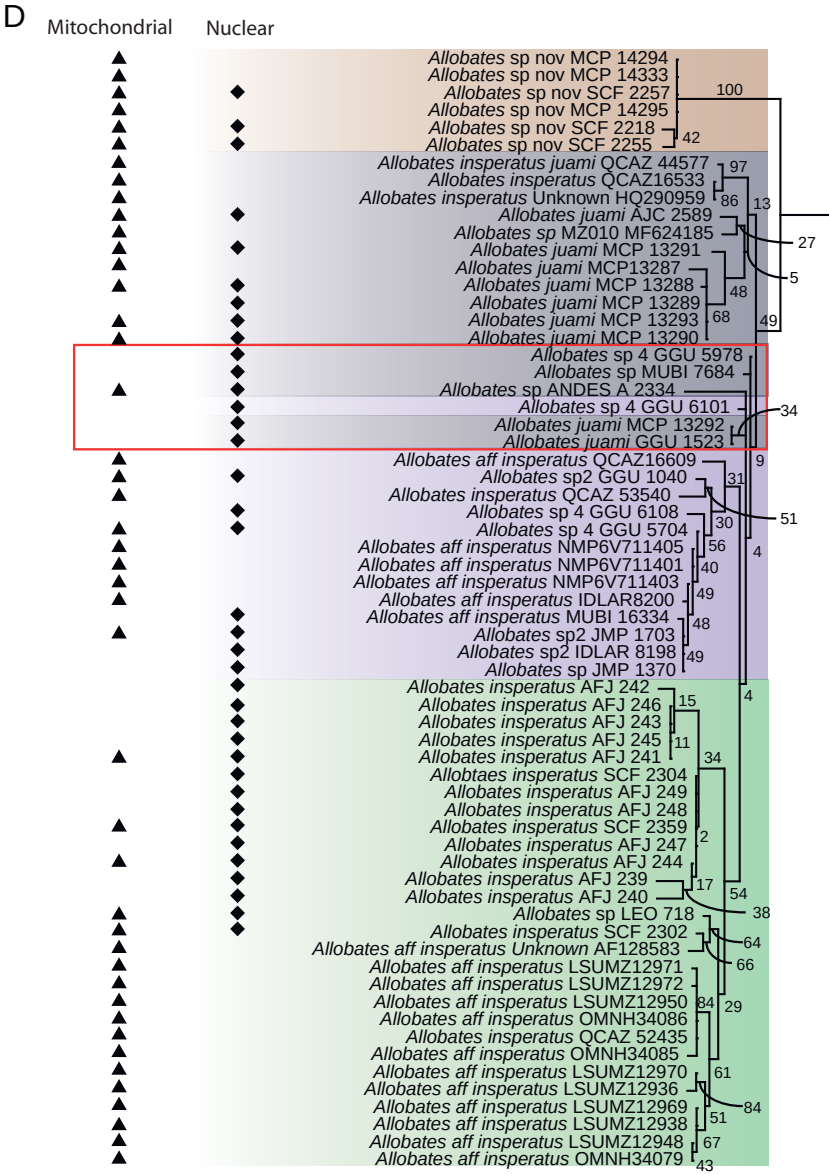
B. nuclear markers dataset [log likelihood score: -3579.4], with the most likely Bayesian phylogenetics and phylo-geography (BPP) and STRUCTURE results (*continues next page*).



C. combined 16S + nuclear and nuclear only [log likelihood score: -5480.9] (continues next page).

interfluvium between the Huallaga and Marañón rivers, a group sister of all other samples (BS = 100). The other three clades form a polytomy. Among them, one clade (BS = 89) contained samples from Ecuador and northern Peru (hereafter labelled *A. aff. juami*). The second (BS = 76) contained the type specimens of *A. juami* from northwestern Brazil and additional samples from Colombia, Ecuador,

and northern Peru. The third clade (BS = 51) contained topotypic *A. insperatus* and samples from neighboring localities in Ecuador and Colombia. The two P value ranges (intraspecific distances) inferred with ABGD were 0.009–0.014 and 0.016–0.023, and resulted in 10 and 5 clusters, respectively (figs. 2A, 4A). The five groups delimitation was fully concordant with the four mitochondrial clades



D. total evidence [log likelihood score: -5863.5], with red rectangle highlighting the samples breaking the monophyly of *A. juami* and *A. aff. juami*.

recovered in the phylogenetic analysis, except that it placed sample ANDES-A 2334 from Finca Bruselas, Caquetá, Colombia (1.1 N, 75.56 W, 225 m), in its own cluster, probably due to its long branch (fig. 2A). The 10-group delimitation resulted from splitting clades *A. aff. juami* into two, *A. juami* into four, and *A. insperatus* into three groups.

The nuclear dataset (fig. 2B) recovered a relatively similar topology, with the Huallaga-Marañón interfluvial clade (BS = 100) as sister of all other samples. While the *A. insperatus* clade (BS = 73) included the same samples evaluated in the mitochondrial tree except one (LEO 718), the other two mitochondrial clades

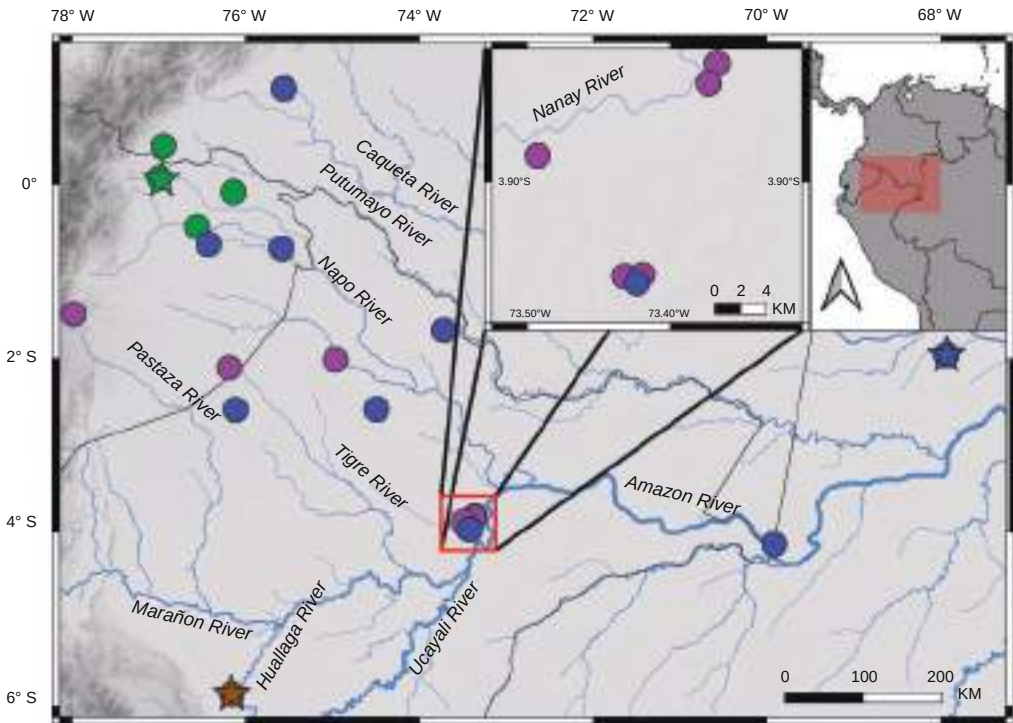


FIGURE 3. Map of northwestern Amazonia with distribution of the *Allobates juami/insperatus* clade (inset map shows the position within South America) with symbols indicating the studied localities of *Allobates insperatus* (green), *A. juami* (blue), *A. aff. juami* (light purple), *A. sp.* Huallaga-Marañón (brown). The area delimited by the red square is augmented to better illustrate the geographic position of localities nearby the Nanay River between Paranapura and Ucayali rivers. Stars represent type localities and black contours country borders. Main rivers are highlighted by blue contours and their thickness is approximately proportional to their flow size.

(*A. juami* and *A. aff. juami*) were part of a complex polytomy in which sample LEO 718 is also embedded. This hierarchical structure is reflected by the BPP and STRUCTURE results regarding the Huallaga-Marañón interfluvial samples and less so by the *A. insperatus* clade samples. The BPP results (fig. 4B) delimited *A. insperatus* as a single unit (including LEO 718) when a “large” N_e was considered, but it split it into three groups (largely congruent with the tree structure of fig. 2B) with “smaller” N_e (fig. 2B). The STRUCTURE result suggests partially shared genetic polymorphism among *A. insperatus* and *A. juami* samples (more so when $K = 7$; fig. 4C, D). Within the *A. juami* and *A. aff. juami* polytomy, a sub-

group of *A. aff. juami* samples from Peru was consistently recovered as a single cluster by both STRUCTURE and BPP (figs. 2B, 3).

Adding the mitochondrial marker to the nuclear dataset made the phylogenetic inference converge with the tree recovered by the 16S-only dataset (fig. 2A, C). However, except for the Huallaga-Marañón clade, bootstrap values dropped for the *A. insperatus*, *A. juami*, and *A. aff. juami* clades, indicating an increase of conflict in the phylogenetic signal. Finally, the phylogenetic analysis of the total evidence dataset (fig. 2D) recovered the *A. insperatus* and Huallaga-Marañón clades, but the *A. juami* and *A. aff. juami* mitochondrial clades were only partially recovered, because six samples formed a polytomy.

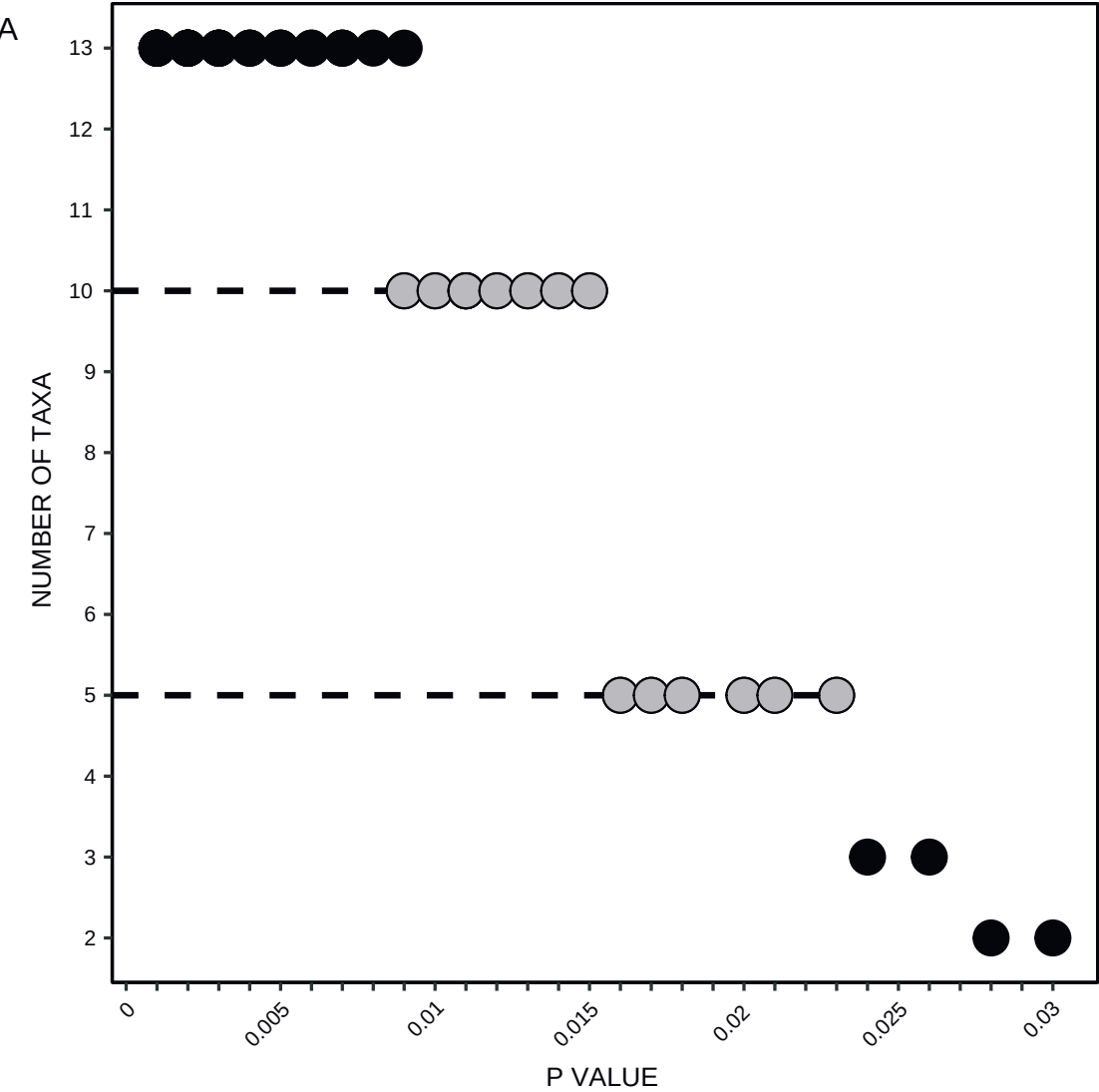
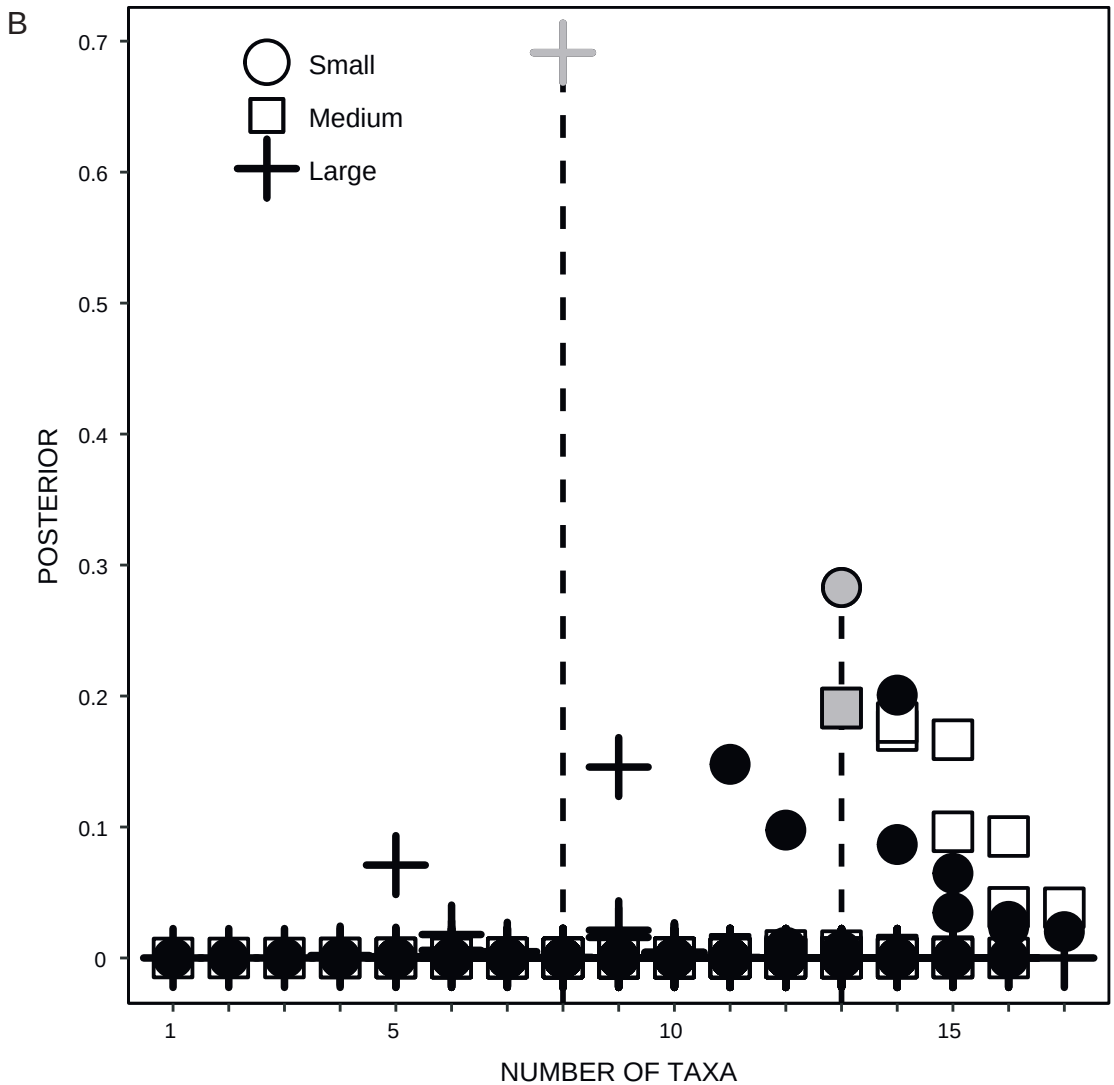


FIGURE 4. (above and following two pages) Cross-validation plots to select the best number of groups (gray symbols) using the: A. middle values of intraspecific distance of ABGD (continues next page);

MORPHOMETRIC AND ACOUSTIC
DIFFERENTIATION

Morphometric variation showed a clear separation between *Allobates insperatus*, *A. juami*, and the Huallaga-Marañón specimens in the morphospace when the raw data was considered (fig. 5A). However, differences between *A. juami* and the Huallaga-Marañón specimens disappeared when we removed the effect of variation in body size

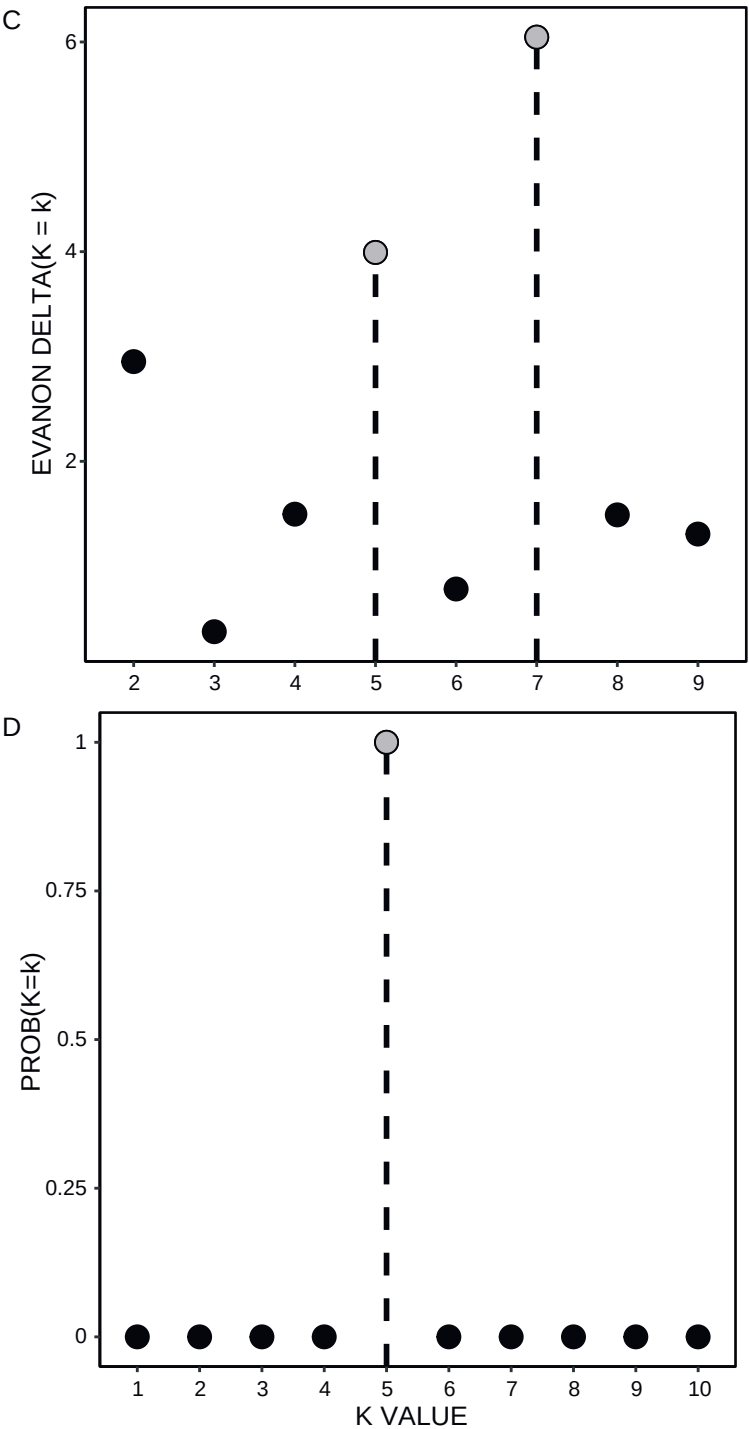
from these variables (fig. 5C). The first two components explained 86.6% and 2.4% of the variance of the raw morphometric data, and the highest loadings for PC1 were SVL, SaL, TL, and FL. For PC2 the highest loadings were attributed to SVL, HAND III, FL, and HW (appendix 5). When the effect of body size was removed, the first two components explained 19.7% and 12.7% of the morphometric variance, and the variables with the



B. posterior probabilities of species delimitation models in BPP under three prior conditions (*continues next page*);

highest loadings were the residuals of FL, TL, HAND I, and HAND III on PC1 and the residuals of FL, TAL, WTIVD, and HW on PC2 (appendix 5). For variables with highest loadings in PCs, we obtained statistically significant differences for raw measurements SVL_{raw} and HW_{raw} between all pairwise comparisons (fig. 5B). All the other variables were significant at least for some of the pairwise comparisons, except for HAND I_{residuals} (fig. 5B, D).

Regarding bioacoustics, *Allobates* sp. individuals from Huallaga-Marañón were not found to emit trills. The first two PCs produced by PCA on acoustic variables explained 97.2% and 1.7%, and 95.0% and 3.8% of the variance for trill and note analyses, respectively (fig. 6A, C). The acoustic variables with the highest loadings on PC1_{trills} and on PC1_{notes} were high, low, and peak frequencies (appendix 6). For PC2_{trills} and PC2_{notes}, the acoustic variables with the highest loadings were, in both cases, low frequency



C. Evanno et al. (2005) method; and D. probability of K parameter given the data in STRUCTURE. (Black circles represent groups not selected by the corresponding analysis.)

and band frequency. At the trill level, only high and low frequencies had statistically significant differences (fig. 6B). However, when comparing notes, all variables (even those with highest loadings in PCs) were significantly different between *Allobates insperatus*, *A. juami*, and Huallaga-Marañón specimens (fig. 6D). The only exception was the note duration between *A. insperatus* and *A. juami*.

DATA INTEGRATION

The results of the analyses of genetic and phenotypic data were fully congruent on singling out the population from the Huallaga-Marañón interfluvium as part of a different and new species of *Allobates*. Although mitochondrial, nuclear, and combined phylogenetic analyses agreed on retrieving a largely congruent *A. insperatus*, all genetic analyses identified at least a discordant sample (LEO 718) for which nuclear genetic variation was partially shared with *A. juami*. This latter species showed conspicuous genetic differences between the analyzed mitochondrial and nuclear DNA sequences. The mitochondrial marker included hierarchical variation from which we inferred two relatively well-supported clades (BS >75): *A. juami* (including type specimens and additional specimens) and *A. aff. juami*. However, the analysis of nuclear DNA sequences revealed nonmonophyly and admixture among specimens of the two mitochondrial clades (fig. 2B, D). Due to this conflict and to the absence of phenotypic data from specimens of *A. aff. juami*, we preferred to be conservative and refer tentatively to all specimens as *A. juami* until a reassessment with more data is available.

Considering these results and the fact that types and topotypes of *A. insperatus* and *A. juami* have nonoverlapping morphometric variation and several statistically significant morphometric and acoustic differences (figs. 5, 6), we recognized them as different species. Below, we describe the population of the Huallaga-Marañón as a new

species and, to provide meaningful comparisons with the closely related but poorly described *A. insperatus*, we redescribe the latter species and carefully diagnose it from *A. juami*.

TAXONOMY

Allobates insperatus (MORALES, 2002)

Unexpected nurse frog

Rana nodriza inesperrada

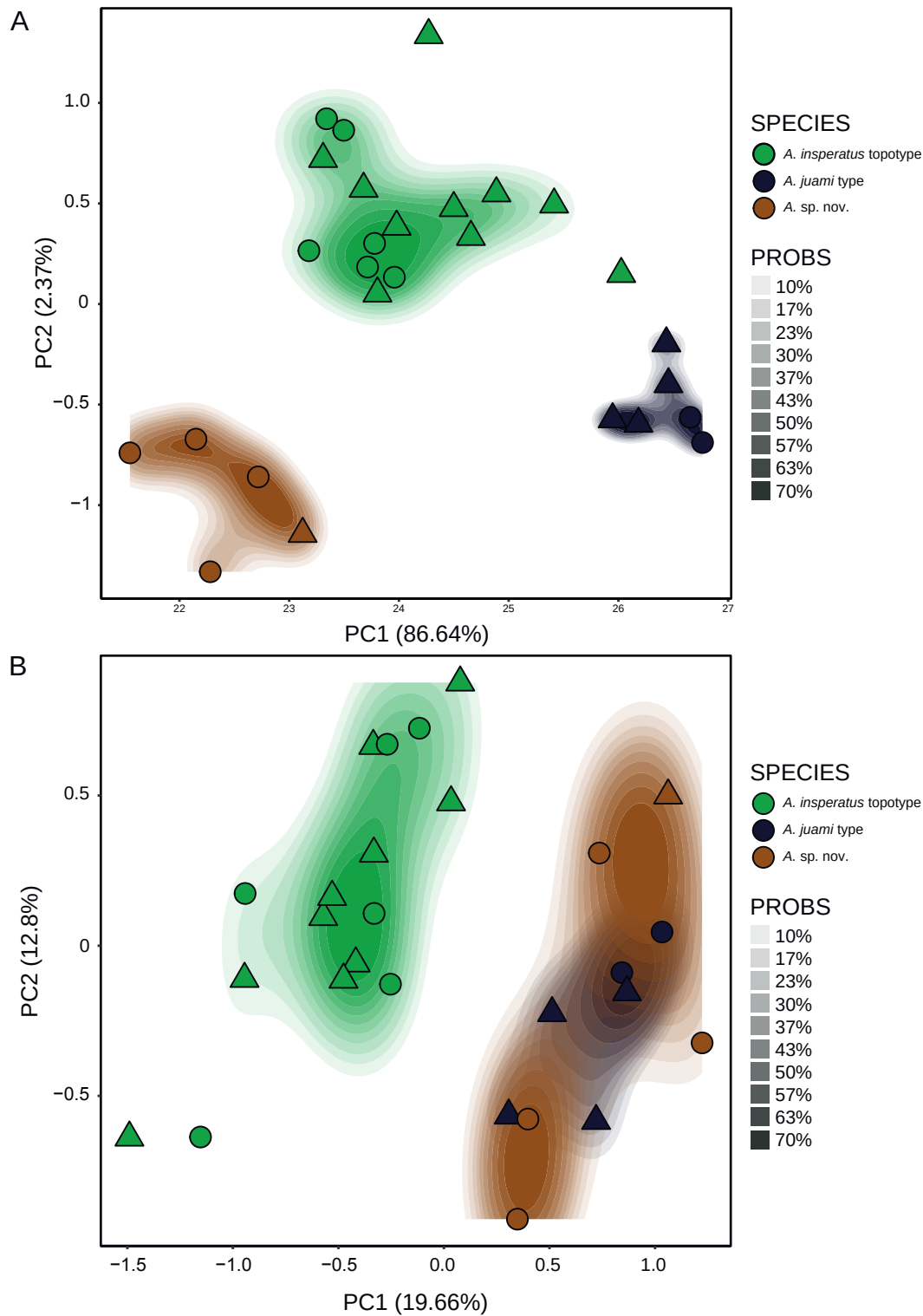
Figures 7, 8

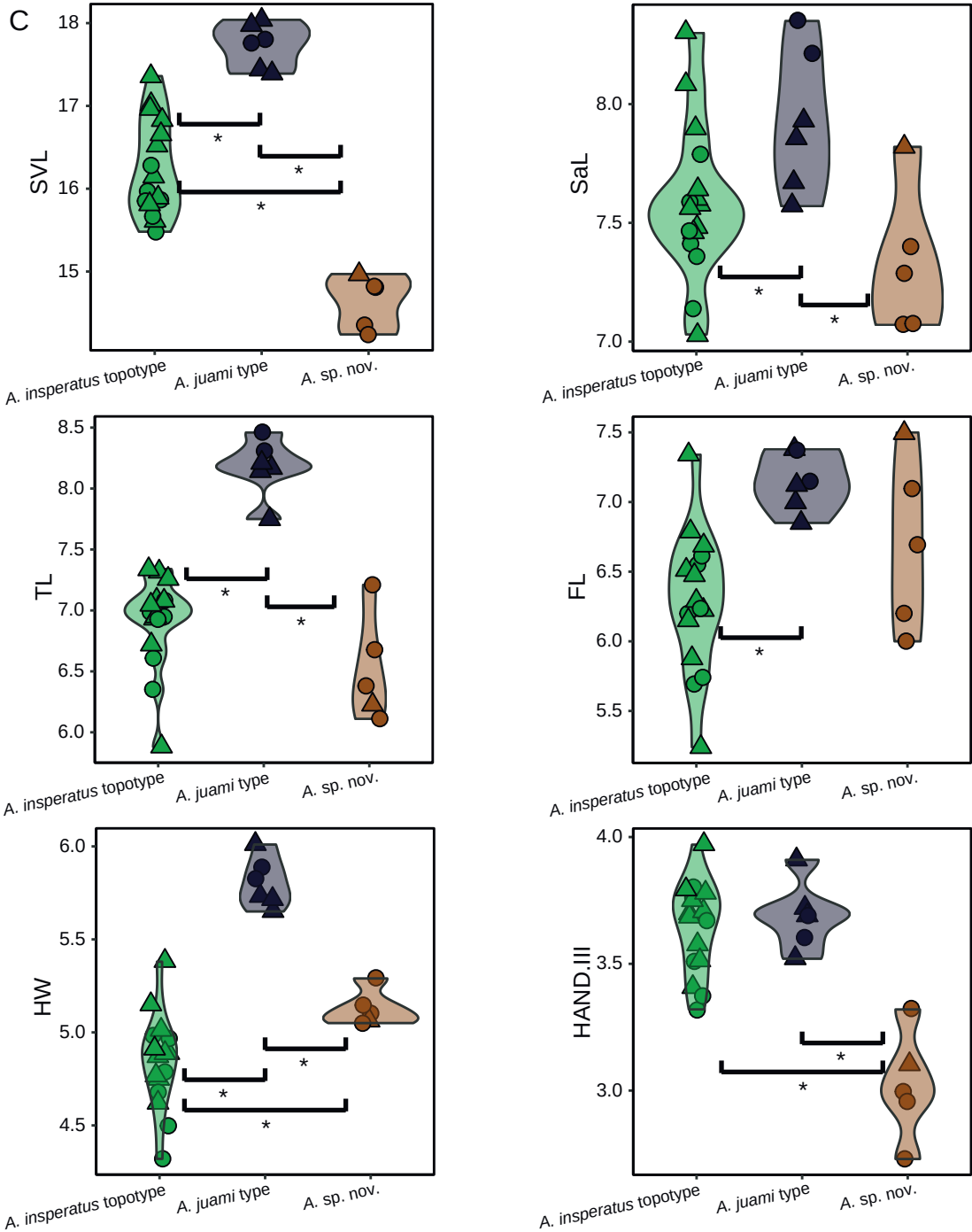
HOLOTYPE: KU 146206, an adult male from Santa Cecilia, Sucumbíos, Ecuador (0.066451° N, 76.992223° W, 319 m).

NEW MATERIAL: Sixteen specimens. Eleven adult females: ZSFQ 6210, 6261, 6268–69, 6272–73, 6274–75, 6279–6280, 6282 (field codes: AFJ 239, AFJ 241, AFJ 242–43, AFJ 246–47, AFJ 248–49, SCF 2301–02, SCF 2304). Five males: ZSFQ 6260, 6270–71, 6281, 6286 (field codes: AFJ 240, AFJ 244–45, SCF 2303, SCF 2359). Five males: ZSFQ 6260, 6270–71, 6281, 6286 (field codes: AFJ 240, AFJ 244–45, SCF 2303, SCF 2359). All specimens collected by Andres Felipe Jaramillo and Malki Inti Bustos on March 1–4, 2019 in Santa Cecilia (type locality), Cantón Lago Agrio, Sucumbíos province, Ecuador (0.07329° N, 76.98927° W, 325 m).

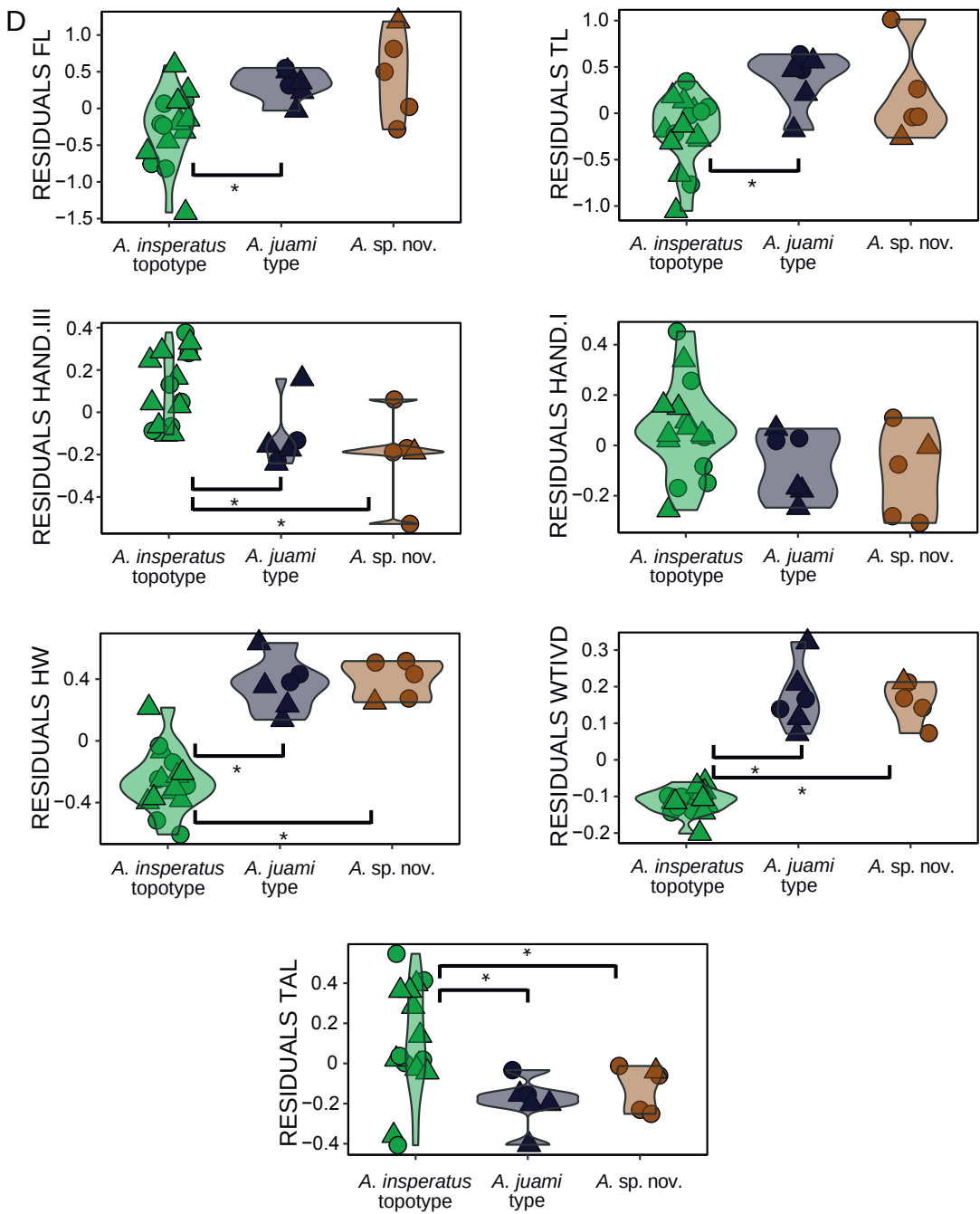
AMENDED CHARACTERIZATION: (1) Small adult body size (SVL: 15.6–17.4 mm in 10 females, 15.5–16.3 mm in 6 males); (2) shagreen dorsal skin of body, forelimbs, thigh, shank, and groin without scattered granules or tubercles; ventral skin smooth; (3) snout subacuminate in dorsal, lateral, and ventral views; (4) nostrils visible in dorsal and ventral views; (5) tympanum small (0.4–0.5× the eye diameter); (6) short teeth present on the maxillary arch; (7) teeth on mandible arch absent; (8) dentigerous process of vomer absent; (9) lingual process absent; (10) vocal sac in males single; (11) paired dorsal digital scutes

FIGURE 5. (next three pages) Comparisons of morphometric variation among specimens of *A. insperatus*, *A. juami*, and *A. sp.* Huallaga-Marañón based on raw and size-removed measurements. Scatter plots (A, B, next page) of the first two components of a PCA, with variance percentage explained by each axis in parenthesis (continues page 22).





(above and next page) Violin plots (C, D) of the main morphometric variables that explain the major loadings of the first two components of the PCA, with asterisks indicating significant pairwise differentiation using a Wilcoxon test ($p < 0.05$).



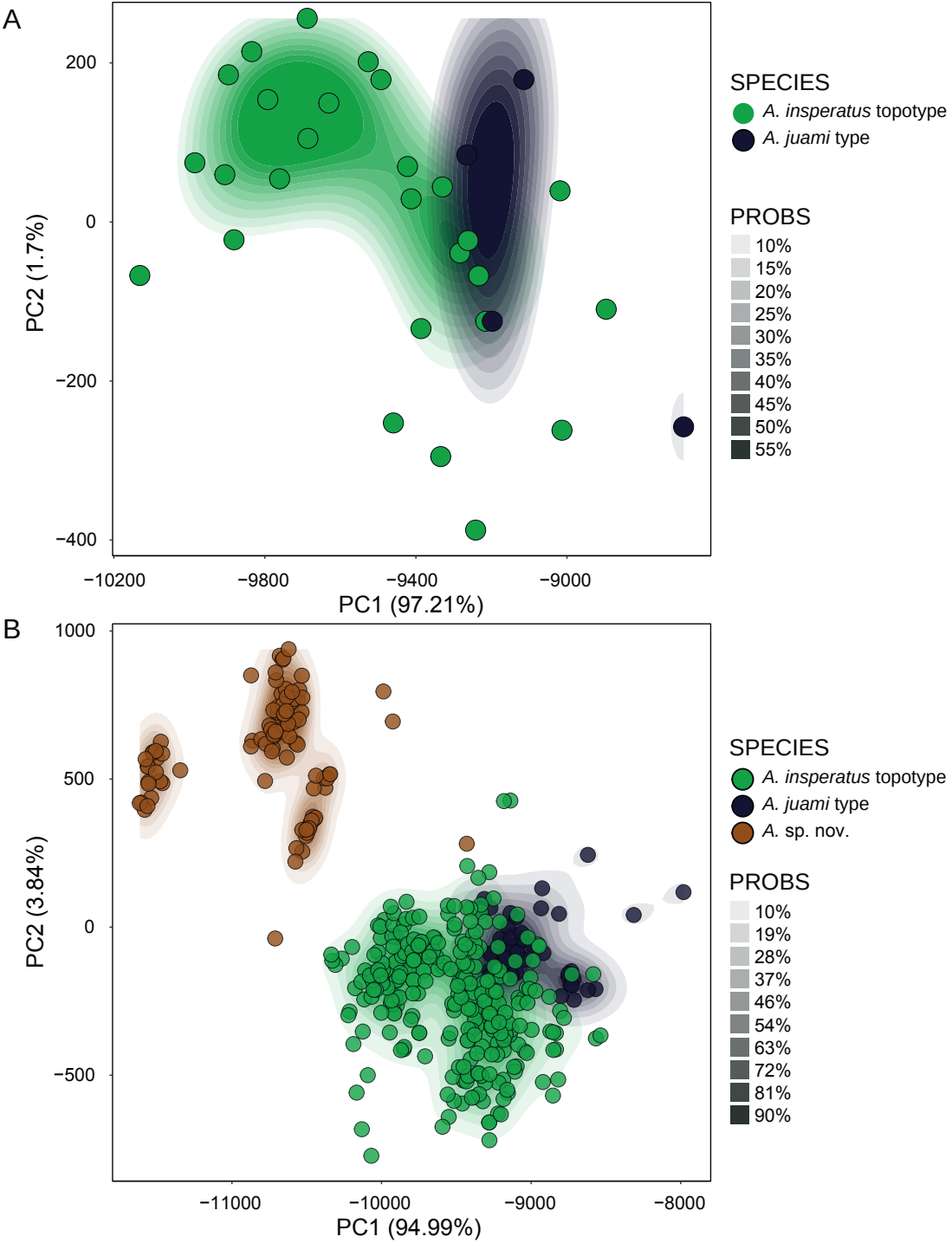
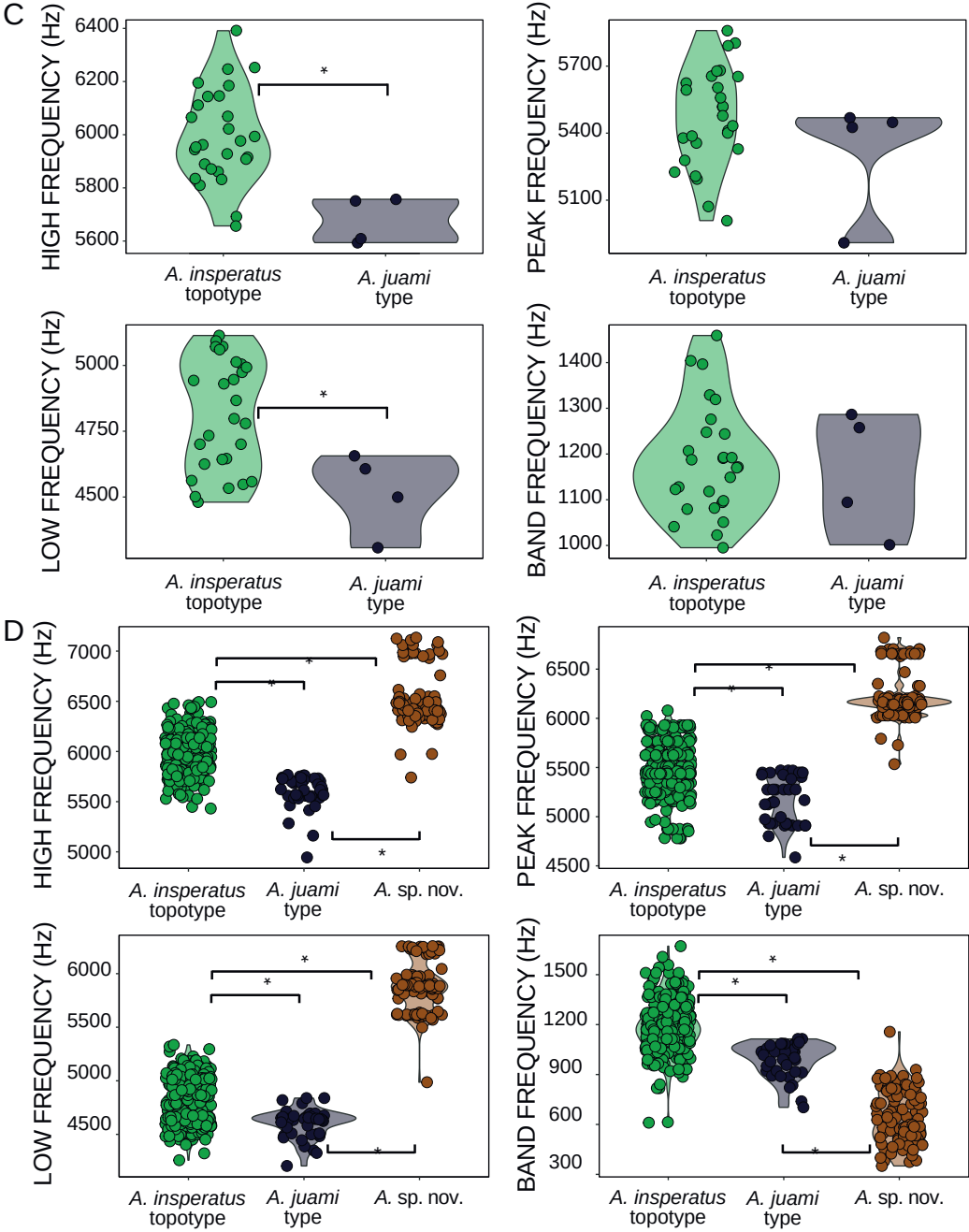


FIGURE 6. (above and next page) Comparisons of acoustic variation among specimens of *A. insperatus*, *A. juami*, and *A. sp.* Huallaga-Marañón based on trills (A, B) and notes (C, D). Only the first two species emit



trills. Scatter plots of the first two components of a PCA, with variance percentage explained by each axis in parenthesis (**A**, **C**). Violin plots of the main acoustic variables that explain the major loadings of the first two components of the PCA (**B**, **D**), with asterisks indicating significant pairwise differentiation from a Wilcoxon test ($p < 0.05$). Each point represents an analyzed trill (**A**, **B**) or note (**C**, **D**), respectively.



FIGURE 7. Dorsal and ventral views of topotypic female specimens of *Allobates insperatus*. **A.** ZSFQ 6269, SVL = 16.5 mm, showing the uniform paler dorsal coloration. **B.** ZSFQ 6272, SVL = 16.3 mm, with irregular dark brown spots. **C.** ZSFQ 6274, SVL = 16.8 mm, note the iridescent pigmentation on pale oblique lateral stripe.

present; (12) metacarpal ridge absent; (13) thenar tubercle conspicuous; (14) nuptial excrescences on thumb absent; (15) webbing on fingers absent; (16) finger III swollen on preaxial side in males; (17) finger II swollen on preaxial side in males; (18) tip of finger IV not reaching distal subarticular tubercle of Finger III; (19) finger II shorter than finger I; (20) lateral keels on preaxial and postaxial sides on fingers absent; (21) discs on fingers I–IV moderately expanded (width of discs on fingers I–IV/width of distal phalanges on fingers I–IV: mean = 1.6 ± 0.2 , range = 1.2–2.4); (22) black gland on arm absent; (23) tarsal keel present, tubercle-like, strongly curved; (24) tarsal fold absent; (25) metatarsal fold present, weak; (26) basal webbing present between toes III and IV, absent between other toes; (27) poorly defined keels on both side of all toes; (28) length of toe III suprasing the proximal margin of central subarticular tubercle of toe IV; (29) Discs of toes I and V moderately expanded ($1.1\text{--}1.4$ and $1.2\text{--}1.8\times$ the width of their adjacent phalanges, respectively). Discs of toes II, III, and IV moderately to greatly expanded ($1.2\text{--}1.9$, $1.5\text{--}2.0$, and $1.5\text{--}2.0\times$ the width of their adjacent phalanges, respectively); (30) background color of dorsum tan brown, becoming yellowish on snout; (31) pale tan brown dorsolateral stripe present, straight, well defined; (32) background color of dorsal surface of arms golden yellow or pink-orange with irregular dark brown spots, cream in preserved specimens; (33) dorsal surface of thigh yellowish brown or tan orange in live and preserved specimens, with a dark brown transverse bar in the middle of dorsal surface of thigh; (34) dark brown lateral stripe surrounding the whole body, reaching leg-body insertion; (35) pale oblique stripe present, diffuse, extending from groin to near mid-body; (36) pale ventrolateral stripe present in live and preserved specimens, irregular, extending from posterior portion of eye to groin, iridescent white in life; (37) pale paraoccal mark present, round or comma shaped; (38) females with yellowish translucent venter, with iridescent pigmentation on throat, chest, and belly in life; (39) males with translucent gray to grayish cream venter with scattered melanophores on throat, chest, and belly, and iridescent pigmentation on belly; (40) iris golden, with dark

brown reticulation; (41) diurnal habits, males vocalizing in daytime; (42) advertisement calls characterized by the emission of trills of notes, with a peak frequency of 4.78–6.08 kHz.

AMENDED DESCRIPTION OF EXTERNAL MORPHOLOGY: All morphometric measurements are in table 2. Body robust; females (SVL: 15.61–17.36 [16.25 ± 0.59] mm) and males (SVL: 15.48–16.28 [15.85 ± 0.27] mm) overlap in body size. Head slightly longer than wide (HL/HW = $1.17\text{--}1.46$ [1.32 ± 0.07]), head length $0.35\text{--}0.42$ (0.39 ± 0.02) times the SVL. Snout subacuminate in dorsal, lateral, and ventral views. Nostrils located laterally to snout, visible in dorsal, lateral, ventral, and anterior views. Distance between nostrils $0.40\text{--}0.47\times$ (0.44 ± 0.02) head width. *Canthus rostralis* slightly convex in cross section. Distance from nostril to anterior corner of eye $0.57\text{--}0.72$ (0.66 ± 0.05) times shorter than eye diameter. Loreal region convex in anterior view and oblique in dorsal view. Eye prominent, diameter $0.13\text{--}0.14$ (0.14 ± 0.004) times SVL. Tympanum round, small, $0.37\text{--}0.46\times$ (0.41 ± 0.03) eye diameter. Tympanic annulus weakly conspicuous, barely visible on the anteroinferior 3/4 of tympanum, under $4\times$ of magnification. Supratympanic fold absent. Maxillary teeth conspicuous under magnification. Dentigerous processes of vomers absent. Choanae round, small, positioned anteriorly to eye bulge. Tongue longer than wide, with anterior third attached to mouth floor. Median lingual process absent. Lateral vocal slits conspicuous. Vocal sac single, extending from middle level of throat to chest.

Forelimb slender, ulnar tubercles absent on ventral-postaxial surface of forearm. Hand length $21\text{--}24$ (22 ± 0.1)% of SVL. Palmar tubercle conspicuous, round. Thenar tubercle elliptical, maximum diameter $1.02\text{--}2.05$ (1.13 ± 0.25) times the maximum diameter of palmar tubercle. Proximal subarticular tubercle of finger IV round, small, not exceeding phalanx width. Distal subarticular tubercle on finger IV absent (see ventral views on figs. 7C and 8A). Finger III with a round, protuberant proximal subarticular tubercle, and a small, round distal subarticular tubercle. Subarticular tubercles on fingers I and

TABLE 2

Summary of Morphometric Variation (in mm) of Specimens of *Allobates*
Minimum and maximum values shown, followed by mean and standard deviation in parenthesis
when sample size is > 3.

<i>Allobates insperatus</i>			<i>Allobates juami</i>		<i>Allobates liniaureum</i>	
Sex	Female	Male	Female	Male	Female	Male
N	10	6	4	2	1	4
SVL	15.61–17.36 (16.5 ± 0.6)	15.48–16.28 (15.9 ± 0.3)	17.39–18.04 (17.7 ± 0.3)	17.76–17.80	14.97	14.24–14.82 (14.6 ± 0.3)
HL	6.10–6.90 (6.5 ± 0.3)	5.60–6.40 (6.2 ± 0.3)	6.70–6.85 (6.8 ± 0.1)	6.68–6.84	5.92	5.53–5.74 (5.6 ± 0.1)
HW	4.62–5.38 (4.9 ± 0.2)	4.32–4.98 (4.7 ± 0.3)	5.65–6.01 (5.8 ± 0.2)	5.83–5.89	5.06	5.05–5.29 (5.1 ± 0.1)
SL	2.50–2.80 (2.7 ± 0.1)	2.20–2.70 (2.5 ± 0.2)	2.63–3.00 (2.8 ± 0.2)	2.78–2.85	2.62	2.07–2.41 (2.2 ± 0.2)
EN	1.33–1.56 (1.5 ± 0.1)	1.28–1.50 (1.4 ± 0.1)	1.45–1.83 (1.6 ± 0.2)	1.50–1.74	1.49	1.28–1.43 (1.4 ± 0.1)
ED	2.10–2.39 (2.2 ± 0.1)	2.07–2.28 (2.1 ± 0.1)	2.20–2.41 (2.3 ± 0.1)	2.30–2.33	2.18	1.85–2.28 (2.0 ± 0.2)
EW	1.20–1.53 (1.4 ± 0.1)	1.20–1.43 (1.3 ± 0.1)	1.31–1.55 (1.4 ± 0.1)	1.32–1.41	1.31	1.19–1.35 (1.3 ± 0.1)
IO	1.80–2.10 (1.9 ± 0.1)	1.80–2.10 (1.9 ± 0.1)	1.99–2.15 (2.1 ± 0.1)	1.98–2.22	1.76	1.63–1.75 (1.7 ± 0.1)
IN	2.06–2.29 (2.2 ± 0.1)	1.96–2.32 (2.0 ± 0.1)	2.17–2.51 (2.4 ± 0.2)	2.27–2.39	2.24	2.08–2.27 (2.2 ± 0.1)
TYM	0.81–1.09 (0.9 ± 0.1)	0.84–0.95 (0.9 ± 0.0)	0.87–1.04 (1.0 ± 0.1)	0.99–1.04	0.9	0.90–1.00 (0.9 ± 0.0)
HAND.I	2.74–3.24 (3.0 ± 0.2)	2.57–3.15 (2.8 ± 0.2)	2.93–3.30 (3.0 ± 0.2)	3.20–3.22	2.53	2.11–2.61 (2.3 ± 0.2)
HAND.II	2.52–2.87 (2.7 ± 0.1)	2.32–2.89 (2.6 ± 0.2)	2.67–2.85 (2.7 ± 0.1)	2.77–2.90	2.22	1.88–2.33 (2.1 ± 0.2)
HAND.III	3.41–3.97 (3.7 ± 0.2)	3.32–3.80 (3.6 ± 0.2)	3.52–3.91 (3.7 ± 0.2)	3.60–3.69	3.1	2.73–3.32 (3.0 ± 0.2)
HAND.IV	2.19–2.65 (2.4 ± 0.2)	2.07–2.54 (2.4 ± 0.2)	2.33–2.62 (2.5 ± 0.1)	2.48–2.70	2.16	1.95–2.27 (2.1 ± 0.1)
WFID	0.35–0.49 (0.4 ± 0.0)	0.29–0.44 (0.4 ± 0.1)	0.51–0.60 (0.5 ± 0.0)	0.41–0.51	0.34	0.32–0.42 (0.4 ± 0.1)
WPFI	0.18–0.33 (0.3 ± 0.0)	0.21–0.28 (0.2 ± 0.0)	0.33–0.36 (0.3 ± 0.0)	0.30–0.33	0.23	0.23–0.33 (0.3 ± 0.0)
WFIID	0.31–0.42 (0.4 ± 0.0)	0.28–0.40 (0.3 ± 0.0)	0.50–0.59 (0.6 ± 0.0)	0.54–0.54	0.39	0.36–0.43 (0.4 ± 0.0)
WPFII	0.19–0.27 (0.2 ± 0.0)	0.17–0.25 (0.2 ± 0.0)	0.30–0.37 (0.3 ± 0.0)	0.32–0.35	0.29	0.27–0.30 (0.3 ± 0.0)
WFIIRD	0.30–0.45 (0.4 ± 0.0)	0.26–0.42 (0.3 ± 0.1)	0.55–0.64 (0.6 ± 0.0)	0.58–0.66	0.48	0.48–0.54 (0.5 ± 0.0)

TABLE 2 *continued*

<i>Allobates insperatus</i>			<i>Allobates juami</i>		<i>Allobates liniaureum</i>	
Sex	Female	Male	Female	Male	Female	Male
N	10	6	4	2	1	4
WPFIII	0.21–0.31 (0.3 ± 0.0)	0.20–0.26 (0.2 ± 0.0)	0.31–0.37 (0.3 ± 0.0)	0.36–0.39	0.31	0.26–0.32 (0.3 ± 0.0)
WFIVD	0.30–0.42 (0.4 ± 0.0)	0.26–0.40 (0.3 ± 0.1)	0.47–0.65 (0.5 ± 0.1)	0.53–0.60	0.47	0.41–0.45 (0.4 ± 0.0)
WPFIV	0.19–0.29 (0.2 ± 0.0)	0.17–0.22 (0.2 ± 0.0)	0.29–0.34 (0.3 ± 0.0)	0.33–0.35	0.27	0.26–0.28 (0.3 ± 0.0)
DPT	0.34–0.48 (0.4 ± 0.0)	0.35–0.49 (0.4 ± 0.1)	0.56–0.65 (0.6 ± 0.0)	0.61–0.69	0.57	0.39–0.53 (0.5 ± 0.1)
DTT	0.35–0.80 (0.5 ± 0.1)	0.38–0.51 (0.4 ± 0.1)	0.36–0.47 (0.4 ± 0.1)	0.47–0.53	0.37	0.25–0.39 (0.3 ± 0.1)
UaL	3.25–3.95 (3.5 ± 0.2)	3.24–3.91 (3.5 ± 0.2)	3.89–4.05 (4.0 ± 0.1)	4.09–4.23	3.54	2.87–3.18 (3.1 ± 0.1)
TL	5.88–7.34 (7.0 ± 0.4)	6.35–7.08 (6.8 ± 0.3)	7.75–8.21 (8.1 ± 0.2)	8.31–8.46	6.23	6.11–7.21 (6.6 ± 0.5)
SaL	7.03–8.30 (7.7 ± 0.4)	7.14–7.79 (7.5 ± 0.2)	7.57–7.93 (7.8 ± 0.2)	8.21–8.35	7.82	7.07–7.40 (7.2 ± 0.2)
Tal	4.17–5.15 (4.7 ± 0.3)	4.19–5.09 (4.6 ± 0.3)	4.50–4.74 (4.6 ± 0.1)	4.70–4.83	4.33	3.99–4.33 (4.2 ± 0.1)
FL	5.24–7.34 (6.4 ± 0.6)	5.69–6.61 (6.2 ± 0.4)	6.85–7.38 (7.1 ± 0.2)	7.15–7.38	7.5	6.00–7.10 (6.5 ± 0.5)
WTID	0.25–0.35 (0.3 ± 0.0)	0.23–0.29 (0.3 ± 0.0)	0.35–0.43 (0.4 ± 0.0)	0.38–0.43	0.33	0.26–0.31 (0.3 ± 0.0)
WTIP	0.18–0.28 (0.2 ± 0.0)	0.16–0.26 (0.2 ± 0.0)	0.31–0.50 (0.4 ± 0.1)	0.29–0.36	0.3	0.23–0.25 (0.2 ± 0.0)
WTIID	0.31–0.43 (0.4 ± 0.0)	0.26–0.36 (0.3 ± 0.0)	0.60–0.70 (0.6 ± 0.0)	0.57–0.63	0.45	0.38–0.45 (0.4 ± 0.0)
WTIIP	0.19–0.29 (0.2 ± 0.0)	0.15–0.26 (0.2 ± 0.0)	0.30–0.37 (0.3 ± 0.0)	0.30–0.35	0.29	0.19–0.29 (0.3 ± 0.0)
WTIID	0.30–0.47 (0.4 ± 0.0)	0.32–0.39 (0.4 ± 0.0)	0.57–0.81 (0.7 ± 0.1)	0.68–0.80	0.56	0.51–0.56 (0.5 ± 0.0)
WTIIP	0.17–0.30 (0.2 ± 0.0)	0.19–0.24 (0.2 ± 0.0)	0.30–0.41 (0.4 ± 0.0)	0.34–0.41	0.32	0.28–0.33 (0.3 ± 0.0)
WTIVD	0.38–0.50 (0.4 ± 0.0)	0.37–0.41 (0.4 ± 0.0)	0.72–0.93 (0.8 ± 0.1)	0.77–0.80	0.67	0.52–0.63 (0.6 ± 0.0)
WTIVP	0.22–0.32 (0.3 ± 0.0)	0.20–0.25 (0.2 ± 0.0)	0.35–0.43 (0.4 ± 0.0)	0.34–0.35	0.34	0.23–0.31 (0.3 ± 0.0)
WTVD	0.29–0.39 (0.3 ± 0.0)	0.27–0.32 (0.3 ± 0.0)	0.48–0.60 (0.5 ± 0.1)	0.59–0.68	0.36	0.34–0.40 (0.4 ± 0.0)
WTVP	0.19–0.27 (0.2 ± 0.0)	0.19–0.23 (0.2 ± 0.0)	0.31–0.53 (0.4 ± 0.1)	0.34–0.38	0.25	0.22–0.27 (0.2 ± 0.0)

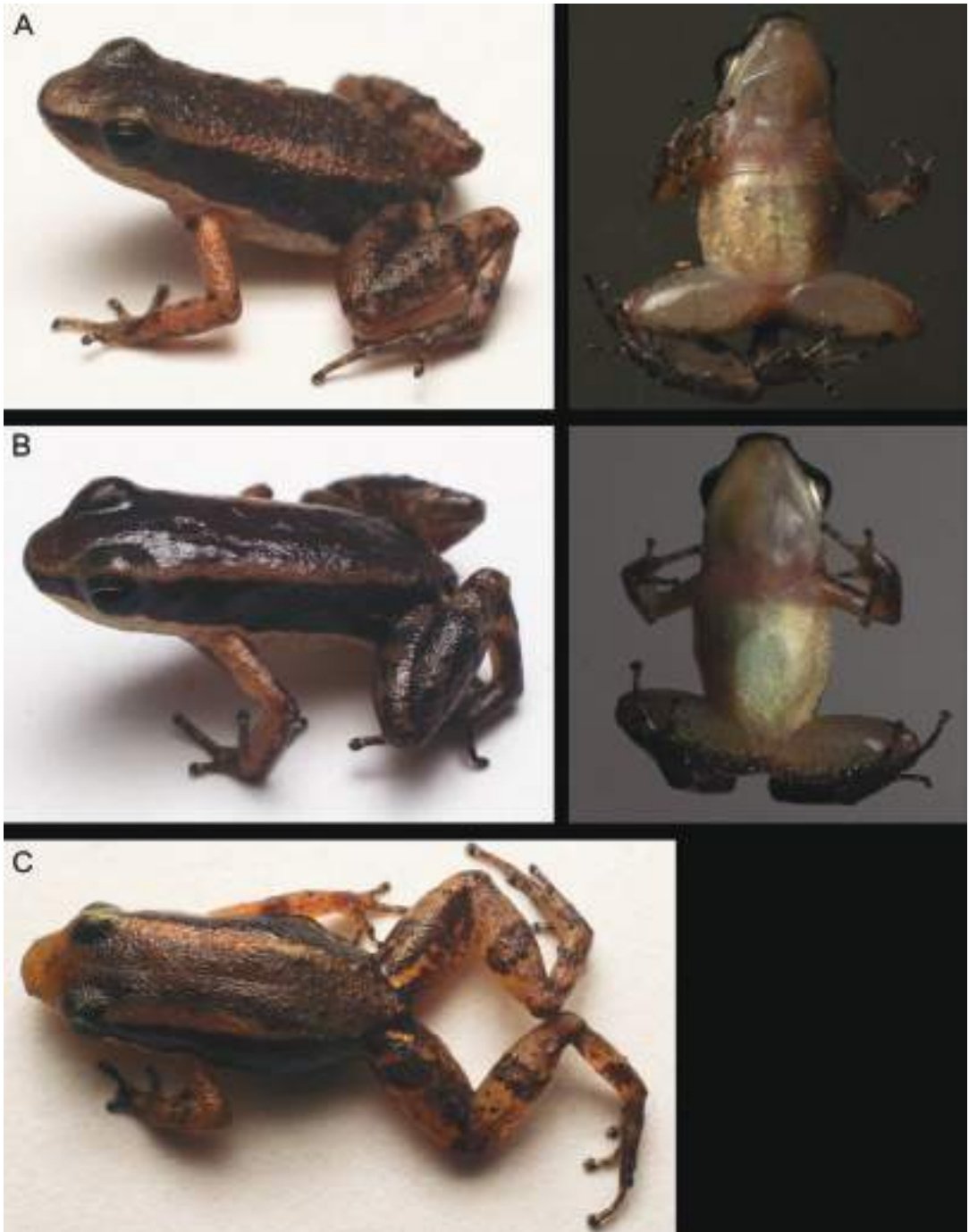


FIGURE 8. Dorsal and ventral views of topotypic male specimens of *Allobates insperatus*. **A.** ZSFQ 6260, SVL = 16.0 mm, dorsum with dark brown uniform dorsal coloration and some scattered tubercles. **B.** ZSFQ 6281, SVL = 15.9 mm, with smooth dorsal skin and slightly paler. **C.** Adult female (ZSFQ 6268, SVL = 15.9 mm), showing the paraclaoal marks, and the transverse bars on thigh, shank, and tarsus.

II very protuberant, elliptical. Supernumerary tubercles absent. Metacarpal ridge absent. Webbing absent between fingers. Length of finger II 84.4–97.2% (91.2 ± 3.8) the finger I length. Tip of finger IV not reaching distal subarticular tubercle of finger III when fingers are juxtaposed. Relative lengths of fingers adpressed: $IV < II < I < III$. Keels absent on the preaxial and postaxial side of fingers. Fingers II and III preaxially swollen in males, finger swelling expanding from the base of the finger III disc to the level of proximal subarticular tubercle. Discs of fingers moderately expanded, width of discs I–IV corresponding to 1.27–1.94 (1.61 ± 0.22), 1.17–1.88 (1.63 ± 0.21), 1.2–1.91 (1.55 ± 0.23), and 1.3–2.35 $\times$ (1.67 ± 0.29) the width of their adjacent phalanges, respectively. Tip of discs rounded. Paired dorsal digital scutes conspicuous and protuberant in all fingers.

Hind limbs robust. Lengths of thigh, shank, and foot similar ($TL/SVL = 37.0\text{--}45.7$ [42.5 ± 2.6]; $SAL/SVL = 43.9\text{--}48.8$ [46.7 ± 1.6]; $FL/SLV = 31.1\text{--}42.8$ [38.7 ± 2.9]). Tarsal keel tubercle-like, short, strongly curved at its proximal end. Inner metatarsal tubercle well-defined, large, elliptical. Outer metatarsal tubercle shorter than inner metatarsal tubercle, round. Metatarsal fold present, weak, extending between outer metatarsal tubercle and the proximal subarticular tubercle of toe V. Basal webbing present between toes III and IV, absent between other toes. Toes slender, all with poorly defined keels. Subarticular tubercles round, smaller than inner metatarsal tubercle in maximum diameter. Plantar supernumerary tubercles absent. Relative lengths of toes: $I < II < V < III < IV$. Length of toe III surpassing the proximal margin of central subarticular tubercle of toe IV. Discs of toes I and V moderately expanded, width of toe discs 1.12–1.44 (1.29 ± 0.12) and 1.17–1.75 (1.51 ± 0.15) times the width of their adjacent phalanges, respectively. Discs of toes II, III, and IV moderately to greatly expanded, width of discs, 1.21–1.87 (1.54 ± 0.22), 1.54–2.0 (1.79 ± 0.15) and 1.46–2.0 (1.71 ± 0.14) times the width of their adjacent phalanges, respectively. Paired dorsal

digital scutes conspicuous and slightly protuberant in all toes.

Dorsal skin of body shagreen, moderately granular from behind the eyes or midbody to the urostyle region. Forelimb surfaces smooth. Dorsal surface of thighs and shanks with small, scattered tubercles. Ventral skin smooth on throat, chest, and belly. Thighs with scattered tubercles in the posterior portion.

COLOR IN LIFE: Dorsum tan brown, becoming yellowish on snout in some individuals (fig. 7A), with dark spots (fig. 7C) or solid areas (fig. 8B) on body. Upper eyelid golden or brown, iridescent background color present among dark brown pigments. Iris golden, with dark brown reticulation. Pale tan brown dorsolateral stripe present, straight, broad, from tip of snout to urostyle region. Lateral stripe, solid, dark brown, extending from the tip of snout to groin. A paler oblique lateral stripe, extending from the groin and becoming diffuse near to midbody, with some white pigments (fig. 7C). Thin, irregular, white iridescent ventrolateral stripe extending from behind the eye and below tympanum to groin, extending ventrally as irregular spots against the dark and yellowish background in some specimens.

Ventral coloration sexually dimorphic (figs. 7, 8). In females the venter is translucent yellow on the throat, becoming yellowish cream posteriorly, bearing iridescent-white patches on throat, chest, and belly reaching the parietal peritoneum (fig. 7). In males, ventral surfaces translucent gray on throat and chest, grayish cream on the posterior portion of venter, with scattered melanophores on throat, chest, and belly; iridescent-white pigmentation confined to belly (fig. 8A, B).

Dorsal and dorsolateral surfaces of upper arm and forearm golden yellow or pink orange, with irregular dark brown spots. Ventral surface of upper arm translucent, with some scattered melanophores in males. Forearm peppered with dark pigments, more densely distributed in males. Fingers golden brown with or without melanophores. Transverse white bars present on fingers in dorsal view, variable in number. Fin-

gers peppered grayish brown in ventral view. Paired scutes on finger discs iridescent white.

Pale paracloacal mark present, round or comma shaped, yellowish brown or tan orange, with iridescent gold or cream parts. In some individuals, pale paracloacal mark diffuse, interrupted by patches of brown melanophores (fig. 8C). Paracloacal mark always surrounded by a dark brown frame, continuous with the posterior surface of thigh. Background color of dorsal surface of thigh yellowish brown or tan orange. An irregular dark brown transverse bar on the middle of the dorsal surface of thigh (fig. 8C). Dark brown longitudinal stripes or patches on anterior and posterior surface of thigh, sometimes extending distally from groin and cloaca and merging at the level of knee (fig. 8C). Dorsal surface of shank with same coloration as thigh, sometimes with a median transverse dark brown bar (75% of examined specimens; fig. 8C), dark brown dots (19% of examined specimens; fig. 7B) or blotches (6% of examined specimens; fig. 8B). Ventral surface of thigh and shank pale gray to yellowish translucent, with scattered brown melanophores marginally, some iridescent white spots on ventroposterior surface of thighs (figs. 7, 8). Tarsal region yellowish brown or tan orange, with dark brown blotches or transverse bars on the middle of the shank and near its articulation with shank (figs. 7, 8). When a median transverse bar is present on both shank and tarsus, they align when posterior limb is in resting position (figs. 7, 8). Ventral surfaces of tarsal region yellowish or grayish translucent, with scattered brown melanophores marginally (figs. 7, 8). Plantar surface of foot densely covered with brown melanophores. Toes brown with a varying number of transverse white bars on dorsal surface, brown on ventral surface. Paired scutes on toe discs iridescent white, black only on toe V.

COLOR IN PRESERVATIVE: Background color of dorsal surface of body tan brown with several patterns; some individuals are uniformly dark brown from tip of snout to cloaca, whereas other bear irregular dark brown patches from snout to

midbody and some scattered dots on the urostyle region. Color of dorsum darker over eyelids. Pale dorsolateral stripe present, well-defined, solid, straight, extending from tip of snout to cloaca; it becomes diffuse in the urostyle region and does not touch its counterpart at the posterior end. Lateral surface of body with a solid dark brown stripe, extending from tip of snout to groin, becoming diffuse on posterior portion of body. Pale oblique lateral stripe present on dark brown stripe at inguinal region, extending anteriorly from groin, but without reaching midbody length, where it becomes diffuse. Irregular or discontinuous ventrolateral stripe extending along the lower edge of lateral dark brown stripe, conformed by tiny mottled iridescent white pigmentation. Scattered dark brown pigmentation present ventrally to white ventrolateral stripe, from upper lips and midbody, reaching the ventral surface with some iridescent patches. Background color of ventral surfaces light cream. In males, scattered melanophores present on throat, chest, and belly, denser in throat. Females lack melanophores on ventral surfaces but have some iridescent patches on the marginal ventral surfaces of mouth.

Dorsal surface of forelimbs tan brown, with dark brown mottling on elbow, forearm and wrist, denser distally. Fingers III and IV more darkly pigmented than fingers I and II. Paired scutes on discs of fingers I, II, and III conspicuous, iridescent white.

Background color of dorsal surface of thigh light brown. Light brown or white cream paracloacal mark conspicuous, comma shaped, broader at its proximal end, bordered with dark brown. Anterior (inner) portion of thigh adjacent to body, dark brown, becoming diffuse near to knee; dorsal portion with a dark brown transverse bar, and posterior portion of thigh dark brown with mottled light brown, especially the area covered by shanks. Dorsal surface of shank same color as thigh, sometimes with a median transverse dark brown bar and dots or blotches. Dorsal surface of tarsal region same color as thigh and shank, with a median transverse dark

brown bar or dark brown dots or blotches. Dorsal surface of toes dark brown, densely pigmented on toes IV and V. Paired scutes white on toes I, II, III, and IV, black on toe V. Ventral surfaces of thigh and shank cream, with densely dark brown mottling on marginal edges. Ventral surface of tarsal region cream, with scattered dark brown pigments. Toes dark brown, densely pigmented on ventral surface.

BIOACOUSTICS: All bioacoustic measurements are in table 3. Advertisement calls of *Allobates insperatus* are characterized by the emission of short notes arranged in trills (fig. 9A). Trills are formed by 36 ± 4 notes (28–46 notes). Call duration is 2.24 ± 0.29 s (1.68–2.87 s) and, within each call, notes are emitted at an average rate of 16.35 ± 0.41 notes/s (15.25–16.84 notes/s). Notes are short and regularly spaced within each call (fig. 9A). Note duration is 0.037 ± 0.004 s (0.026–0.052 s) and the duration of the silent interval between consecutive notes is 0.025 ± 0.004 s (0.015–0.044 s). Notes have an ascending frequency modulation. Average peak frequency is 5.471 ± 0.271 kHz (4.781–6.079 kHz). Average lower and upper frequencies of notes are 4.810 ± 0.229 kHz (4.258–5.335 kHz) and 5.994 ± 0.216 kHz (5.431–6.492 kHz), respectively. The peak frequency corresponds to the second harmonic. In calls recorded with best quality and less background noise, the fundamental frequency was observed at about 2.990 kHz, and a third harmonic at 7.910 kHz.

PHYLOGENETIC RELATIONSHIPS AND GENETIC DISTANCES: The most closely related taxon to *Allobates insperatus* is *A. juami* (figs. 1, 2), with some specimens having shared nuclear alleles (e.g., LEO 718). We provide a summary of 16S genetic distances in appendix 7. Uncorrected pairwise genetic distances within *A. insperatus* samples are 0–4.4%, while distances between *A. insperatus* and *A. juami* and *A. aff. juami* are 3.4–6.0% and 2.8–6.8%, respectively.

DIAGNOSIS: *Allobates insperatus* as defined herein is found in the lowlands (<500 m) of northwestern Amazonia, around the headwaters of the Napo and Putumayo rivers in Colombia

and Ecuador (fig. 3), relatively close to the border with Peru and Brazil. These four countries harbor a great diversity of *Allobates* species. Hence, we focus our morphological comparisons on lowland Amazonian species—excluding mountain (i.e., *A. pacaas* Melo-Sampaio et al., 2020, and *A. carajas* Simões et al., 2019) and savanna-dweller (i.e., *A. brunneus* [Cope, 1887] and *A. goianus* [Bokermann, 1975]) taxa—from these four countries, with particular emphasis on the most closely related species. We organized the diagnosis into morphological and bioacoustic characters. Character states of *A. insperatus* are in parenthesis throughout the diagnosis.

Morphological diagnosis. The most similar and closely related species is *Allobates juami*, but in life males and females have a yellow throat, chest, and belly without melanophores (throat, chest, and belly translucent gray to grayish cream with scattered melanophores in males, with yellow coloration restricted to throat and chest of females) (figs. 7, 8, 10, 11); dark brown lateral stripe about the same width from behind the eye to the inguinal region (conspicuously narrower behind the eye than at the inguinal region); pre-axial side of fingers II and III not swollen (swollen); larger body, SVL = 17.4–18.0 mm (SVL = 15.5–17.4 mm); and wider head, HW = 5.7–6.0 mm (HW = 4.3–5.4 mm) (table 2).

Excluding the new species described in this study (see next species account for comparisons), the most closely related nominal species are *Allobates conspicuus* and *A. subfolionidificans* (fig. 1). They can be diagnosed by males having a venter without melanophores (present) and preaxial side of fingers II and III not swollen (swollen). Furthermore, *A. subfolionidificans* lacks defined pale dorsolateral and ventrolateral stripes (well defined and conspicuous).

Other species can be differentiated by: absence of melanophores on throat, chest and belly of males in *A. crombiei* (Morales, 2002), *A. grillicantus* Moraes and Lima, 2021, *A. paleci* Silva et al., 2022, *A. sumtuosus* (Morales, 2002), and *A. tapajos* Lima et al., 2015 (melanophores present on ventral surfaces of males); melanophores on

TABLE 3
Temporal and Spectral Acoustic Parameters of the Advertisement Call of *Allobates*

Calls from male specimens recorded at the species type localities in Santa Cecilia, Provincia Subumbíos, Ecuador, in March 2019, Estación Ecológica Juami-Japurá, State of Amazonas, Brazil, in February 2017, and Yurimaguas, Loreto, Peru, in February 2019, respectively. Values are provided as min-max (average $\pm$ standard deviation). N = number of analyzed units of bioacoustic signal (i.e., note trills or single notes) recorded and analyzed for each male.

	<i>Allobates insperatus</i>				<i>Allobates juami</i>				<i>Allobates liniaureum</i>					
	AFJ0235	AFJ0236	AFJ0237		SCF 2027	SCF2028	AFJ_0197	AFJ_187	GGU_A_0295	GGU_A_0315	GGU_A_0317			
Voucher	ZSFQ 6270	Uncollected	Uncollected		MCP 13287	MCP 13290	MCP 14296	MCP 14294	MCP 14332	Uncollected	Uncollected			
FonoZoo	14942	14943	14944		14949	14948	14941	14940	14947	14945	14946			
N (call)	4	8	15		3	1	20	20	20	20	20			
No. notes/call	38.00-41.00	35-46	28-40		60-73	65	1	1	1	1	1			
Call duration (s)	2.40-2.61 (2.5 $\pm$ 0.1)	2.09-2.87 (2.3 $\pm$ 0.2)	1.68-2.46 (2.1 $\pm$ 0.3)		4.23-4.99 (4.7)	4.56	-	-	-	-	-			
Call silent (s)	0.02-0.03 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)		0.03-0.03 (0.0)	0.03	-	-	-	-	-			
Call silent/Call duration	0.57-0.77 (0.7 $\pm$ 0.1)	0.68-0.86 (0.8 $\pm$ 0.1)	0.54-0.81 (0.7 $\pm$ 0.1)		0.89-1.01 (0.9)	0.59	-	-	-	-	-			
Call Lower Frequency (kHz)	4.48-4.56 (4.5 $\pm$ 0.0)	4.50-4.78 (4.6 $\pm$ 0.1)	4.70-5.11 (5.0 $\pm$ 0.1)		4.31-4.66 (4.5)	4.61	-	-	-	-	-			
Call Upper Frequency (kHz)	5.81-5.99 (5.9 $\pm$ 0.1)	5.66-5.98 (5.8 $\pm$ 0.1)	5.87-6.39 (6.1 $\pm$ 0.1)		5.59-5.76 (5.7)	5.61	-	-	-	-	-			
Call Peak Frequency (kHz)	5.07-5.62 (5.3 $\pm$ 0.2)	5.01-5.41 (5.3 $\pm$ 0.1)	5.28-5.86 (5.6 $\pm$ 0.2)		4.91-5.47 (5.3)	5.43	-	-	-	-	-			
Call Delta Frequency (kHz)	1.33-1.46 (1.4 $\pm$ 0.1)	1.09-1.28 (1.2 $\pm$ 0.1)	1.00-1.32 (1.1 $\pm$ 0.1)		1.09-1.29 (1.2)	1	-	-	-	-	-			
N (notes)	41	81	153		30	10	-	-	-	-	-			
Note emission rate (notes/s)	15.58-15.85 (15.7 $\pm$ 0.1)	16.01-16.84 (16.6 $\pm$ 0.3)	15.25-16.74 (16.4 $\pm$ 0.3)		12.03-17.27 (14.6 $\pm$ 2.2)	14.26-14.26 (14.3 $\pm$ 0.0)	0.56	1.01	0.2	0.64	0.42			
Note duration (s)	0.03-0.05 (0.0 $\pm$ 0.0)	0.03-0.05 (0.0 $\pm$ 0.0)	0.03-0.05 (0.0 $\pm$ 0.0)		0.03-0.04 (0.0 $\pm$ 0.0)	0.04-0.06 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)	0.01-0.02 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)			
Silent interval between notes (s)	0.02-0.04 (0.0 $\pm$ 0.0)	0.02-0.04 (0.0 $\pm$ 0.0)	0.01-0.04 (0.0 $\pm$ 0.0)		0.02-0.05 (0.0 $\pm$ 0.0)	0.02-0.03 (0.0 $\pm$ 0.0)	1.04-2.84 (1.8 $\pm$ 0.4)	0.58-1.91 (1.0 $\pm$ 0.4)	0.44-12.54 (5.1 $\pm$ 3.6)	0.48-2.52 (1.7 $\pm$ 0.5)	0.36-12.98 (2.7 $\pm$ 2.9)			
Note silent/Note duration	0.41-1.09 (0.7 $\pm$ 0.2)	0.35-1.19 (0.8 $\pm$ 0.2)	0.40-1.23 (0.7 $\pm$ 0.2)		0.57-1.48 (0.9 $\pm$ 0.2)	0.37-0.74 (0.6 $\pm$ 0.1)	38.44-108.86 (68.7 $\pm$ 20.1)	21.46-76.56 (41.8 $\pm$ 17.4)	21.00-627.25 (285.0 $\pm$ 200.2)	17.85-102.90 (66.1 $\pm$ 21.3)	17.87-590.14 (116.8 $\pm$ 130.1)			

TABLE 3 continued

	<i>Allobates insperatus</i>			<i>Allobates juami</i>		<i>Allobates liniaureum</i>				
Note Lower Fre-	4.32-4.64	4.26-5.00	4.58-5.34	4.21-4.84	4.56-4.66	6.18-6.26	5.81-6.04	5.77-5.92	5.59-5.65	4.99-5.99
quency (kHz)	(4.5 ± 0.1)	(4.6 ± 0.1)	(5.0 ± 0.2)	(4.6 ± 0.2)	(4.6 ± 0.0)	(6.2 ± 0.0)	(5.9 ± 0.0)	(5.9 ± 0.0)	(5.6 ± 0.0)	(5.8 ± 0.2)
Note Upper Fre-	5.53-6.12	5.43-6.06	5.57-6.49	4.94-5.77	5.28-5.65	6.93-7.13	6.35-6.57	6.32-6.48	6.27-6.76	5.74-6.61
quency (kHz)	(5.9 ± 0.1)	(5.8 ± 0.1)	(6.1 ± 0.2)	(5.6 ± 0.2)	(5.5 ± 0.1)	(7.0 ± 0.1)	(6.5 ± 0.1)	(6.4 ± 0.0)	(6.4 ± 0.1)	(6.3 ± 0.2)
Note Peak Fre-	4.78-5.72	4.88-5.62	4.85-6.08	4.59-5.47	4.97-5.45	6.47-6.82	6.14-6.35	6.12-6.22	6.01-6.05	5.53-6.22
quency (kHz)	(5.3 ± 0.3)	(5.3 ± 0.2)	(5.6 ± 0.2)	(5.2 ± 0.3)	(5.2 ± 0.2)	(6.7 ± 0.1)	(6.2 ± 0.1)	(6.2 ± 0.0)	(6.0 ± 0.0)	(6.1 ± 0.2)
Note Delta Fre-	1.21-1.56	0.96-1.38	0.61-1.67	0.74-1.11	0.70-1.00	0.69-0.89	0.45-0.73	0.45-0.65	0.66-1.16	0.35-0.75
quency (kHz)	(1.4 ± 0.1)	(1.2 ± 0.1)	(1.1 ± 0.1)	(1.0 ± 0.1)	(0.9 ± 0.1)	(0.8 ± 0.1)	(0.6 ± 0.1)	(0.5 ± 0.0)	(0.8 ± 0.1)	(0.5 ± 0.1)

chin or extending to belly in females of *A. albiventris* Souza et al., 2023, *A. bacurau* Simões, 2016, *A. caeruleodactylus* (Lima and Caldwell, 2001), *A. fratisenescus* (Morales, 2002), *A. kamilae* Ferrão, Hanken, and Lima, 2022, *A. siegreenae* Gagliardi-Urrutia et al., 2021, *A. tinae* Melo-Sampaio, Oliveira, and Prates, 2018, *A. trilineatus* (Boulenger, 1884), and *A. velocicantus* Souza et al., 2020 (melanophores absent on chin or belly of females); throat and chest solid dark-gray in males of *A. masniger* (Morales, 2002), *A. melanolaemus* (Grant and Rodríguez, 2001), and *A. paleovarzensis* Lima et al., 2010 (throat and chest with scattered melanophores); preaxial side of fingers II and/or III not swollen in males of *A. amissibilis* Kok et al., 2013, *A. bacurau*, *A. caeruleodactylus*, *A. caldwella* Lima et al., 2020, *A. crombiei*, *A. flaviventris* Melo-Sampaio et al., 2013, *A. fratisenescus*, *A. fuscillus* (Morales, 2002), *A. gasconi* (Morales, 2002), *A. granti* (Kok et al., 2006), *A. grillisimilis* Simões et al., 2013, *A. kamilae*, *A. magnussoni* Lima et al., 2014, *A. marchesianus* (Melin, 1941), *A. masniger*, *A. nidicola* (Caldwell and Lima, 2003), *A. nunciatus* Moraes et al., 2019, *A. ornatus* (Morales, 2002), *A. paleci*, *A. paleovarzensis*, *A. ripicolus* Fouquet et al., 2023, *A. tapajos*, *A. tinae*, *A. trilineatus*, *A. vanzolinus* (Morales, 2002), *A. velocicantus*, and *A. vicinus* Fouquet et al., 2023 (fingers II and III swollen in males); dorsolateral stripe broadened posteriorly to eyelids forming an hourglass pattern in *A. albiventris*, *A. crombiei*, *A. flaviventris*, *A. gasconi*, *A. kamilae*, *A. magnussoni*, *A. ornatus*, *A. tapajos*, and *A. vicinus* (dorsolateral stripe with constant width, not forming an hourglass pattern); adults of *A. fratisenescus*, *A. kingsburyi* (Boulenger, 1918), *A. masniger*, *A. melanolaemus*, *A. paleovarzensis*, *A. nidicola*, *A. nunciatus*, and *A. vanzolinus* are larger, from all this species the smaller recorded adult male is *A. masniger* with SVL = 17.9 mm (SVL up to 16.3 mm in males and up to 17.4 mm in females); in life, *Allobates femoralis*, *A. hodli* Simões et al., 2010, *A. myersi* (Pyburn, 1981), and *A. zaparo* (Silverstone, 1976) have bright yellow, orange or red

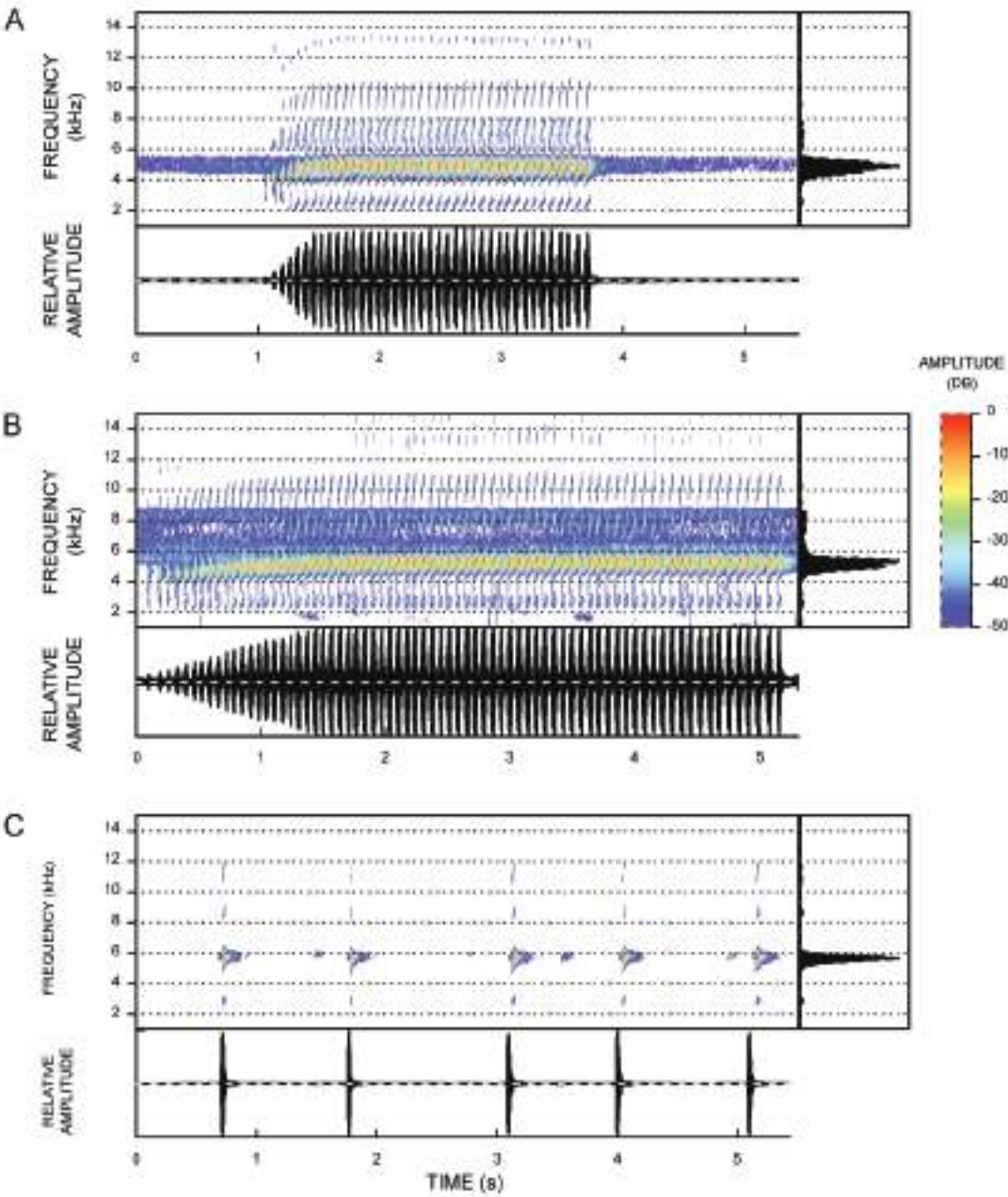


FIGURE 9. Advertisement calls of **A.** *Allobates insperatus* [topotype male, AFJ 245], **B.** *A. juami* [holotype male, MCP 13287], and **C.** *A. liniaureum* [paratype, MCP 14294]. Showing the spectrogram (upper graph), waveform (lower graph) and power spectrum (right graph).

flash marks on dorsal surfaces of thighs, and black and white marbling on belly and ventral surface of thighs in preserved and live specimens (yellow, orange or red flash marks, and black and white marbling absent).

Acoustic diagnosis. We compare the advertisement call of *A. insperatus* with the available call description of 43 *Allobates* species. The call structure of *A. algorei* (Barrio-Amorós and Santos, 2009), *A. caeruleodactylus* (Lima and Caldwell, 2001), *A. chalcopis* (Kaiser et al., 1994), *A. magnussoni* (Lima et al., 2014), *A. masniger* (Moraes et al., 2019), *A. nidicola* (Caldwell and Lima, 2003), *A. niputidea* Grant, Acosta-Galvis, and Rada, 2007 (Ospina-L. et al., 2019), *A. offersioides* (Lutz, 1925) (Forti et al., 2017), *A. ripicolus* (Fouquet et al., 2023), and *A. subfolionidificans* (Lima et al., 2007) is composed by continuous emission of single notes (trills of single notes).

The advertisement calls of *A. albiventris* (Souza et al., 2023) *A. femoralis* (Moraes et al., 2019), *A. hodli* (Simões et al., 2010), *A. myersi* (Simões and Lima, 2011), *A. tapajos* (Lima et al., 2014), *A. trilineatus* (Jaramillo et al., 2021), *A. undulatus* (Myers and Donnelly, 2001), *A. zaparo* (Santos et al., 2014), and *A. vicinus* (Fouquet et al., 2023) is arranged in series or trills of note pairs, or trills of three, four, or six notes.

The calls of *A. bacurau* (Simões, 2016), *A. brunneus* (Lima et al., 2009), *A. velocicantus* (Souza et al., 2020) contain more notes per trill: 60–81, 60–73, 66–138 notes/trill, respectively (less notes: 28–46 notes/trill). The trills with few notes in *A. amissibilis*: 1–19 notes/trill (Kok et al., 2013), *A. caldwellaie*: 3–7 notes/trill (Lima et al., 2020), *A. carajas*: 4–22 notes/trill (Simões et al., 2019), *A. flaviventris*: 2–10 notes/trill (Melo-Sampaio et al., 2013), *A. granti*: 1–7 notes/trill (Kok et al., 2006), *A. grillicantus*: 4–15 notes/trill (Moraes and Lima, 2021), *A. grillisimilis*: 3–15 notes/trill (Simões et al., 2013), *A. juanii*: 9–19 notes/trill (Grant and Rodríguez, 2001), *A. kamilaie*: 1–4 notes/trill (Ferrão et al., 2022), *A. marchesianus*: 21–24 notes/trill (Caldwell et

al., 2002), *A. nunciatus*: 4 notes/trill (Moraes et al., 2019), *A. paleovarzensis*: 3–20 notes/trill (Lima et al., 2010), *A. sieggreenae*: 5–16 notes/trill (Gagliardi-Urrutia et al., 2021), *A. talamancae* (Cope, 1875): 5–15 notes/trill (Lechelt et al., 2014), and *A. tinae*: 5–13 notes/trill (Melo-Sampaio et al., 2018) (more notes: 28–46 notes/trill). The emission rate of notes is slower in *A. amissibilis*: 2.4–3.5 notes/s, *A. brunneus*: 0.5–2.3 notes/s, *A. caldwellaie*: 5.8–11.6 notes/s, *A. carajas*: 2.7–3.1 notes/s, *A. crombiei*: 13.0–13.1 notes/s (Lima et al., 2012), *A. flaviventris*: 11.1–13.04 notes/s, *A. goianus*: 3.1–3.9 notes/s (De Carvalho et al., 2016), *A. granti*: 3.8–7.1 notes/s, *A. ignotus* Anganoy-Criollo, 2012: 2.3–4.5 notes/s (Granda-Rodríguez et al., 2018), *A. juami*: 13.0–14.0 notes/s, *A. juanii*: 3–4 notes/s, *A. marchesianus*: 5.4–6.2 notes/s, *A. nunciatus*: 2.7–6.5 notes/s, *A. paleovarzensis*: 4.2–6.6 notes/s, *A. sieggreenae*: 0.92–3.3 notes/s, *A. sumtuosus*: 6.0–7.7 notes/s, *A. talamancae*: 2.1–3.1 notes/s, *A. tinae*: 3.7–5.9 notes/s (high note emission: 15.25–16.84 notes/s). *A. grillisimilis*, *A. grillicantus*, *A. paleci* (Silva et al., 2022), and *A. velocicantus* emit notes at high rates: 25.0–49.2, 22.1–26.3, 16.3–19.1, and 34.7–47.6 notes/s, respectively (low note emission: 15.25–16.84 notes/s). Note duration is longer in the advertisement call of *A. talamancae*, 0.072–0.083 s (short note duration: 0.026–0.052 s). *Allobates flaviventris*, *A. nunciatus* and *A. talamancae* emit calls at lower peak frequencies: 3.62–4.65, 3.84–4.31, 4.06–4.53 kHz, respectively (high peak frequency: 4.78–6.08 kHz).

GEOGRAPHIC DISTRIBUTION: We confirm the presence of *Allobates insperatus* in four localities in northwestern Amazonia, three within Ecuador and one in Colombia (fig. 3). These localities are on terra-firme forests (222–346 m) in the headwater regions of the Napo and Putumayo rivers. Considering the availability of similar continuous habitat, we expect that *A. insperatus* is present in other localities of the Napo-Putumayo and Putumayo-Caquetá interfluvies to the east.

Allobates juami Simões et al., 2018

Juami's nurse frog

Rana nodriza de Juami

Figures 10, 11

COMMENTS: The original phenotypic description is accurate, and reexamination of the specimens and associated data did not reveal relevant unknown variation. The analyses of DNA sequences of additional specimens from other localities revealed a complex hierarchical and populational structure, with some specimens from Ecuador and Peru carrying highly distinct mitochondrial haplotypes from those of the type locality (fig. 2). We labelled this matrilineal lineage as *Allobates* aff. *juami* and present potential explanations to this genetic variation in the Discussion. We recognized an *Allobates juami* sensu stricto that includes all samples with haplotypes more closely related to those of the topotypes than to any of the other sampled individuals (fig. 2A, C).

OSTEOLOGY: Because there are no published complete osteological descriptions of any *Allobates* species closely related to the ones addressed in our study, we included a description of the mineralized skeleton of the adult female paratype MCP 13288 (figs. 12, 13). This allowed us to explore interspecific variation of osteological characters in the genus and discuss the potential use of this character system in the systematics of *Allobates*.

Cranium (fig. 13A–D). Skull well ossified; almost as wide as long ($w/l = 0.9$). Paired septomaxillary bones very small, partially mineralized (only some portions of their arcs visible in ventral view). Nasals small, rounded in dorsal view, not articulated with each other medially and only partially covering the lateroposterior roof of snout; articulated medially with (or barely separated from) anterolateral margins of the sphenethmoid; nasals narrow in lateral view, with a strongly keeled maxillary process posteroventrally oriented, posteriorly extending past the level of planum antorbitale, and ventrally articulated with preorbital process of maxilla. Sphen-

ethmoid wider than long in dorsal view; anterodorsal border trilobed; posteriorly investing the anterior borders of frontoparietals and extending posterolaterally to the posterior third of these roofing bones; border of optic foramen not completely ossified (dorsal margin cartilaginous); lacrimal foramen visible in lateral view, located at the superior portion of the angle of sphenethmoid with planum antorbitale; the anterolateral portion that forms the anterior wall of orbit (planum antorbitale) projecting laterally toward nasal of each side but without reaching them. Posteroventral surface of sphenethmoid articulating with anterodorsal surface of the cultriform process of parasphenoid. Frontoparietals paired, large, flat, smooth, slightly narrower anteriorly; articulated medially with each other along their entire length, without fusing; frontoparietals posteriorly fused with otoccipitals; orbital margins of frontoparietals straight in dorsal view. Prootic completely fused with exoccipital in a single piece (otoccipital); epiotic eminences visible; crista parotica (= prootic) short, not articulated with otic ramus of squamosal; posterior wall of otic capsule oriented anterolaterally (in ventral view); exoccipitals sagittally fused to each other around foramen magnum; occipital condyles weakly protuberant, positioned lateroventrally at the edge of foramen magnum. Neopalatines and dentigerous processes of vomers (and thus vomerine teeth) absent. Parasphenoid large, T-shaped, parasphenoid-otoccipital articulation fused (margin of posterior process not evident in ventral view); tip of cultriform process deeply bifid; anterior third of cultriform process articulating dorsally with the ventral surface of sphenethmoid; alary processes fused to otoccipitals but discernible, tapering toward tips, weakly oriented posterolaterally. Premaxillae well-developed, medial articulation between them mediated by cartilage; with 7–8 nonpedicellate conical teeth; alary process of premaxillae narrower at its tips, tilted anterolaterally; tips of inner processes of pars palatina pointed or truncated, outer processes truncated and notably wider than inner ones. Maxillae with



FIGURE 10. Variation in male life coloration in dorsal and ventral views. **A.** *Allobates insperatus* [ZSFQ 6286, SVL = 15.48 mm], **B.** *A. juami* [MCP 13292, SVL = 17.80 mm] and **C.** *A. liniaureum*, sp. nov. [MCP 14296, SVL = 14.24 mm].

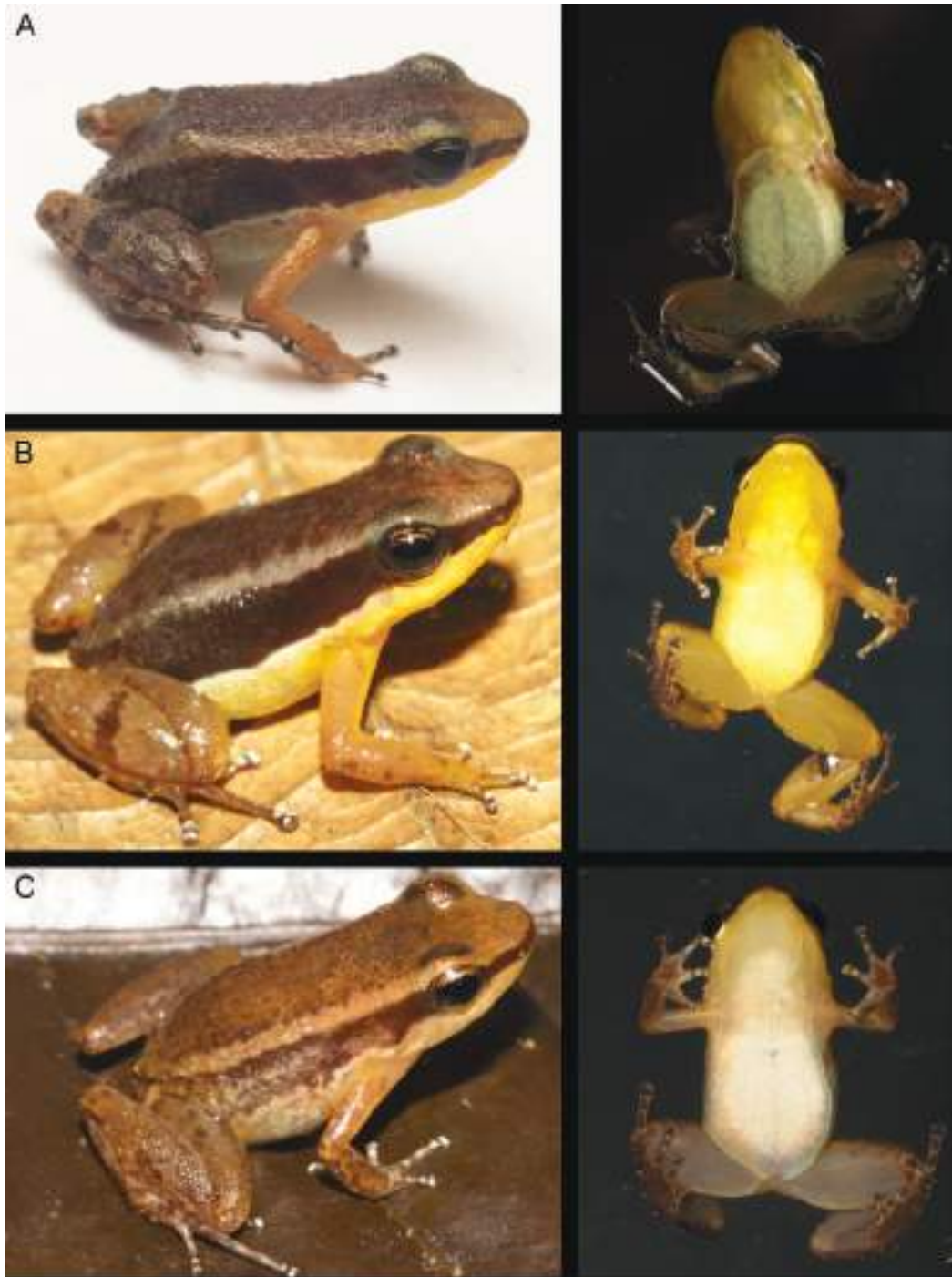


FIGURE 11. Variation in female life coloration in dorsal and ventral views. **A.** *Allobates insperatus* [ZSFQ 6282, SVL = 16.15 mm], **B.** *A. juami* [MCP 13291, SVL = 18.04 mm] and **C.** *A. liniaureum* [MCP 14333, SVL = 14.82].

31–33 nonpedicellate conical teeth each; articulating anteriorly with premaxilla, medially with the anterior ramus of pterygoid, and posteriorly with the anterior tip of quadratojugal; pars facialis of maxilla with a well-differentiated preorbital process; orbital border of pars facialis progressively lower toward back; postorbital process absent; pars palatina of maxilla slightly wider toward front, notched at its anterior margin. Quadratojugal fully ossified, acicular, narrower toward front, articulated with ventral surface of squamosal, barely overlapping with maxilla anteriorly. Pterygoid triradiate, Y-shaped; anterior ramus with a deep and continuous lateral sulcus; margo orbitalis of anterior ramus without dorsal lobes; medial ramus very short, robust, conical in ventral view, dorsal-posteriorly oriented but not reaching the crista parotica; posterior ramus of pterygoid almost twice the length of medial ramus. Squamosal triradiate, T-shaped; zygomatic ramus short, robust, truncated or pointed, anteroventrally oriented in lateral view, anterolaterally oriented in dorsal view; otic ramus fusiform, laterolaterally flattened toward tip, not articulated with crista parotica; ventral ramus directed posteroventrally, broader, flatter, and laterally convex toward tip. Mandible edentate; mentomeckelians short, cylindrical, narrowly separated medially by soft tissue, posterolaterally fused to anterior tip of dentary on each side; dentary thin, laterolaterally flattened, tapering toward back, reaching about the mid-point of angulosplenial; angulosplenial truncated at its anterior tip, not reaching the mentomeckelian, wider toward back, lateral surface deeply sulcate; coronoid process absent; retroarticular process long, posteriorly extending past mandibular articulation.

Axial skeleton (fig. 12). Vertebral column with eight procoelous presacral vertebrae, sacrum, and urostyle. Atlas with two elliptical concave cotyles (type I sensu Lynch, 1969), ventrolaterally located at the anterior border of the vertebra and weakly oriented anterolaterally. Neural crest of atlas present, strong; neural crest of vertebrae II–VIII weak; neural arc of verte-

brae II–III weakly projected posterior to the level of postzygapophyses, reaching the level of postzygapophyses in vertebra IV, and anterior to the level of postzygapophyses in vertebrae V–VIII. Articular surfaces of prezygapophyses and postzygapophyses of all vertebrae flat; posterior border of postzygapophyses of vertebrae II–V weakly convex to straight, convex between VI–VIII (in dorsal view). All vertebrae free (not fused). Ventral surface of vertebrae III–VII flat to weakly concave, convex in vertebrae I, II, and VIII. Transverse processes of vertebrae II not expanded distally; transverse processes of vertebrae II, VII, and VIII directed anterolaterally, III and VI laterally, and IV–V posterolaterally; relative length of transverse processes of vertebrae: III>IV>V=VI=VII>VIII>II. Centrum of sacrum with two small posterior condyles very close to each other; sacral diapophyses barely expanded to unexpanded distally, posterolaterally oriented in dorsal view, slightly upturned in lateral view; sesamoids present on lateral surface of sacral diapophyses. Urostyle long, slender, weakly expanded distally (1.5× wider than its middle width); anterodorsal process of urostyle uniformly wide from base to tip in anterior view; with an anterolateral foramen present at the base of the process; urostyle without anterolateral crest; dorsal crest of urostyle almost reaching the posterior end, decreasing posteriorly in height, with a short dorsal groove located between the first and second anterior thirds of urostyle; distal end of urostyle strongly elliptical in cross-section (3.3× wider than height).

Pectoral girdle (fig. 13E). Epicoracoids entirely fused, not overlapping (firmisternal pectoral girdle). Omosternum present, with a simple (not bifurcated) posterior terminus; mineralized portion of posterior terminus of omosternum pointed and cylindrical anteriorly, wider and flattened posteriorly. Clavicles acicular, subtly wider medially, directed laterad (perpendicular to sagittal plane); medial end of clavicles in contact with anteromedial portion of coracoids, lateral end mineralized or fused with posterior portion of pars acromialis of scapula. Coracoids

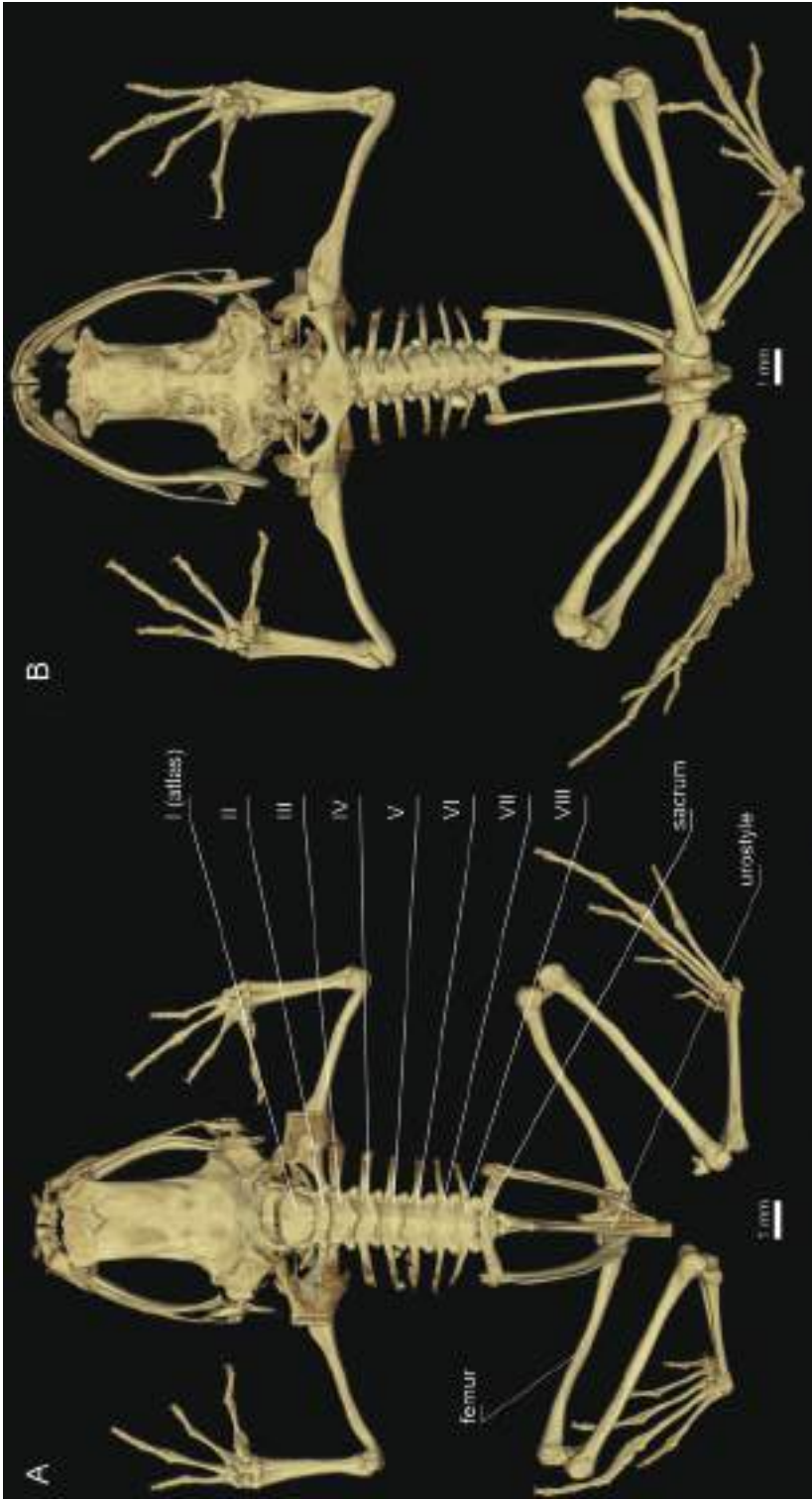


FIGURE 12. Computed microtomography reconstruction of the skeleton of a paratype (MCP 13288, field number SCF 2058, adult female) of *Allobates juami*, in dorsal **A**. and ventral **B**. views. Roman numerals indicate presacral vertebrae.

wider and dorsoventrally flattened medially, wide and cylindrical laterally, anterior surface of coracoids markedly concave in ventral view, anterior portion of lateral head of coracoid fused or mineralized in medial portion of pars glenoidalis of scapula. Acromion process fully mineralized, projecting slightly inward in ventral view. Cleithrum bifurcated, V-shaped, anterior ramus longer and narrower than posterior. Suprascapular anterior projection mineralized.

Pelvic girdle (figs. 12, 13F). Ilium straight, slightly divergent anteriorly, anterior end elliptical in cross section; ilial crest very high (>100% of ilial shaft height), anteriorly stepped and subtly tilted laterally; ilio-sacral articulation Type II B of Emerson (1982) with mineralized sesamoid element between ilio-sacral articulations; posteriorly, bears ilium protuberance, with a lateral transversal process; ilium posteriorly fused with ischium, forming the dorsal margin of acetabulum, and with acetabular expansion ventrally (= pubis). Ischium synostotically fused to one another forming posterodorsal interischial crest. Preacetabular expansion well developed.

Forelimbs (figs. 12, 13G). Humerus with expanded glenoid and distal heads, crista ventralis of humerus extending distally over almost the entire proximal third of the bone; proximal end of paraventral crest of humerus weakly raised (forming a small bulge). Radioulnar distally wider and flatter, deeply grooved distally on dorsal and ventral surfaces; ventral groove longer and deeper than dorsal. Prepollex formed by two mineralized elements (base + one segment). Postaxial surface of metacarpal IV smooth, slightly convex in dorsal view. Large mineralized sesamoid present on ventral surface of fingers I–IV metacarpal-proximal phalanx articulation; absent on ventral surface of fingers III–IV proximal-medial phalanx articulation. Phalangeal formula: 2-2-3-3; terminal phalanx T-shaped, with weakly expanded laterodistal processes.

Hind limbs (figs. 12, 13H). Femur sigmoid shaped; proximal-posterodorsal crest of femur

weak; proximal-ventral crest inconspicuous. Tibiofibula softly sulcate longitudinally on dorsal and ventral surfaces of its proximal and distal ends; nutrient foramina on dorsal and ventral surfaces of mid-portion of tibiofibula conspicuous. Tibiale-fibulare fusion involving only the proximal and distal ends of both bones; fused portions deeply grooved ventrally. Four tarsal elements: element Y, distal tarsal 1, distal tarsal 2–3, and prehallux. Mineralized sesamoids absent on ventral surface of metatarsal-proximal phalanx articulations and proximal-medial phalanx articulations of all toes. Phalangeal formula: 2-2-3-4-3. Terminal phalanx T-shaped, with weakly expanded laterodistal processes.

Allobates liniaureum, new species

Golden line nurse frog

Rana nodriza de línea dorada

Figures 14–23

HOLOTYPE: MCP 14332 (field code SCF 2255). An adult male collected by Giuseppe Gagliardi-Urrutia, Andrés Felipe Jaramillo, Lourdes Y. Echevarría, and Santiago Castroviejo-Fisher on February 12, 2019, in Carretera a Jeberillos, Puerto Nuevo Yurimaguas, municipality of Yurimaguas, province of Alto Amazonas, department of Loreto, Peru (5.85724° S, 76.16201° W, 202 m).

PARATOPOTYPES: Four specimens. One adult female, MCP 14333 (field code: SCF 2256), and three adult males, MCP 14294–96 (field codes: SCF 2216–18, respectively), collected on February 11 and 12, 2019. Same collectors as the holotype.

ETYMOLOGY: The specific name is a noun in apposition, derived from the combination of the Latin words *linum*, a neuter noun meaning “thread or string,” and *aureum*, the neuter form of the Latin adjective meaning “golden” (the connective vowel between the two roots is the traditional -i-). The specific epithet refers to the

in males); (2) dorsal skin of body, forelimbs, thigh, shank, and groin smooth; ventral skin smooth; (3) snout subacuminate in dorsal and ventral views, rounded in lateral view; (4) nostrils visible in dorsal and ventral views; (5) tympanum small ($\sim 0.5 \times$ the eye diameter); (6) short teeth present on the maxillary arch, visible under magnification; (7) teeth on mandible arch absent; (8) dentigerous process of vomer absent; (9) lingual process absent; (10) vocal sac in males single; (11) paired dorsal digital scutes present; (12) metacarpal ridge absent; (13) thenar tubercle conspicuous; (14) nuptial excrescences on thumb absent; (15) webbing absent on fingers; (16) finger III swollen on preaxial side in males; (17) finger II swollen on preaxial side in males; (18) tip of finger IV not reaching the distal end of distal subarticular tubercle of finger III; (19) finger II shorter than finger I; (20) lateral keels on preaxial and postaxial sides on fingers absent; (21) discs on fingers I–IV moderately expanded (width of discs on fingers I–IV/width of distal phalanges on fingers I–IV: mean = 1.7 ± 0.2 , range = 1.3 – 2.2); (22) black gland absent on arm; (23) tarsal keel present, tubercle-like, strongly curved; (24) tarsal fold absent; (25) metatarsal fold weak and poorly defined; (26) basal webbing present between toes III and IV, absent between other toes; (27) lateral keels on both sides in all toes; (28) length of toe III surpassing the proximal margin of central subarticular tubercle of toe IV; (29) discs of toes I and II moderately expanded (1.2 – $1.4 \times$ and 1.1 – $1.6 \times$ the width of their adjacent phalanges, respectively), discs of toes III, IV, and V moderately to greatly expanded (1.7 – $2.3 \times$, 1.8 – $1.9 \times$, and 1.0 – $1.7 \times$ the width of their adjacent phalanges, respectively); (30) background color of dorsum yellowish brown or cream with scattered dark brown spots; (31) pale tan brown dorsolateral stripe present, straight, well defined, with golden iridescent tones in life; (32) background color of dorsal surface of arms golden yellow in life, cream in preserved specimens; (33) dorsal surface of

thigh tan brown or cream in live and preserved specimens, with dark brown irregular spots; (34) dark brown lateral stripe surrounding the whole body, reaching leg-body insertion; (35) pale oblique lateral stripe present, extending from groin to near posterior portion of the forearms; (36) pale ventrolateral stripe present in live and preserved specimens, irregular, extending from posterior portion of tympanum to groin, iridescent white in life; (37) pale paracloacal mark present, round or comma shaped; (38) color of ventral surfaces yellowish anteriorly and white to translucent posteriorly in life with sexual dimorphism, the light cream with iridescent pigmentation extends from belly to chest in males or throat in females; (39) iris golden, with dark brown reticulation; (40) diurnal habits, males vocalizing in daytime; (41) advertisement calls characterized by irregular emission of single notes, with a peak frequency of 5.53 – 6.82 kHz.

DESCRIPTION OF THE HOLOTYPE: Adult male (figs. 14–16), SVL = 14.4 mm, well preserved, with a longitudinal incision on the ventral surface of left thigh (fig. 16B). Measurements of the holotype are provided in table 2. Body robust, head slightly longer than wide (HL/HW = 1.1), head length $0.4 \times$ the SVL. Snout subacuminate in dorsal and ventral views, rounded in lateral view. Nostrils located posterolaterally to snout, visible in dorsal, lateral, anterior, and ventral views. Distance between nostrils $0.4 \times$ the head width. *Canthus rostralis* rounded in cross section, slightly convex or straight in dorsal view. Distance from nostril to anterior corner of eye $0.7 \times$ shorter than eye diameter. Loreal region straight and oblique. Eyelid smooth, lacking tubercles, dorsal width $0.6 \times$ eye diameter. Eye prominent, diameter $0.1 \times$ the SVL. Supratympanic fold absent. Tympanum round, small, $0.5 \times$ eye diameter. Tympanic annulus visible under magnification. Maxillary teeth conspicuous under magnification. Dentigerous processes of vomers absent. Choanae round, small, positioned anteriorly to eye bulge. Teeth on mandible arch absent. Tongue longer than wide, with ante-



FIGURE 14. Ventral view of hand of type specimens of *Allobates liniaureum*. **A.** Right hand of the holotype (MCP 14332, adult male), showing the preaxially swollen fingers II and III. **B.** Right hand of a paratopotype (MCP 14333, adult female), without swollen fingers.

rior third attached to the mouth floor; median lingual process absent. Lateral vocal slits conspicuous. Vocal sac single, extending from middle level of throat to chest.

Forelimb slender, ulnar tubercles absent on external surface of forearm. Black arm gland absent. Hand length 28.6% of SVL. Palmar tubercle conspicuous, round. Thenar tubercle elliptical, maximum diameter 66.7% of maximum diameter of palmar tubercle. Proximal subarticular tubercle of finger IV round, small, not exceeding the width of phalanx. Distal subarticular tubercle on finger IV absent (fig. 4). Finger III with a round, protuberant proximal subarticular tubercle and a small, round, distal subarticular tubercle. Subarticular tubercles on fingers I and II very protuberant, elliptical. Supernumerary tubercles absent. Metacarpal ridge absent. Webbing absent between fingers. Nuptial excrescences on thumb (= finger 1) absent. Length of finger II 96.7% of finger I length. Tip of finger IV not reaching the distal end of distal subarticular

tubercle of finger III when fingers are juxtaposed. Relative lengths of fingers adpressed: $II < IV < I < III$. Keels or fringes absent in all fingers (fig. 14A). Fingers II and III weakly swollen preaxially, expanding from the base of disc to the base of proximal subarticular tubercle (fig. 14A). Discs of fingers moderately expanded, width of discs on fingers I–IV corresponding to 1.4×, 1.8×, 2.2×, and 2.0× the width of their adjacent phalanges, respectively. Tip of discs rounded. Paired dorsal digital scutes conspicuous and protuberant in all fingers.

Hind limbs robust. Lengths of thigh, shank, and foot similar ($TL/SVL = 50.2\%$, $SAL/SVL = 50.8\%$; $FL/SLV = 46.7\%$). Tarsal keel tubercle-like, short, strongly curved at its proximal end. Inner metatarsal tubercle well defined, large, elliptical. Outer metatarsal tubercle shorter, round (fig. 15A). Metatarsal fold weak and poorly defined. Basal webbing present between toes III and IV, absent between other toes. Toes slender, with poorly defined keels on both sides



FIGURE 15. Ventral view of the right foot of preserved types of *Allobates liniaureum*. **A.** Holotype adult male (MCP 14332). **B.** Paratopotype adult female (MCP 14333).

of all toes, but in toes III and IV extending from distal end of basal webbing to 1/4 the length between distal or proximal and central subarticular tubercles respectively (fig. 15A). Subarticular tubercles round, smaller than inner metatarsal tubercle in maximum diameter, the number varies between toes: I = 1, II = 1, III = 2, IV = 3, V = 2. Plantar supernumerary tubercles absent. Relative lengths of toes: $I < II < V < III < IV$ (fig. 15A).

Length of toe III surpassing the proximal margin of central subarticular tubercle of toe IV. Discs of toes I and II moderately expanded, width of toe discs $1.3\times$ and $1.7\times$ the width of their adjacent phalanges, respectively. Discs of toes III, IV, and V moderately to greatly expanded, width

of discs, $1.7\times$, $1.7\times$ and $1.8\times$ the width of their adjacent phalanges, respectively. Paired dorsal digital scutes conspicuous and slightly protuberant in all toes.

Skin smooth on head and upper eyelid, dorsum with small, scattered tubercles. Dorsal surfaces of forelimbs smooth. Dorsal surfaces of thigh smooth, shank with scattered tubercles in life. Groin smooth. Ventral skin smooth on throat, chest, belly, and thighs.

COLOR OF HOLOTYPE IN ALCOHOL: The description is based on the observation of the specimen after three years of preservation. Background color of dorsal surface of body uniformly brown, with sparse dark brown mottling more densely concentrated along the inner margin of

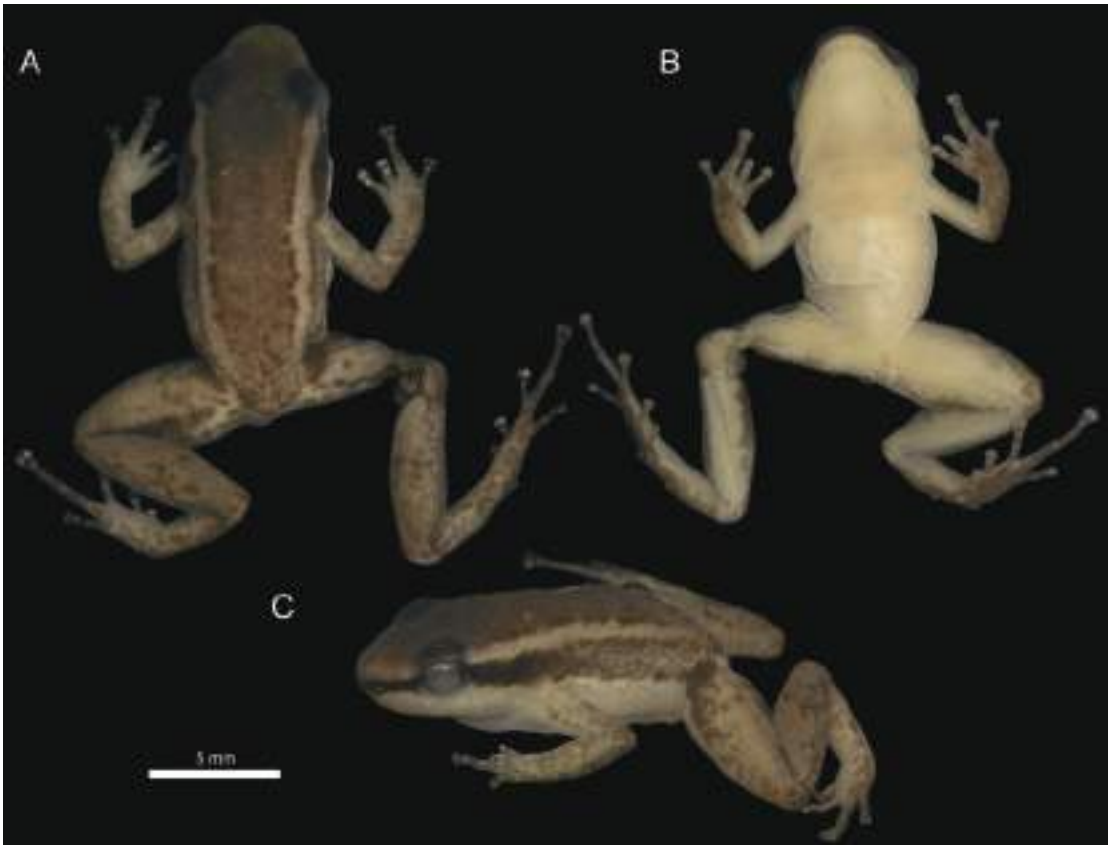


FIGURE 16. Preserved holotype of *Allobates liniaureum* (MCP 14332, adult male, SVL = 14.35 mm) in **A.** dorsal, **B.** ventral and **C.** anterolateral views.

pale dorsolateral stripe (fig. 16A). Color of dorsum darker over eyelids. Pale dorsolateral stripe present, well-defined, solid, straight, extending from tip of snout to the urostyle region, not merging with its counterpart over urostyle (fig. 16A). Lateral surface of body with a solid dark brown stripe, extending from tip of snout to groin becoming diffuse from midbody to groin (fig. 16C). Pale oblique lateral stripe present on dark brown stripe at inguinal region, extending from groin to midbody becoming diffused before forelimbs. Ventrolateral stripe inconspicuous, noticed only by mottled iridescent white pigmentation present along the ventral edge of dark brown lateral stripe (fig. 16C). Scattered dark brown pigmentation present ventrally to white ventrolateral stripe, from upper lips to midbody,

without reaching the ventral surface (fig. 16B). Background color of ventral surfaces light cream, with iridescent pigmentation on belly (fig. 16B).

Dorsal surface of forelimbs cream to translucent, with dark brown mottling on elbow, forearm, and wrist, denser on distal portion of forearm (fig. 16A). Fingers III and IV more darkly pigmented than fingers I and II. Paired scutes conspicuous on discs of all fingers, iridescent white on fingers I, II, and III (fig. 16A).

Dorsal surface of thigh light brown. Pale paracloacal mark conspicuous, comma shaped, broader at its proximal end, bordered dark brown (fig. 16A). Anterior (= inner or medial) edge of thigh dark brown adjacent to groin, diffuse near to knee; dorsally, the thigh bears scattered irregular dark brown spots without forming

a transverse bar; posterior edge of thigh dark brown with mottled light brown, especially the area covered by shanks. Dorsal surface of shank same color as thigh, with irregular dark brown spots not forming a transverse bar. Dorsal surface of tarsal region same color as thigh and shank, with dark brown irregular spots. Dorsal surface of toes dark brown, densely pigmented on toes IV and V. Paired scutes white on toes I, II, III, and IV, black on toe V. Ventral surfaces of thigh and shank cream, with dense dark brown mottling on marginal edges. Ventral surface of tarsal region cream, with scattered dark brown pigments. Toes dark brown, densely pigmented in ventral view (fig. 16B).

TYPE SERIES VARIATION: Variation in morphometric measurements of adult male and female paratypes are presented in table 2. Body size of the only female paratype is larger than that of all male paratypes but remaining morphometric measurements generally overlap. The two paratypes have swollen finger II and III, strongly swollen in MCP 14294 and weakly swollen in MCP 14296. Background brown color of dorsum variable in lightness (fig. 17). Paler oblique lateral stripe varies in length, in some individuals it extends to the midbody (fig. 18B) or near to forelimbs (fig. 18D). Dark brown irregular spots below ventrolateral stripe varies from absent (fig. 18A) to present (fig. 18D). A transverse brown bar or diffuse brown blotch may be present on dorsal surface of shank (fig. 17A, B). Paracloacal mark not comma shaped in some specimens, with a diffuse distal edge (fig. 17A). Color of paired scutes on fingers and toes variable, black, gray, or iridescent white. In male specimens, iridophores are present only on belly (fig. 19A, B). In female, iridophores are present on belly, chest, and throat (fig. 19C, D).

COLOR IN LIFE: Dorsum yellowish brown or cream with scattered dark brown spots (figs. 20, 21). In some individuals, dark brown spots form a thin stripe on the lateral edge of dorsal surface. Golden, iridescent background color present among dark brown pigments over the eyelids. Background color of iris golden, with

dark brown reticulation. Pale tan brown dorso-lateral stripe present, broad, extending from tip of snout to the urostyle region, with golden iridescent colors only on its posterior portion (fig. 20A) or along its whole length (fig. 20B). Lateral dark brown stripe extending from the tip of snout to groin. A pale oblique lateral stripe, extended from de groin to near the arm insertion, with some golden iridescent (fig. 21D). Thin irregular iridescent white ventrolateral stripe present from snout to groin, interrupted in the posterior portion of tympanum. Small iridescent spots and blotches, same color as that of ventrolateral stripe, present from ventral edge of ventrolateral stripe to marginal surface of belly, on yellowish background.

Lower lip yellow. Throat and chest translucent yellow. Saturation of yellow iridescent white pigments and on throat sexually dimorphic, uniformly solid bright yellow in males, paler yellow and restricted to the marginal surfaces of throat in female. Also, female with iridescent-white patches on belly, throat and chest (fig. 21B), or only in belly in males.

Dorsal and dorsolateral surfaces of upper arm and forearm golden yellow, with irregular dark brown (figs. 20A, 21A) or iridescent (fig. 20C) spots. Ventral surface of upper arm translucent. Forearm peppered with sparse light brown pigments. Fingers brown with a varying number of transverse white bars on dorsal surface, peppered grayish brown on ventral surface. Paired scutes on finger discs iridescent white.

Paracloacal mark iridescent gold or cream, diffuse in some individuals (fig. 21C). Paracloacal mark always surrounded by a dark brown frame, continuous in the posterior portion of thigh. White tubercles present ventrally around cloaca (fig. 21C). Background color of dorsal surface of thigh uniformly light tan brown. Dark brown irregular spots may be present on the middle of the dorsal surface of thigh. Dark brown blotches on anterior and posterior portion of thigh sometimes extending laterally and forming a longitudinal dark brown stripe or patch. Dorsal surface of shank same color as

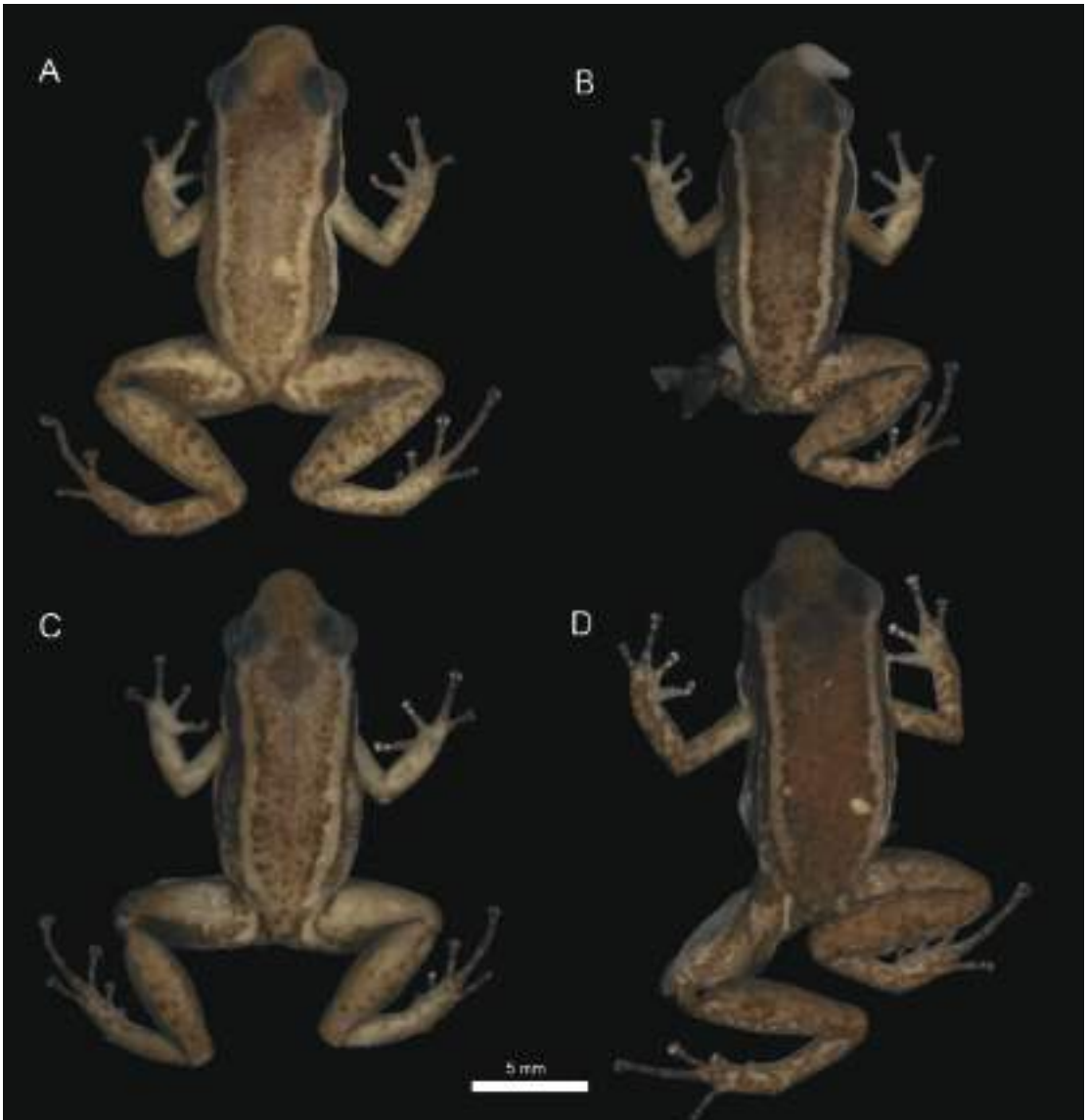


FIGURE 17. Dorsal coloration pattern variation of *Allobates liniaureum*, paratopotypes A. adult male MCP 14294 [SVL = 14.82 mm], B. adult male MCP 14296 [SVL = 14.24 mm], C. adult male MCP 14295 [SVL = 14.81 mm], D. adult female MCP 14333 [SVL = 14.97 mm].

thigh, sometimes with a diffuse median transverse dark brown bar. Smaller dark brown dots or blotches may be present on dorsal surface of shank. Ventral surface of thigh and shank pale gray to translucent, with scattered brown melanophores present marginally, some iridescent white spots present on the proximal ventrolat-

eral surface of thigh. Tarsal region tan brown, with an irregular dark brown blotch or transverse stripe present dorsal medially. When the median transverse stripe is well defined on both shank and tarsal regions, they align when posterior limb is in resting position. Ventral surfaces of tarsal region pale gray to translucent, with

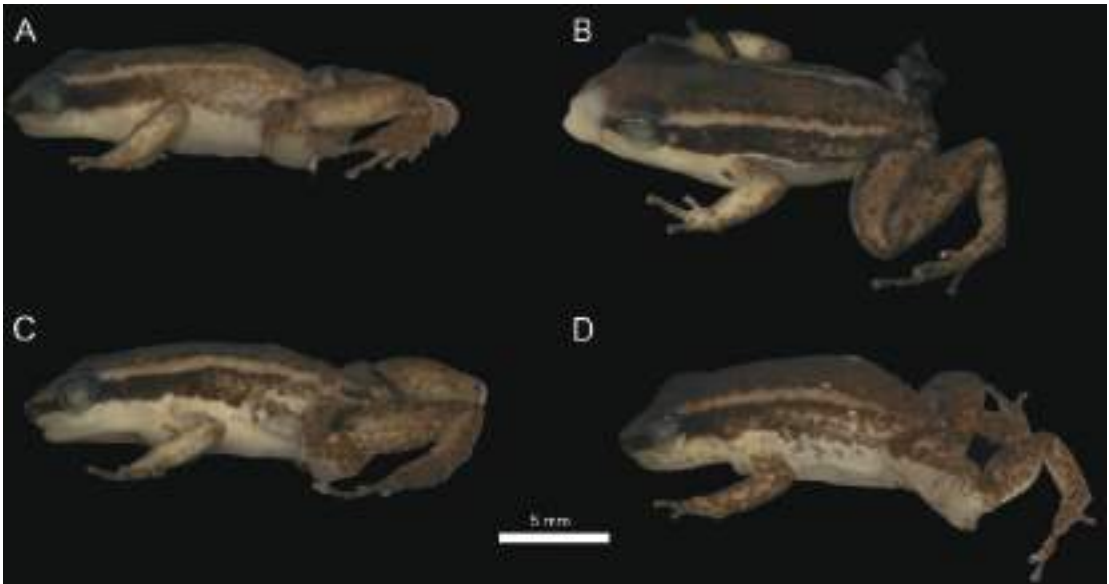


FIGURE 18. Lateral color pattern variation of *Allobates liniaureum* paratopotypes A. adult male MCP 14294 [SVL = 14.82 mm], B. adult male MCP 14296 [SVL = 14.24 mm], C. adult male MCP 14295 [SVL = 14.81 mm], D. adult female MCP 14333 [SVL = 14.97 mm].

scattered brown melanophores present marginally. Plantar surface of foot densely covered with brown melanophores. Toes brown with a varying number of transverse white bars on dorsal surface. Toes uniformly brown on ventral surface. Paired scutes on toe discs iridescent white, black only on toe V.

CALL DESCRIPTION: All bioacoustic measurements are in table 3. Advertisement calls of *Allobates liniaureum* were characterized by continuous emission of short single notes (fig. 9C). Notes are spaced by irregular silent intervals, with an average duration of 2.50 ± 2.54 s (range 0.36–12.98 s). The average rate of note emission was 0.60 ± 0.33 notes/s (range 0.20–1.00 notes/s). The average note duration was 0.02 ± 0.004 s (range 0.01–0.03). Notes have an ascending frequency modulation. Average peak frequency is 6.228 ± 0.245 kHz (range 5.534–6.820 kHz). Average lower and upper frequencies of notes were 5.876 ± 0.231 kHz (4.986–6.256 kHz) and 6.522 ± 0.276 kHz (range 5.740–7.134 kHz) respectively. The peak frequency corresponds to the second harmonic.

PHYLOGENETIC RELATIONSHIPS AND GENETIC DISTANCES: All optimal trees, regardless of dataset, unambiguously found that *Allobates liniaureum* is the sister group to a clade formed by *A. insperatus* and *A. juami*. Intraspecific uncorrected-pairwise genetic distances based on a fragment of the 16S rRNA mitochondrial marker within *A. liniaureum* vary between 0%–0.2% (appendix 7). Genetic distances between *A. liniaureum* and *A. insperatus*, *A. juami*, and *A. aff. juami* vary between 5.5–8.1%, 6%–8.9%, and 6%–8.2%, respectively.

OSTEOLOGY: We describe osteological characters from the adult male paratype MCP 14295, the skeleton is illustrated in figures 22 and 23.

Cranium (fig. 23A–C). Skull well ossified, longer than wide ($w/l = 0.8$). Septomaxilla small, U-shaped, flattened at the base. Nasals separated, rounded anteromedially, articulated posteromedially with the sphenomoid; laterally, narrow, keel shaped, extending to the posterior portion of the pars facialis of the maxilla without articulating with it; each nasal laterally bears a small foramen. Sphenomoid dorsally cup

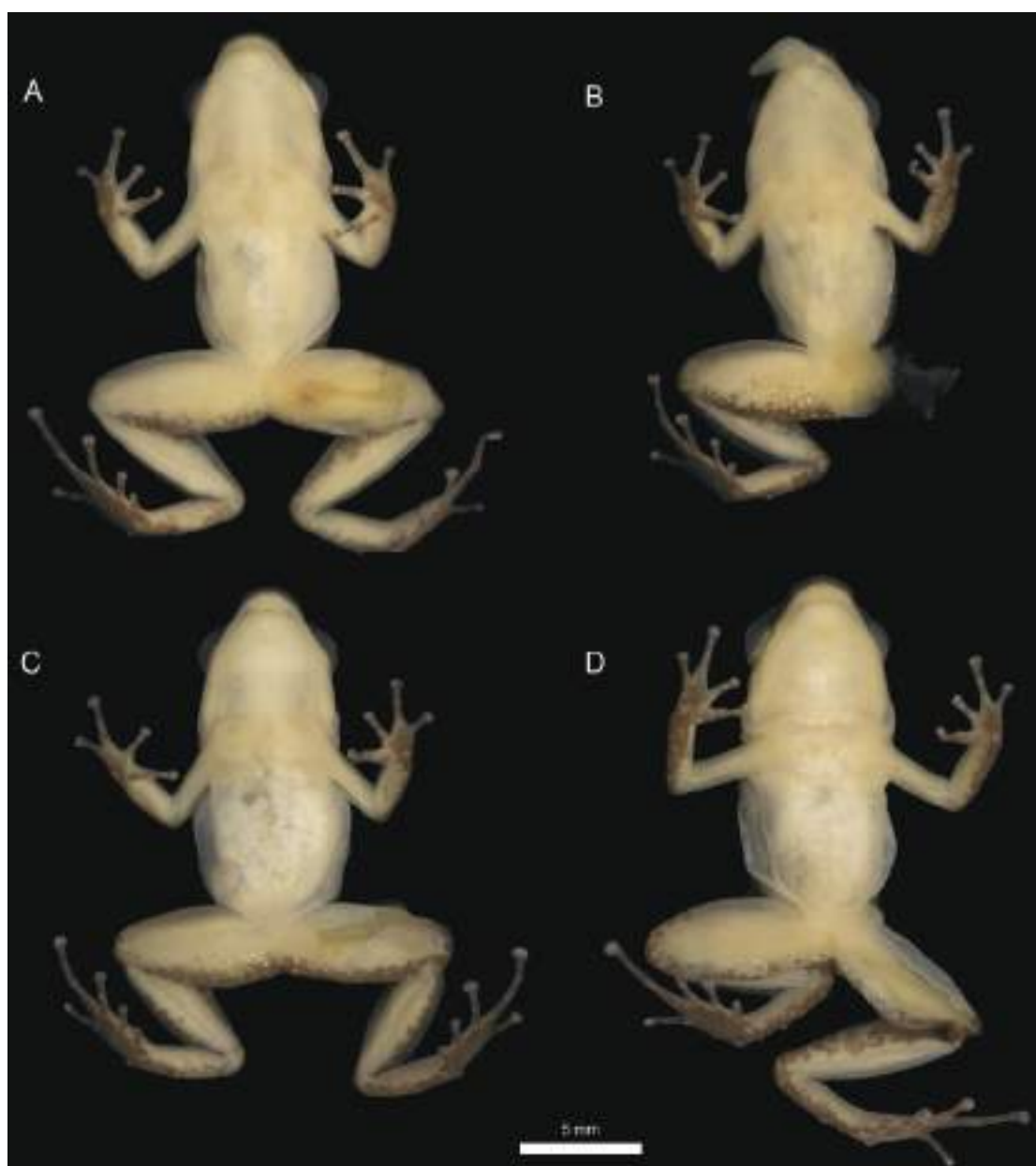


FIGURE 19. Ventral color pattern variation of *Allobates liniaureum* paratopotypes **A.** adult male MCP 14294 [SVL = 14.82 mm], **B.** adult male MCP 14296 [SVL = 14.24 mm], **C.** adult male MCP 14295 [SVL = 14.81 mm], **D.** adult female MCP 14333 [SVL = 14.97 mm].

shaped, articulated posteriorly with the anterior margins of frontoparietals; extends posterolaterally along 1/3 of frontoparietals, with lacrimal foramen located on the dorsolateral anterior edge and oriented posteriorly; anterolateral processes of sphenethmoid extending laterally toward the maxillary process of nasals without reaching them; ventrally rounded, articulating posteroventrally with the dorsal surface of the cultriform process of parasphenoid. Frontoparietals large, flat, smooth, articulated to each other medially, fused along their posterior half; fused posteriorly with the otoccipital; orbital edges of frontoparietals straight in dorsal and lateral view; lamina perpendicularis (= tectum supraorbitale) poorly developed or absent. Prootic portion of otoccipital completely fused; epiotic eminence of prootic area well developed; crista parotica posterolaterally not articulated with the otic ramus of squamosal; exoccipital portion of otoccipital fused, surrounding the foramen magnum; occipital condyles widely separated. Dentigerous process of vomers and neopalatines absent. Parasphenoid T-shaped, extending from the posterior portion of otoccipital, to one third of posterior portion of sphenethmoid, with which it is fused; cultriform process bifid; alary processes fused to the otoccipital, borders not differentiated. Premaxilla well developed and not articulated laterally with maxilla; alary process of premaxilla tilted anterolaterally, projected laterodorsally forming a V-shape, also extends along the inner process of pars palatina; pars dentalis of premaxilla bears 5–6 recurved teeth; pars palatina of premaxilla extends posteriorly and bears two (inner and outer) processes that do not articulate in the middle forming a U-shaped cavity. The maxilla (fig. 23D), with 23–24 recurved teeth on each side, articulates anteriorly with premaxilla, medially with the anterior ramus of pterygoid, and posteriorly with anterior tip of quadratojugal; pars facialis of the maxilla triangular; pars palatina of maxilla truncated in anterior portion. Pterygoid Y-shaped, with anterior ramus of pterygoid forming a single rod; bonny middle ramus shorter and not reach the base of crista parotica;

posterior ramus invests the quadratojugal and ventral ramus of squamosal without reaching or surpassing the pars articularis of mandible. Squamosal T-shaped, its anterior (= zygomatic) ramus short, projected anteroventrally, and ending in a tip; posterior (= otic) ramus flattened, its length three times that of anterior ramus, and not articulate with prootic; ventral ramus of squamosal longest and projected posteroventrally, the tip expanded and curved. Mandible without teeth, curved in the prearticular region. Mentomeckelian cylindrical, not articulated medially to each other, in frontal view placed below and fused with the anterior portion of dentary, given impression of concave dorsal surface; short ramus projected dorsally. Dentary thin, taller anteriorly and flat, pointed posteriorly extending to middle of angulosplenial. Angulosplenial truncated in anterior tip, does not articulate with mentomekelian, middle and posterior portions of the outer surface sulcated; coronoid process absent; posteriorly, articulated region becomes taller, flattened, and rounded tip.

Axial skeleton (fig. 22). Vertebral column consists of eight procoelous presacral vertebrae, a sacrum, and the urostyle. Atlas with cuplike cotyles displaced laterally and widely separated (Type I sensu Lynch, 1969), prezygapophyses with rectangular shape and moderately differentiated; neural crest poorly developed and decrease in height progressively from anterior to posterior vertebrae. The prezygapophyses of presacral vertebrae II placed above the postzygapophyses of cervical vertebra, for other vertebrae the prezygapophysis placed below the postzygapophysis of the previous vertebra. The relative length of transverse processes of presacrals is: IV=VII>V>VI=VIII>III>II, cervical lacks transverse processes. The processes of presacral II and VIII directed anteriorly, presacral III–VI directed nearly posteriorly, and presacral VII directed laterally. The sacrum bears two well-developed diapophyses, slightly flattened dorsoventrally along their length and directed posteriorly; anterior and posterior borders of centrum of sacrum straight in dorsal view,



FIGURE 20. Dorsal and ventral views of live *Allobates liniaureum* adult males **A.** paratopotype MCP 14294 [SVL = 14.82 mm], **B.** paratopotype MCP 14296 [SVL = 14.24 mm], **C.** holotype MCP 14332 [SVL = 14.35 mm].



FIGURE 21. Dorsal and ventral views of live *Allobates liniaureum*. **A.** paratopotype MCP 14295 [SVL = 14.81 mm] adult male, **B.** paratopotype MCP 14333 [SVL = 14.97 mm] adult female. **C.** Lateral and posterior views of live *A. liniaureum* adult male MCP 14296 [SVL = 14.24 mm].

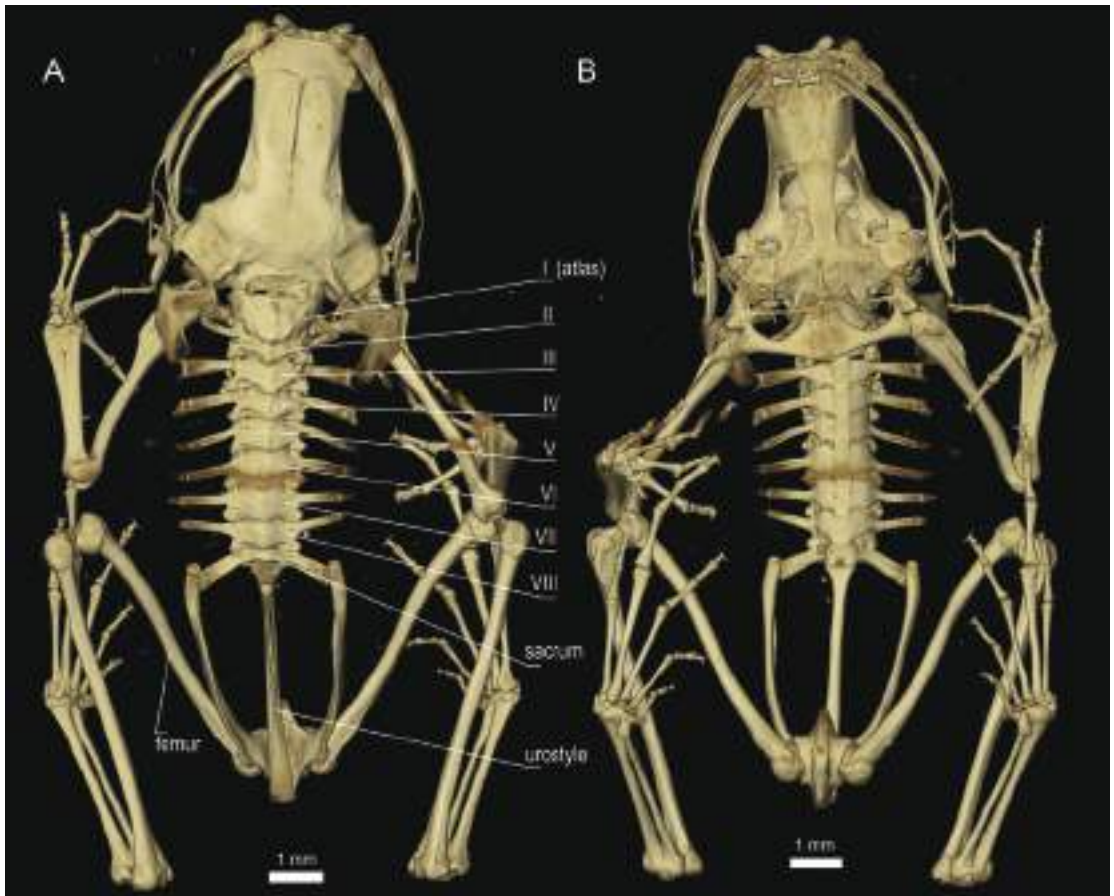


FIGURE 22. Computed microtomography reconstruction of the skeleton of a paratopotype (MCP 14295, adult male) of *Allobates liniaureum* in dorsal **A.** and ventral **B.** views. Roman numerals indicate presacral vertebrae.

bearing two separated condyles in ventral view. Urostyle long, slender, equal or slightly longer than the length of presacral portion of vertebral column, weakly expanded distally ($1.6\times$ wider than its middle width); dorsal crest starts after an anterior knob, crest taller anteriorly and gradually decreases in height posteriorly, with a small groove in the middle; distal end of urostyle strongly elliptical in cross section ($3.4\times$ wider than height).

Pectoral girdle (figs. 22, 23E). Epicoracoids entirely fused, not overlapping (firmisternal pectoral girdle). Omosternum present, with a simple (not bifurcated) posterior terminus; mineralized portion of posterior terminus of

omosternum pointed and cylindrical anteriorly, wider and flattened posteriorly. Clavicle straight and slightly directed anteriorly; lateral edge of clavicle mineralized or fused in posterior portion of pars acromialis of scapula, and medial edge articulates with anterior portion of coracoids. Coracoids cylindrical at its mid-portion, medial head wider than lateral head, anterior portion of lateral head of coracoid fused or mineralized with pars glenoidalis of scapula. Scapula slightly larger than adjacent coracoid; pars acromialis of scapula expanded anteriorly, and dorsal edge of scapula expanded. Cleithrum bifurcated, V-shaped, both ramus with same length and partially ossified in outer surface.

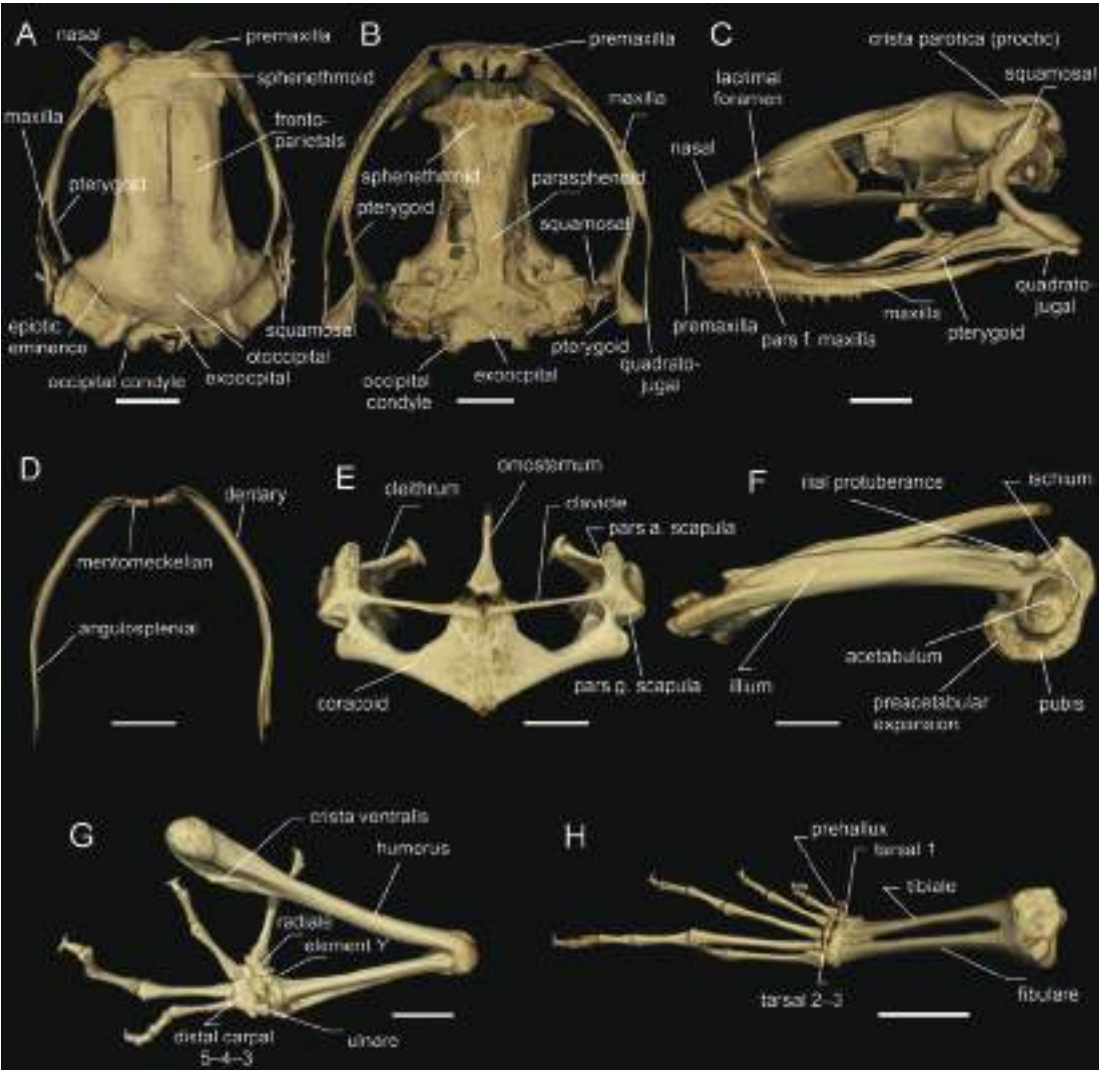


FIGURE 23. Segmentation of some osteological elements of *Allobates liniaureum* (MCP 14295, adult male). **A.** Dorsal, **B.** ventral, and **C.** lateral views of skull. **D.** Dorsal view of the maxillary arch. **E.** Ventral view of pectoral girdle. **F.** Lateral view of pelvic girdle, including the sacrum and urostyle bones of the axial skeleton. **G.** Dorsal view of the left forelimb. **H.** Dorsal view of the left hind limb. Scale bar = 1 mm. Abbreviations: **pars f. maxilla** = pars facialis of maxilla, **pars a. scapula** = pars acromialis of scapula, **pars g. scapula** = pars glenoidalis of scapula.

Pelvic girdle (figs. 22, 23F). Ilium straight and cylindrical, with well-developed ilial crest (>100% of ilial shaft height) along posterior 3/4 of its length; ilio-sacral articulation Type II B of Emerson (1982) with mineralized sesamoid element between ilio-sacral articulations; posteriorly, bears ilial protuberance, with a lat-

eral transversal process; ilium posteriorly fused with ischium forming the dorsal margin of acetabulum, with an acetabular expansion ventrally (= pubis). Ischium synostotically fused to one another forming posterodorsal interischadic crest. Preacetabular expansion well developed.

Forelimbs (fig. 23G). Humerus with expanded glenoid and distal heads, short and well-developed crista ventralis next to glenoidal head. Radioulnar flattened showing a short groove in distal portion. Five carpal elements: radiale, ulnare, element Y + distal carpal 2, distal carpal 5-4-3, and prepollex. Prepollex formed by two mineralized elements (base + one segment). Postaxial surface of metacarpal IV smooth, slightly convex in dorsal view. Large mineralized sesamoid present on articulation of radioulna with radiales in the dorsal view and ventral surface of FI–FIV metacarpal-proximal phalanx articulation; absent on ventral surface of FIII–FIV proximal-median phalanx articulation. Phalangeal formula: 2-2-3-3. Terminal phalanges T-shaped, with transverse processes curved and directed ventrolaterally.

Hind limbs (fig. 23H). Femur sigmoid shaped, with low crest in inner portion. Tibio-fibulae has deep sulci next to its heads, nutrient foramina small in the middle of the bone, both on dorsal and ventral surfaces. Tibiale and fibulare fused only in heads, showing a middle separation; a rounded sesamoid element present near articulation of tibio-fibulae with tibiale/tibulare. Four tarsal elements: element Y, distal tarsal 1, distal tarsal 2-3, and prehallux. A rounded sesamoid element present in articulation between metatarsal and proximal phalanx of toes I, II, III, and IV, also between proximal and distal phalanx of toe IV. Phalangeal formula: 2-2-3-4-3. Terminal phalanges T-shaped, with transverse processes curved and directed ventrolaterally.

DIAGNOSIS: As with *Allobates insperatus*, we focus our comparisons on Amazonian species from Brazil, Colombia, Ecuador, and Peru, with particular emphasis on the most closely related species. Character states of *A. liniaureum* are in parenthesis throughout the diagnosis.

Morphological diagnosis. The most similar and closely related species are *Allobates insperatus* and *A. juami*; both have a shorter pale oblique lateral stripe, extending from the inguinal region but never reaching the lateral midbody (pale oblique lateral stripe longer, from groin to or

surpassing lateral midbody) (figs. 10, 11); shanks with well-defined transverse dark brown blotch (absent or not well defined); larger size, smallest adult specimen of *A. insperatus* was a male with a SVL of 15.5 mm (largest individual and only known female, 15.0 mm SVL). *Allobates insperatus* is further differentiated by males having scattered melanophores on the ventral surfaces of throat, chest, and belly (absent). Specimens of *A. juami* are further diagnosed by having ventral surfaces of belly and thighs yellow in life (translucent); fingers II and III not swollen (swollen on the preaxial side); basal webbing between toes II and III (webbing absent); dark brown lateral stripe about the same width from behind the eye to the inguinal region (conspicuously narrower behind the eye than in the inguinal region). Other morphometric diagnostic variables are summarized in table 2 and illustrated in figure 5.

Excluding *Allobates insperatus* and *A. juami*, the most closely related nominal species are *A. conspicuus* and *A. subfolionidificans* (fig. 1) but these are readily differentiated by having a dark brown lateral stripe about the same width from behind the eye to the inguinal region (conspicuously narrower behind the eye than in the inguinal region); pale oblique lateral stripe absent or hardly apparent as small patches never reaching the lateral midbody (pale oblique lateral stripe longer, from groin to or surpassing lateral midbody); shanks with well-defined transverse dark brown blotch (absent or not well defined); adult males with finger III not swollen on the preaxial side (swollen). Additionally, *A. subfolionidificans* has paler ill-defined dorsolateral and ventrolateral stripes (well defined).

Other species can be differentiated by: melanophores on the ventral surface, varying from scattered only on the chin to solid black spots on throat and chest with mottled gray on belly or uniform light gray in males of *Allobates albiventris*, *A. amissibilis*, *A. bacurau*, *A. caeruleodactylus*, *A. caldwella*, *A. flaviventris*, *A. fratisenesculus*, *A. fuscillus*, *A. gA. asconi*, *A. granti*, *A. grillisimilis*, *A. kamillae*, *A. kingsburyi*, *A. magnussoni*, *A. marchesianus*, *A. masniger*, *A. melanolae-*

mus, *A. nidicola*, *A. nunciatus*, *A. ornatus*, *A. pacaas*, *A. paleovarzensis*, *A. ripicolus*, *A. sieggreenae*, *A. sumtuosus*, *A. tinae*, *A. trilineatus*, *A. vanzolinus*, *A. velocicantus*, and *A. vicinus*, also in females of *A. albiventris*, *A. bacurau*, *A. caeruleodactylus*, *A. kamilae*, *A. melanolaemus*, *A. sieggreenae*, *A. tinae*, *A. trilineatus*, and *A. velocicantus* (absent); dorsolateral stripe broadens posteriorly to eyelid forming an hourglass, wavy or X pattern in *A. albiventris*, *A. crombiei*, *A. flaviventris*, *A. gasconi*, *A. juanii*, *A. kamilae*, *A. magnussoni*, *A. ornatus*, *A. paleci*, *A. tapajos*, and *A. vicinus* (absent); preaxial side of fingers II and/or III not swollen in males of *A. amissibilis*, *A. bacurau*, *A. caeruleodactylus*, *A. caldwella*, *A. crombiei*, *A. flaviventris*, *A. fratisenescus*, *A. fuscillus*, *A. gasconi*, *A. granti*, *A. grillisimilis*, *A. kamilae*, *A. magnussoni*, *A. marchesianus*, *A. masniger*, *A. nidicola*, *A. nunciatus*, *A. ornatus*, *A. paleci*, *A. ripicolus*, *A. tapajos*, *A. tinae*, *A. trilineatus*, *A. vanzolinus*, *A. velocicantus*, and *A. vicinus* (swollen); adults of *A. fratisenescus*, *A. kingsburyi*, *A. masniger*, *A. melanolaemus*, *A. nidicola*, *A. nunciatus*, *A. paleovarzensis*, and *A. vanzolinus* are larger, smaller recorded SVL = 18.3 mm (SVL = 14.2–15.0 mm); rudimentary basal webbing between toes II and III in *A. tapajos* (absent); pale oblique lateral stripe short, extending from inguinal region but never reaching the lateral midbody in *Allobates sumtuosus* (pale oblique lateral stripe longer, from groin to or surpassing lateral midbody); in life, *Allobates femoralis*, *A. hodli*, *A. myersi*, and *A. zaparo* have bright yellow, orange, or red flash marks on dorsal surfaces of thighs, and black and white marbling on belly and ventral surface of thighs in preserved and live specimens (yellow, orange, or red flash marks, and black and white marbling absent).

Acoustic diagnosis. The call structure in *Allobates albiventris*, *A. amissibilis*, *A. bacurau*, *A. brunneus*, *A. caldwella*, *A. crombiei*, *A. femoralis*, *A. flaviventris*, *A. granti*, *A. grillicantus*, *A. grillisimilis*, *A. hodli*, *A. ignotus*, *A. insperatus*, *A. juami*, *A. juanii*, *A. kamilae*, *A. marchesianus*, *A. myersi*, *A. nunciatus*, *A. paleci*, *A. paleovarzensis*,

A. sieggreenae, *A. sumtuosus*, *A. talamancae*, *A. tapajos*, *A. tinae*, *A. trilineatus*, *A. undulatus*, *A. velocicantus*, *A. vicinus*, and *A. zaparo* is arranged in trills or series of single notes, note pairs, or groups of three, four, or six notes (continuous emission of a single note, not arranged in note trill or note series).

Among specimens with calls characterized by the continuous emission of a single note, *Allobates algorei*, *A. caeruleodactylus*, *A. chalcopis*, *A. magnussoni*, *A. masniger*, *A. nidicola*, *A. niputidea*, *A. olfersioides*, and *A. subfolionidificans* emit notes or calls at a relative high rate: 2.0–3.0 notes-call/s, 2.5 notes-call/s, 2.5 notes or calls, 1.6–2.4 notes-call/s, 2.1–4.0 notes-call/s, 2.0–4.0 notes-call/s, 1.4–1.7 notes-call/s, 1.1–1.6 notes-call/s, respectively (low rate, 0.6–1.0 notes-call/s). The peak frequency in *A. algorei*, *A. chalcopis*, *A. magnussoni*, *A. masniger*, *A. nidicola*, *A. niputidea*, and *A. sieggreenae* is lower: 5.07 kHz, 4.00–5.20 kHz, 4.27–5.45 kHz, 4.03–4.41 kHz, 3.76–4.69 kHz, 5.30 kHz, 4.90–5.70, respectively (high peak frequency, 5.53–6.82 kHz). Note-call duration long in *A. caeruleodactylus*, *A. chalcopis*, *A. magnussoni*, *A. masniger*, *A. nidicola*, and *A. niputidea*: 0.062 s, 0.06 s, 0.027–0.104 s, 0.031–0.089 s, 0.034–0.060 s, 0.041–0.052 s, respectively (short note-call duration, 0.013–0.034 s).

Osteological differences between *Allobates liniaureum* and *A. juami*. Although we acknowledge that our sampling is limited to one individual per species, we focus our comparisons on characters that are less prone to variation such as presence/absence of structures or contact/not in contact. Additionally, we have scanned the largest individual available of each species on an attempt to reduce differences due to poor mineralization. Character states of *A. juami* in parentheses: maxillary process of nasal does not contact preorbital process of maxilla (in contact); crista parotica contacting the otic ramus of squamosal (not in contact); posterior wall of otic capsule oriented laterad, in ventral view (oriented anteriad); posteromedial tip of parasphenoid inconspicuous (conspicuous); border of optic foramina completely mineralized

(dorsal margin cartilaginous); premaxilla with 5–6 recurved teeth (7–8 conical teeth); outer processes of pars palatina of premaxilla hardly wider than inner ones (notably wider); zygomatic ramus of squamosal anteriorly oriented in dorsal view (anterolaterally oriented); clavicles slightly directed anteriorly (laterally oriented); acromion process projecting slightly outward in ventral view (projecting slightly inward); anterior end of ilial crest sloped (stepped).

GEOGRAPHIC DISTRIBUTION: The new species is known only from the type locality, about 6 km northwest of Yurimaguas airport and about 100 m south of Terminal Portuario de Yurimaguas. The type locality is a small patch (about 0.1 km²) of secondary-growth rainforest, which was very degraded, with signs of recent logging. The landscape alongside the type locality is formed by a continuous stretch of terra-firme (nonflooded) environments delimited by the Huallaga and Paranapura rivers, currently dominated by patches of altered or secondary-growth rainforest and open habitats resulting from deforestation (fig. 24). *Allobates liniaureum* may be present at other localities in lowland terra-firme forests delimited by the Cordillera Escalera to the west, the Huallaga River to the east and south, and the Marañón River to the north.

DISCUSSION

Our field and laboratory work have uncovered phenotypic and genetic variation that reveals a complex diversification scenario. We have detected the gamut of species divergence, ranging from a clearcut example of a well-delimited species (*Allobates liniaureum*) supported by the results of all analyses—likely the product of complete geographic isolation for many generations—to strong incongruence among data within a widely distributed species (*A. juami*), and with the somewhat intermediate case of *A. insperatus*, which has mild incongruence among some data and/or analyses due to a few samples, indicating partial isolation and secondary contact.

We inferred *Allobates liniaureum* as an independent evolutionary lineage in all analyses that consider genealogy, regardless of dataset (fig. 2). This result is congruent with our inferences derived from mitochondrial genetic distances (ABGD) and admixture analysis of nuclear SNPs (STRUCTURE). Furthermore, we discovered various phenotypic characters related to morphometrics, coloration, osteology, and behavior that are diagnostic (i.e., nonoverlapping) in relation to the most closely related taxa. These congruent results obtained from different data and analyses are compatible with the cessation of alleles sharing (i.e., complete lineage sorting), migration, and gene flow for many generations (Yang, 2015; Luo et al., 2018; Chan et al., 2021). Within a geographic context, this result suggests strong isolation facilitated by physical and ecological barriers such as the Cordillera Escalera to the west, the Marañón River to the north, and the Huallaga River to the east and south (fig. 25). Even in the unlikely case of sympatry between *A. liniaureum* and *A. insperatus* or *A. juami*—perhaps in the poorly explored interfluves between the Morona and Tigre rivers, given that the Huallaga-Ucayali interfluve consists of *várzea* forests (i.e., seasonably flooded), an unsuitable environment for *Allobates*—gene flow seems unlikely given the structural and quantitative differences found in their advertisement calls, a behavior linked to mate choice and reproductive isolation (Ryan, 1980; Gerhardt, 1991; Ryan and Keddy-Hector, 1992; Boul et al., 2007).

Like *Allobates liniaureum*, *A. insperatus* is also restricted to a relatively small area of terra-firme forest near the Andean foothills, in this case between the headwaters of the Napo and Putumayo rivers (fig. 3). But contrary to *A. liniaureum*, we found samples from three localities on the margins of this area with clearly mixed genetic affinities in nuclear markers to *A. insperatus* and *A. juami* (figs. 2, 25). Two of these localities are in Colombia, between the headwaters of the Putumayo and Caquetá rivers, while the other one is in Peru, south of the Napo River. This shared genetic signal could be caused by

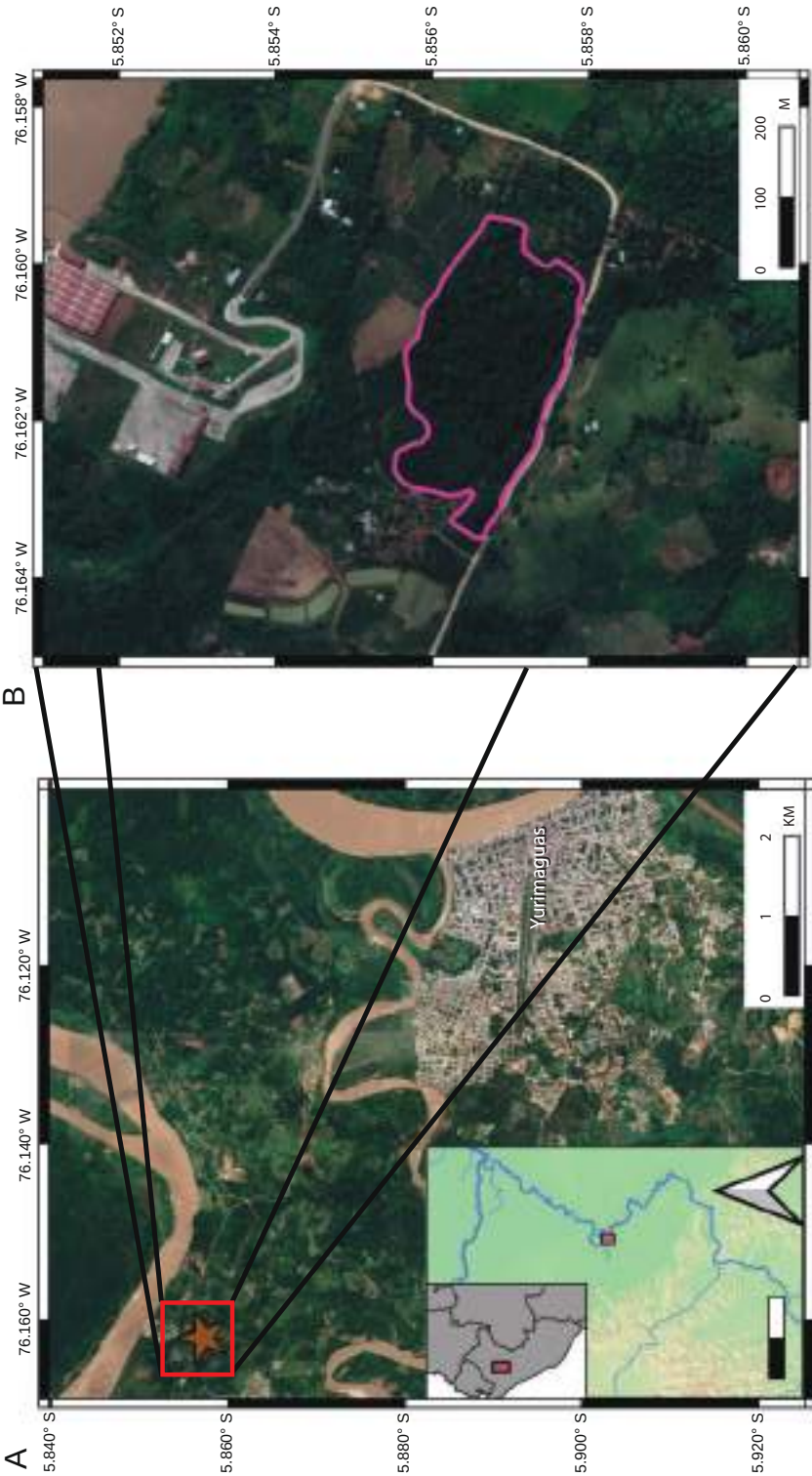


FIGURE 24. **A.** Satellite images of the type locality of *Allobates lineiaureum*; note the position of Yurimaguas city and Terminal Portuario de Yurimaguas. The area delimited by the red square on the left is augmented to better illustrate its geographic position. Dark green areas correspond to secondary growth forest. Light green and dark brown correspond to deforested areas, and light brown to rivers. The violet polygon delimits the patch of secondary forest where we found individuals of *A. lineiaureum*. **B.** Photograph of secondary forest Amazonian rainforest, which *A. lineiaureum* inhabits. Satellite images from Google Earth, Image © 2023 Airbus.

incomplete lineage sorting, gene flow, or a combination of both (Funk and Omland, 2003; Toews and Brelsford, 2012; Zheng and Janke, 2018). Although formal testing with a larger number of genetic markers and samples is needed to discern between these and other possible causes (e.g., homoplasy), the examination of the geographic setting, geological history of the area, phenotypes, behavior related to reproduction, and patterns of shared genetic variation allows us to put forward a hypothesis regarding the origin of the putative admixed lineage. The genetic diversity in both mitochondrial and nuclear markers exhibited by samples of *A. insperatus* within the Napo-Putumayo interfluvium is impressive (fig. 2), considering that most samples were collected within a small range. This high level of variation combined with the isolation created by the Andes to the west and the Napo and Putumayo rivers to the south and north, is compatible with this region acting as long-lasting refuge. Réjaud et al. (2020) dated the divergence between the clades *A. conspicuus/subfollionidificans* and *A. insperatus/juami* at 8–12 Mya (fig. 26). Around that time, most of central western Amazonia was covered by water (the Pebas megawetland) and the Andean foothills might have served as a refuge for *Allobates* and other terra-firme organisms (fig. 26B; Santos et al., 2009; Réjaud et al., 2020). As the Pebas system started to gradually recede in central western Amazonia about 4.5 Mya (see Albert et al., 2018), newly emerged habitats that favored the migration of *A. insperatus* became available (fig. 26C). However, at about the same time the current configuration of riverine barriers such as the Napo and Putumayo rivers started to build up, purportedly limiting *A. insperatus* geographic expansion (fig. 26D). The presence of genetic variation associated with *A. insperatus* outside the Napo-Putumayo interfluvium is restricted to a couple of nearby localities and is compatible with this model of limited and recent migration. This pattern is also consistent between mitochondrial and nuclear markers (figs. 3, 25). However, it is also relevant that all samples from outside the

Napo-Putumayo interfluvium that exhibit a clear *A. insperatus* genetic signal also have a 40%–60% *A. juami* genetic component (fig. 26E). We interpret this pattern as more compatible with relatively recent gene flow, with *A. juami* creating a hybrid zone rather than with incomplete lineage sorting. If shared ancestral polymorphism was the leading cause of the lack of genetic differentiation between the two lineages, one would not observe a combination of genetic admixture geographically limited to a narrow zone. The scenario of a hybrid zone around the core distribution of *A. insperatus* is further supported by our bioacoustic comparisons between *A. insperatus* and *A. juami*. Our results clearly show that the acoustic space inferred from structural, temporal, and spectral variables is partially shared between both species, arguably facilitating reproduction events when specimens of the two lineages share the same habitat. We conclude that the data at hand favor a scenario of geographic isolation and secondary contact without hard reproductive barriers and, thus, hybridization (Harrison, 1990). Although this hypothesis needs rigorous testing, we want to highlight that confirmation of hybridization per se does not reject species hypotheses as defendants of the evolutionary species concept have argued (Mayr, 1942; Coyne and Orr, 2004). Under the evolutionary species concept (Simpson, 1951; Wiley, 1978; de Queiroz, 1998), allopatric speciation is not necessarily coupled with the evolution of intrinsic reproductive barriers (Wiens, 2004), and Escalona-Sulbarán et al. (2019) showed that acoustic differentiation is not necessarily synchronous with the evolution of other phenotypic characters. Thus, if hybridization does not blur the footprints of divergent evolution that allow us to infer *A. insperatus* and *A. juami* as segments of independent metapopulation lineages, our species hypotheses are not rejected.

Interpreting the variation within *Allobates juami* is more challenging than in the previous two cases. First, there are largely incongruent results between the inferences made from mitochondrial and nuclear DNA sequences; second,

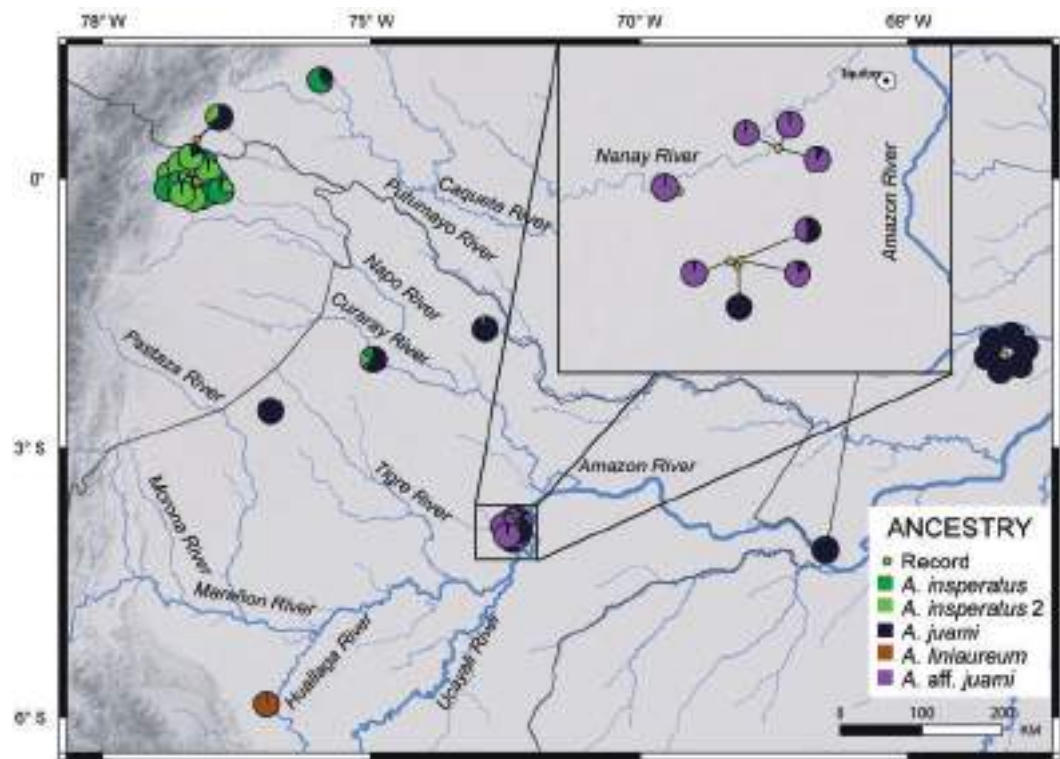


FIGURE 25. Map of central western Amazonia showing the spatial distribution of ancestry coefficient proportions of the most optimal grouping with $K = 5$. Each circle represents a specimen and the colors the proportion of genetic variation assigned to each group. Country borders and main rivers are delimited by black and blue contours, with thicker blue contours indicating larger water flow. When several samples originate from the same or very close localities, the exact collection site is marked by an orange dot. Inset map shows the distribution of samples and their genetic composition in the Amazon-Nanay interfluvium.

we lack nuclear DNA sequences and phenotypic data from the upper Pastaza and Tigre rivers; and third, we do not have samples from between the type locality of *A. juami* and the upper course of the Caquetá River. Nonetheless, by considering the geological history of the region, the available genetic information, and its geographical distribution, we argue for the following scenario: The mitochondrial group *A. aff. juami* is restricted to the large interfluvium delimited by the Napo, Ucayali, and Marañón rivers to the north, east, and south respectively, while the Andes constitute the western barrier. Although this area harbors several smaller rivers such as the Curaray, Pastaza, and Tigre, they are relatively short and small, so that their upper courses allow an ample region of

connected terra-firme rainforest (fig. 3). It is also relevant that when considering nuclear markers, there is hardly any genetic signature of this group among the samples of *A. juami* outside of this region. Considering this, we hypothesized that the Pebas megawetland isolated populations on both the northeast side, which correspond to the Guiana Shield, and the upper Pastaza River around 10 Mya (fig. 26B). Later, while the Iquitos Arch separating the Pebas and Acre systems could have allowed the expansion of the Guiana Shield populations toward the southwest before 4.5 Mya, the migration of the upper Pastaza populations toward the east was probably delayed until more recently, when the Pebas system was drained into the Atlantic with the establishment of the current

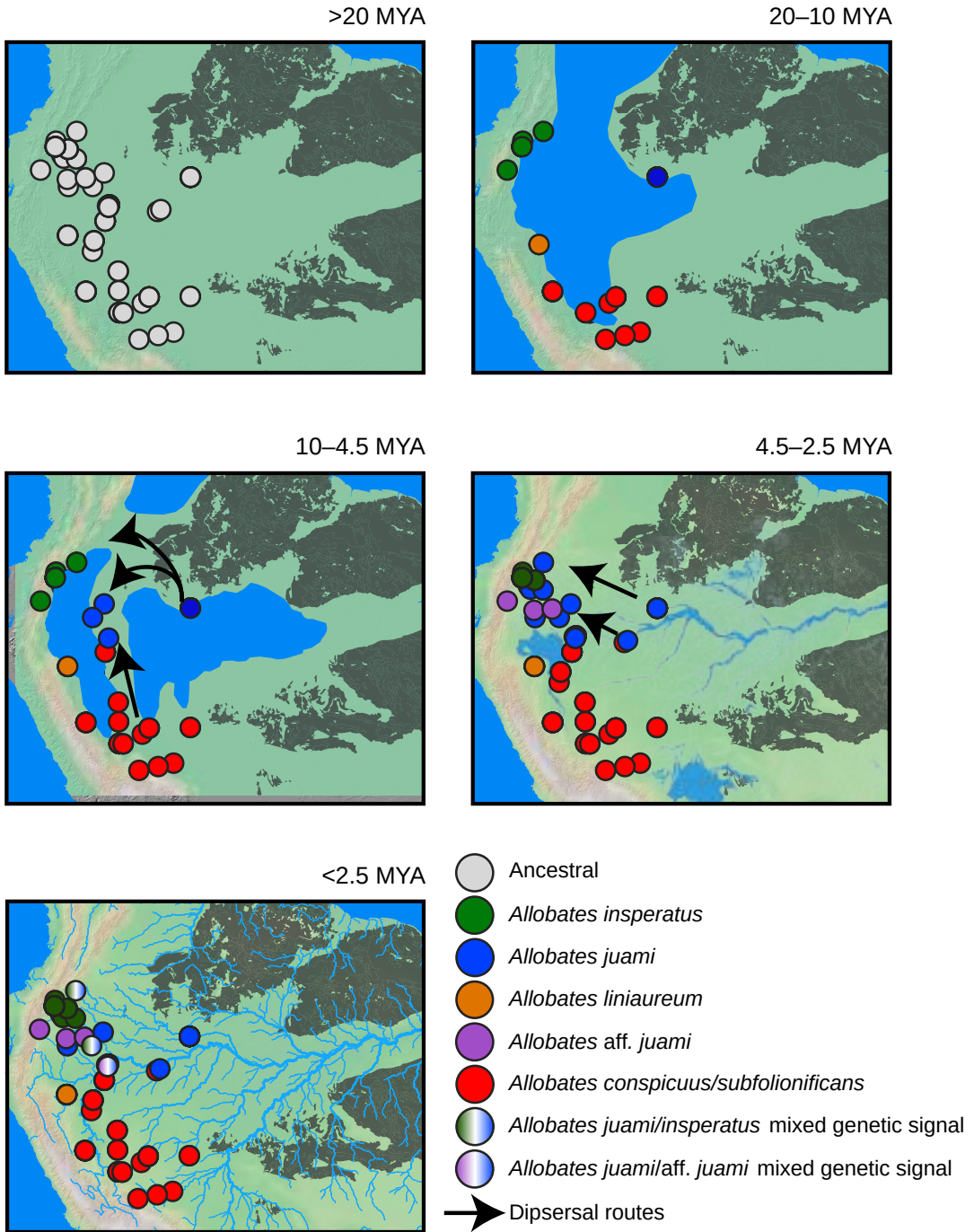


FIGURE 26. Hypothetical schematic representation of the biogeographical scenario of the *Allobates insperatus/juami* species complex over time following the major landscape modifications in western Amazonia.

Amazonian watershed configuration (fig. 26C). As mentioned above, while more land became available for *A. aff. juami* due to the drainage of the Pebas system, the current configuration of riverine barriers such as the Mara  n, Napo, and Ucayali rivers started to build up, limiting its expansion to other interfluves (fig. 26D, E). The different properties of the mitochondrial and nuclear genomes could then explain the hierarchical pattern of the former and the lack of resolution of the latter. Briefly, nuclear markers have larger effective population size and thus tend to show older coalescent times—approximately four times that of mitochondrial DNA—and slower fixation rates (Zink and Barrowclough, 2008). From a systematic perspective, one could use this last argument to support the inferred mitochondrial lineages *A. aff. juami* and *A. juami* as different species. However, we prefer to be conservative and consider these two groups as a single species due to the (1) absence of phenotypic data from key localities, (2) poor sampling around the headwaters of the Curaray, Pastaza, and Tigre rivers, (3) evidence of possible hybridization (e.g., sample GGU 6108, figs. 2B, 25), and (4) the absence of hierarchical structure on the total evidence phylogenetic analysis (fig. 2D).

At small spatial scales (grids $\leq 10 \times 10$ km), western Amazonia contains the highest species richness in the world (Jenkins et al., 2013). Among amphibians, species diversity is highest in central western Amazonia (< 500 m), roughly east of the Andes between the Caquet   and Huallaga rivers and extending to the Ucayali. Within this area, reports of amphibian diversity are astonishing, documenting more than 100 amphibian species in 10 km^2 (Lynch, 2005; Bass et al., 2010). Local species richness depends on abiotic and biotic factors controlling coexistence, but also dramatic habitat changes in recent geologic times have favored dispersal of previously isolated lineages (e.g., Harrison and Cornell, 2008; Wiens et al., 2011). Our study of the systematics of a small clade of frogs has allowed us to suggest a model for diversity build up in this biodiversity hotspot that might be applicable to

other groups of organisms. This model allows several testable predictions:

(1) The number of diagnosable evolutionary units (e.g., species, operational taxonomic units, mitochondrial clades) should be larger and with smaller geographic ranges west of the Ucayali and Amazonas rivers and along the Andes foothills than on neighboring emerged lowlands on southern and northeastern Amazonia (R  jaud et al., 2020). Temporally, the older splits along Andean foothills should roughly coincide with the height of the Pebas megawetland 10–20 Mya. This would be the result of higher fragmentation of emerged lowlands and for longer times along the foothills of the Andes by the Pebas megawetland than on southern or northeastern Amazonia. For example, within our study group *Allobates liniaureum*, *A. insperatus*, and *A. aff. juami* are restricted to small areas within this region while to the northeast we detected only *A. juami* and to the south *A. conspicuus* and *A. subfolionidificans*.

(2) Dispersion across and along interfluves of evolutionary units resulting from isolation on the foothills of the Andes should be absent or very limited and with a west-to-east pattern. Temporally, dispersions should be < 10 Mya and with a stronger signal < 5 Mya. The limited dispersion 5–10 Mya would be caused by the presence of a smaller Pebas system. Starting at about 5 Mya, the gradual drainage of the Pebas system was concomitant with the building up of current riverine barriers, limiting migration across interfluves and favoring a west-to-east pattern. For example, the genetic signature of *A. insperatus* is concentrated closer to the Andes and within the Napo-Putumayo interfluve and diminishing with geographic distance. The furthest genetic signature of *A. insperatus* is detected within the same interfluve.

(3) Within the evolutionary units formed along the Andean foothills, those found on the Mara  n-Huallaga interfluve have been isolated more continuously and efficiently, reflecting the permanence of megawetlands around this area to the present day (i.e., Ucamara mega-wetland sys-

tem). For example, we did not find genetic signals of *A. liniaureum* outside this interfluvium.

(4) Northeastern and southern groups may have migrated more efficiently to the west and are present in several interfluviums. This pattern would be the result of migration through emerged land across the north to south Iquitos Arch 5–10 Mya. When the current riverine barriers started to cross the emerged lands of the Iquitos Arch <5 Mya on a west to east direction, the north to south position of the Iquitos Arch allowed migration along several interfluviums without dispersal across major rivers. For example, the presence of *A. juami* across several interfluviums (e.g., Japura-Putumayo-Napo-Pastaza).

Besides most-needed empirical support for this model, several important limitations should be kept in mind. For example, the paleogeographic reconstructions are coarse-grained and simplified at both temporal and spatial scales; this model is most likely restricted to lowland and terrestrial organisms with limited dispersal capacities; other historical events such as Andean uplifts, landscape dynamics from Miocene, and glaciation cycles are also likely contributors to the diversity of the region; and variation of other intrinsic (e.g., migration rates, generation time, antipredatory adaptations) and extrinsic variables (e.g., distribution of predators and parasites, soil and vegetation types, rainfall, and temperature) are likely important aspects to be considered.

CONCLUSION

We have uncovered a complex evolutionary history within the *Allobates juami/insperatus* clade through an interdisciplinary approach. The congruence of all genetic and phenotypic datasets strongly supports the recognition of *A. liniaureum*, a new and narrowly distributed species. On the other hand, we have found that although *A. juami* and *A. insperatus* show congruent morphological characters and mitochondrial haplotypes supporting their species status, some individuals of putative *A. insperatus* have mixed genetic signals compatible with recent

gene flow with *A. juami*. We also detected mitochondrial genetic variation within *A. juami* that could be indicative of two evolutionary lineages under this name. However, our limited geographic and genetic sampling, combined with substantial incongruence between mitochondrial versus nuclear markers, does not allow us to test this hypothesis and our taxonomic proposal remains conservative by recognizing *A. juami* as a widespread species with substantial genetic variation. We interpret these results in the context of the paleodynamics of western Amazonia (e.g., Pebas megawetland and the formation of the main Amazonian rivers) during the last 10 Mya allowing dispersal routes of isolated evolutionary lineages and outline a biogeographic scenario of diversification for this region that goes beyond the traditional vicariance hypothesis by physical barriers. Our model proposes predictions that could be tested with other low-vagility terra-firme taxa.

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APPENDIX 1

EXAMINED SPECIMENS

Additional specimens examined. An asterisk indicates individuals examined only by photographs. We compared species not listed here using images and descriptions included in the original publication of each species, which are cited in the main text and included in the list of references.

Allobates bacurau—BRAZIL: Amazonas: Manicoré: INPA-H 35397–35409. *Allobates fratisenes-cus*—ECUADOR: Pastaza: El Siguin: KU 182205*; Comunidad Simón Bolívar Sacha Yacu: MZUTI 187, 224, 290. *Allobates gasconi*—BRAZIL: Amazonas: Rio Juruá, Jainu: INPA-H 3082, 3085, 3087, 3090, 3150, 3151, 3172, 3249, 3406, 3415, 3483, 3484, 3491, 3494, 3496, 3512, 3513. *Allobates grilli-similis*—BRAZIL: Amazonas: Borba: INPA-H 30779–30808; Nova Olinda do Norte: INPA-H 30809–30823. *Allobates hodli*—BRAZIL: Rondônia: Cachoeira do Jirau: INPA-H 16541–16569. *Allobates insperatus*—ECUADOR: Sucumbíos: Santa Cecilia: KU 146206*. *Allobates juami*—BRAZIL: Amazonas: Estação Ecológica Juami-Japurá: MCP 13287–93. *Allobates magnussoni*—BRAZIL: Pará: Itaituba, Parque Nacional da Amazônia: INPA-H 32960–32976; Belterra, Fazenda Treviso: INPA-H 10105–10109, 33930–33934. *Allobates masniger**—BRAZIL: Pará: Itaituba, Parque Nacional da Amazônia: USNM 303584, 303585, 303587, 303589, 303590. *Allobates mcdiarmidi**—BOLIVIA: Cochabamba: Cochabamba: USNM 257804–257806. *Allobates myersi*—BRAZIL: Amazonas: São Gabriel da Cachoeira: INPA-H 26396–26372, 26374, 26376, 26377, 26379. *Allobates nidicola*—BRAZIL: Rondônia: Porto Velho: INPA-H 28602–28612. *Allobates sumtuosus*—BRAZIL: Pará: Reserva Biológica do Rio Trombetas: INPA-H 31952–31956, INPA-H 31958–31960. *Allobates tapajos*—BRAZIL: Pará: Itaituba, Parque Nacional da Amazônia: INPA-H 34402–34406, 34410–34412, 34414–34416, 34418, 34423, 34407–34409, 34413, 34417, 34419–34422, 34424–34425. *Allobates trilineatus*—PERU: Loreto: Alto Amazonas, Munichis: MCP 14303–04, 14307, 14309–10, 14312, 14315–16, 14305–06, 14311, and 14317.

APPENDIX 2

OUTGROUP GENBANK ACCESSION NUMBERS

Outgroup terminals, voucher codes, and GenBank accession numbers of 16S mtDNA sequences used in this study.

Taxon	Voucher Code	16S
<i>Allobates</i> aff. <i>hodli</i>	OMNH36066	DQ502091
<i>Allobates</i> aff. <i>juanii</i>	AJC3383	KJ130697
<i>Allobates</i> aff. <i>juanii</i>	AJC3385	KJ130698
<i>Allobates</i> aff. <i>magnussoni</i>	MTR28013	KDQF01003644
<i>Allobates</i> aff. <i>magnussoni</i>	MTR977126	KDQF01003760
<i>Allobates</i> aff. <i>magnussoni</i>	MTR977138	KDQF01003761
<i>Allobates</i> aff. <i>olfersioides</i>	JFT959	KDQF01002701
<i>Allobates</i> aff. <i>tapajos2</i>	MPEG13383	DQ502241
<i>Allobates</i> aff. <i>tapajos3</i>	MTR10084	JN690935
<i>Allobates</i> aff. <i>tapajos4</i>	AG092	EU201069
<i>Allobates</i> aff. <i>tinae</i>	LSUMZ17601	DQ502136
<i>Allobates</i> aff. <i>trilineatus</i>	LSUMZ13690	EU342562
<i>Allobates</i> aff. <i>trilineatus</i>	MNRJ 91664	KY886584
<i>Allobates</i> aff. <i>trilineatus</i>	MNRJ 91676	KY886595
<i>Allobates</i> aff. <i>trilineatus</i>	OMNH 36026	DQ502198
<i>Allobates</i> <i>alagoanus</i>	MTR06031	DQ502126
<i>Allobates</i> <i>albiventris</i>	OMNH36637	DQ502202
<i>Allobates</i> <i>albiventris</i>	APL14410	KJ747333
<i>Allobates</i> <i>albiventris</i>	APL14411	KJ747334
<i>Allobates</i> <i>albiventris</i>	MNRJ 91679	KY886578
<i>Allobates</i> <i>albiventris</i>	MNRJ 91681	KY886572
<i>Allobates</i> <i>algoei</i>	TNHCF5551	HQ290950
<i>Allobates</i> <i>amissibilis</i>	MTD47884	KC520680
<i>Allobates</i> <i>bacurau</i>	INPA-H35399	KU195703
<i>Allobates</i> <i>bacurau</i>	INPA-H35404	KU195702
<i>Allobates</i> <i>bacurau</i>	INPA-H35406	KU195698
<i>Allobates</i> <i>caeruleodactylus</i>	LSUMZ16926	EU342542
<i>Allobates</i> <i>caeruleodactylus</i>	MPEG13808	DQ502186
<i>Allobates</i> <i>caeruleodactylus</i>	OMNH37411	DQ502212
<i>Allobates</i> <i>caldwellae</i>	MPEG13827	DQ502240
<i>Allobates</i> <i>caldwellae</i>	MTR19227	KDQF01003431
<i>Allobates</i> <i>carajas</i>	TG3264	MF624180
<i>Allobates</i> cf. <i>flaviventris</i>	APL15179	KJ747341
<i>Allobates</i> cf. <i>flaviventris</i>	OMNH36959	DQ502184
<i>Allobates</i> <i>chalcopis</i>	Alca1	KC520676

APPENDIX 2 continued

Taxon	Voucher Code	16S
<i>Allobates chalcopis</i>	Alca2	KC520678
<i>Allobates conspicuus</i>	MPEG12321	DQ502134
<i>Allobates conspicuus</i>	OMNH35997	DQ502135
<i>Allobates crombiei</i>	APL13462	KF250509
<i>Allobates crombiei</i>	APL14118	KJ747345
<i>Allobates crombiei</i>	APL14124	KJ747346
<i>Allobates femoralis</i>	AJC3598	KJ130696
<i>Allobates femoralis</i>	LSU12798	DQ502228
<i>Allobates femoralis</i>	MJH3976	DQ502113
<i>Allobates femoralis</i>	OMNH34102	DQ502093
<i>Allobates flaviventris</i>	MTR 25726	KY886569
<i>Allobates fratisenescus</i>	QCAZ54377	MF624172
<i>Allobates goianus</i>	SAMA8574	KDQF01004209
<i>Allobates goianus</i>	SAMA8575	KDQF01004210
<i>Allobates granti</i>	CM185	EU201066
<i>Allobates granti</i>	CM231	JN690927
<i>Allobates granti</i>	PG125	JN690931
<i>Allobates grillisimilis</i>	APL12674	KF250506
<i>Allobates grillisimilis</i>	APL12747	KF250504

APPENDIX 3

LOCALITY DATA AND GENBANK ACCESSION CODES OF INGROUP EMPLOYED IN THIS STUDY

Ingroup terminals, voucher codes, localities, and GenBank accession numbers of mitochondrial and nuclear sequences used in this study. Newly generated sequences are in bold face.

Taxon	Terminal Name	Voucher Code	Locality	Country	State	Latitude	Longitude	16S	DOLK	LIG4	LRRC8D	MED13	PPL	SALL1
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12948	Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502239	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	Unknown	-	Ecuador	Orellana	-0.491	-76.559	AFI28583	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates insperatus_juani	QCAZ_52435	Cuyabeno	Ecuador	Ecuador	-0.0886	-76.1419	MT524121.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates sp.	LEO_718	Guadalitos	Colombia	Putumayo	0.441535	-76.943628	MT524122.1	PQ723380	PQ723403	PQ723427	PQ723455	PQ723478	PQ723501
<i>A. insperatus</i>	Allobates aff. insperatus	OMNH34085	Estacion Cientifica de Universidad Catolica near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502187.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12971	Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502044.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12972	Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502045.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12950	Dantas Trail near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502097.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	OMNH34086	Estacion Cientifica de Universidad Catolica near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502041.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12938	Estacion Cientifica de Universidad Catolica near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502096.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12969	Palmas Rojas Trail near Estacion Cientifica de Universidad Catolica	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502042.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	OMNH34079	Estacion Cientifica de Universidad Catolica near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502208.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates aff. insperatus	LSUMZ12936	Estacion Cientifica de Universidad Catolica near Reserva Faunistica Cuyabeno	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502195.1	-	-	-	-	-	-

APPENDIX 3 continued

Taxon	Terminal Name	Voucher Code	Locality	Country	State	Latitude	Longitude	IGS	DOLK	LIG4	LRRC8D	MED13	PPL	SALL1
<i>A. insperatus</i>	Allobates_aff_insuperatus	LSUMZ12970	Palmas Rojas Trail near Estacion Cientifica de Universidad Catolica	Ecuador	Sucumbios	-0.0886	-76.1419	DQ502043.1	-	-	-	-	-	-
<i>A. insperatus</i>	Allobates	AFI 239/ZSFQ 6210	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	-	-	-	PQ723428	-	-	PQ723502
<i>A. insperatus</i>	Allobates	AFI 240/ZSFQ 6260	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	-	-	-	PQ723429	-	-	PQ723503
<i>A. insperatus</i>	Allobates	AFI 241/ZSFQ 6261	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	PQ722449	-	-	PQ723430	-	-	PQ723504
<i>A. insperatus</i>	Allobates	AFI 242/ZSFQ 6268	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	-	-	-	PQ723431	-	-	PQ723505
<i>A. insperatus</i>	Allobates	AFI 243/ZSFQ 6269	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	-	-	-	PQ723432	-	-	PQ723506
<i>A. insperatus</i>	Allobates	AFI 244/ZSFQ 6270	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	PQ722450	-	-	-	-	-	PQ723507
<i>A. insperatus</i>	Allobates	AFI 245/ZSFQ 6271	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.07329	-76.98927	-	-	-	PQ723433	-	-	PQ723508
<i>A. insperatus</i>	Allobates	AFI 246/ZSFQ 6272	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	-	-	-	PQ723509
<i>A. insperatus</i>	Allobates	AFI 247/ZSFQ 6273	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	PQ723434	-	PQ723479	PQ723510
<i>A. insperatus</i>	Allobates	AFI 248/ZSFQ 6274	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	PQ723435	-	-	PQ723511
<i>A. insperatus</i>	Allobates	AFI 249/ZSFQ 6275	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	PQ723436	-	-	PQ723512
<i>A. insperatus</i>	Allobates	SCF 2302/ ZSFQ 6280	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	PQ723437	-	-	PQ723513
<i>A. insperatus</i>	Allobates	SCF 2359/ ZSFQ 6286	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	PQ722451	-	-	PQ723438	-	-	PQ723514
<i>A. insperatus</i>	Allobates	SCF 2304/ ZSFQ 6282	Santa Cecilia, Lago Agrio	Ecuador	Sucumbios	0.073	-76.989	-	-	-	PQ723439	-	-	PQ723515
<i>A. juani</i>	Allobates_insuperatus	QCAZ16533	Comunidad Santa Maria de Huiririma, margen S del Rio Napo	Ecuador	Orellana	-0.736	-75.586	AY364557	-	-	-	-	-	-
<i>A. juani</i>	Allobates	Unknown	Estacion Cientifica Yasuni, km 1	Ecuador	Orellana	-0.689	-76.429	HQ290959	-	-	-	-	-	-
<i>A. juani</i>	Allobates sp.	MZ010	Alto Mazan	Peru	Loreto	-2.586	-74.492	MF624185	-	-	-	-	-	-
<i>A. juani</i>	Allobates_juani	MCP 13287	Japura, Estacao Ecologica Juami-japura	Brazil	Amazonas	-1.965	-67.936	MG243349	-	-	-	-	-	-
<i>A. juani</i>	Allobates_sp	ANDES_A_2334	Finca Bruselas	Colombia	Caqueta	1.1	-75.56	PQ722452	PQ723381	PQ723404	-	PQ723456	PQ723480	PQ723516
<i>A. juani</i>	Allobates_insuperatus_juani	QCAZ_44577	Comunidad Santa Maria de Huiririma, margen S del Rio Napo	Ecuador	Orellana	-0.736	-75.586	MT524120.1	-	-	-	-	-	-
<i>A. juani</i>	Allobates_juani	AJC_2589	Rio Takana, km 9 carretera	Colombia	Amazonas	-4.136	-69.924	MT524165.1	PQ723382	PQ723405	-	PQ723457	PQ723481	PQ723517

APPENDIX 3 continued

Taxon	Terminal Name	Voucher Code	Locality	Country	State	Latitude	Longitude	IGS	DOLK	LIG4	LRRCD	MED13	PPL	SALL1
<i>A. juami</i>	Allobates juami	MCP13290	Japura, Estacao Ecologica Juami-Japura	Brazil	Amazonas	-1.965	-67.936	MG243348	PQ723383	PQ723406	PQ723440	PQ723458	PQ723482	PQ723518
<i>A. juami</i>	Allobates juami	MCP13291	Japura, Estacao Ecologica Juami-Japura	Brazil	Amazonas	-1.965	-67.936	MG243351	PQ723384	PQ723407	PQ723441	PQ723459	PQ723483	PQ723519
<i>A. juami</i>	Allobates_ juami	MCP_13292	Estacao Ecologica Juami-Japura	Brasil	Amazonas	-1.965	-67.936	-	-	PQ723408	PQ723442	PQ723460	PQ723484	PQ723520
<i>A. juami</i>	Allobates_ juami	MCP_13293	Estacao Ecologica Juami-Japura	Brasil	Amazonas	-1.965	-67.936	PQ722453	PQ723385	PQ723409	PQ723443	PQ723461	PQ723485	PQ723521
<i>A. juami</i>	Allobates juami	MCP13288	Japura, Estacao Ecologica Juami-Japura	Brazil	Amazonas	-1.965	-67.936	MG243350	PQ723386	PQ723410	-	PQ723462	PQ723486	PQ723522
<i>A. juami</i>	Allobates_ juami	MCP_13289	Estacao Ecologica Juami-Japura	Brasil	Amazonas	-1.965	-67.936	-	PQ723387	PQ723411	-	PQ723463	PQ723487	PQ723523
<i>A. juami</i>	Allobates_sp	MUBI_7684	Teniente Lopez	Peru	Loreto	-2.592	-76.117	-	PQ723388	PQ723412	-	PQ723464	PQ723488	PQ723524
<i>A. juami</i>	Allobates_sp_4	GGU_5978	Estacion Biologica Jose Alvarez Alonso - EB/AA, Reserva Nacional Allpahuasi	Peru	Loreto	-3.971	-73.423	-	PQ723389	PQ723413	PQ723444	PQ723465	PQ723489	PQ723525
<i>A. juami</i>	Allobates_ juami	GGU_1523	huayo - Mishana Trocha T6, Campamento C2, Cabecera Rio Ere, Distrito Putumayo, Provincia Maynas	Peru	Loreto	-1.679	-73.720	-	PQ723390	PQ723414	-	PQ723466	PQ723490	PQ723526
<i>A. liniaureum</i>	Allobates sp nov	MCP 14294 (SCF 2216)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722454	-	-	-	-	-	-
<i>A. liniaureum</i>	Allobates sp nov	MCP 14295 (SCF 2217)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722455	-	-	-	-	-	-
<i>A. liniaureum</i>	Allobates sp nov	MCP 14332 (SCF 2255)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722456	PQ723391	PQ723415	PQ723445	-	PQ723491	PQ723527
<i>A. liniaureum</i>	Allobates sp nov	MCP 14333 (SCF 2256)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722457	PQ723392	PQ723416	PQ723446	PQ723467	PQ723492	PQ723528
<i>A. liniaureum</i>	Allobates sp nov	MCP 14334 (SCF 2257)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722458	PQ723393	PQ723417	PQ723447	PQ723468	PQ723493	PQ723529
<i>A. liniaureum</i>	Allobates sp nov	MCP 14296 (SCF 2218)	Carretera a Jeberillo, Puerto nuevo Yurimaguas	Peru	Loreto	-5.857	-76.162	PQ722459	PQ723394	PQ723418	PQ723448	PQ723469	PQ723494	PQ723530
<i>A. aff. juami</i>	Allobates aff. insperatus	QCAZI6609	Pastaza Puyo-Macas	Ecuador	Pastaza	-1.49	-77.98	AY364561	-	-	-	-	-	-
<i>A. aff. juami</i>	Allobates aff. insperatus	IDLAR 8200	Arboretum de la UNAM, camino hacia Puerto Almendras	Peru	Loreto	-3.831	-73.373	MTS24136	-	-	-	-	-	-

APPENDIX 3 continued

Taxon	Terminal Name	Voucher Code	Locality	Country	State	Latitude	Longitude	16S	DOLK	LIG4	LRRCD	MED13	PPL	SALL1
A. aff. <i>juani</i>	Allobates aff. <i>insperatus</i>	NMP6V711401	Arboretum de la UNAM, camino hacia Puerto Almendras	Peru	Loreto	-3.831	-73.373	MT524146	-	-	-	-	-	-
A. aff. <i>juani</i>	Allobates aff. <i>insperatus</i>	NMP6V711405	Puerto Almendras	Peru	Loreto	-3.817	-73.367	MT524149	-	-	-	-	-	-
A. aff. <i>juani</i>	Allobates aff. <i>insperatus</i>	NMP6V711403	Camino a Zungarococha y Arboreto de la Universidad de Iquitos, Camino a Puerto Alendras	Peru	Loreto	-3.831	-73.373	MT524147	-	-	-	-	-	-
A. aff. <i>juani</i>	Allobates_ <i>insperatus_</i> <i>juani</i>	QCAZ_53540	Juyu Iniza, 4.5 km SE	Ecuador	Pastaza	-2.108	-76.189	MT524122.1	-	-	-	-	-	-
A. aff. <i>juani</i>	Allobates_sp2	GGU_1040	Rio Curaray, Maynas	Peru	Loreto	-2.018	-74.970	MT524167	PQ723395	PQ723419	-	PQ723470	PQ723495	PQ723531
A. aff. <i>juani</i>	Allobates_sp_4	GGU_6108	Estacion Biologica Jose Alvarez Alonso	Peru	Loreto	-3.965	-73.419	-	PQ723396	PQ723420	-	PQ723471	PQ723496	PQ723532
A. aff. <i>juani</i>	Allobates_sp	GGU_5704	Jose Alvarez Alonso - EBJAA, Reserva Nacional Allpa-huayo - Mishana	Peru	Loreto	-3.966	-73.432	PQ722460	PQ723397	PQ723421	PQ723449	PQ723472	-	PQ723533
A. aff. <i>juani</i>	Allobates_sp	JMP_1370	Estacion Biologica Jose Alvarez Alonso - EBJAA, Reserva Nacional Allpa-huayo - Mishana	Peru	Loreto	-3.831	-73.373	-	PQ723398	PQ723422	PQ723450	PQ723473	PQ723497	PQ723534
A. aff. <i>juani</i>	Allobates_sp2	IDLAR_8198	Arboretum de la UNAM, camino hacia Puerto Almendras	Peru	Loreto	-3.831	-73.373	-	PQ723399	PQ723423	PQ723451	PQ723474	PQ723498	PQ723535
A. aff. <i>juani</i>	Allobates_sp2	JMP_1703	Camino a Zungarococha y Arboreto de la Universidad de Iquitos, Camino a Puerto Alendras	Peru	Loreto	-3.831	-73.373	MT627193.1	PQ723400	PQ723424	PQ723452	PQ723475	PQ723499	PQ723536
A. aff. <i>juani</i>	Allobates aff. <i>trilineatus</i>	MUBI_16334	Mishana, Cerca del poblado	Peru	Loreto	-3.882	-73.492	-	PQ723401	PQ723425	PQ723453	PQ723476	PQ723500	PQ723537
A. aff. <i>juani</i>	Allobates_sp_4	GGU_6101	Estacion Biologica Jose Alvarez Alonso - EBJAA, Reserva Nacional Allpa-huayo - Mishana	Peru	Loreto	-3.965	-73.419	-	PQ723402	PQ723426	PQ723454	PQ723477	-	PQ723538
-	Allobates_femoralis	TNHG-GDC 5344	-	-	-	-	-	-	KX201496	KX205233	KX205820	-	KX208541	KX209675

APPENDIX 4

PARTITION SCHEME

Best partition scheme found for each molecular dataset.

Partition (no.)	Only 16S	Only Nuclear	Excluding Terminals with Only 16S	Combined Including All Terminals
1	GRT+G+I	DOLK_3: HKY	16S: GTR+G	16S: GTR+G
2		SALL1_3, MED13_3, LIG4_1: HKY	DOLK_1: HKY	DOLK_1: HKY
3		LRRC8D_2, LIG4_2, MED13_1: HKY	DOLK_2: TRN+G	DOLK_2: GTR
4		MED13_2, LIG4_3, SALL1_2: HKY	DOLK_3: TRNEF+I	DOLK_3, LRRC8D_1: TRNEF+I
5		LRRC8D_1: JC+I	SALL1_2, LIG4_1, MED13_3: HKY	SALL1_3, LIG4_1, MED13_3: HKY
6		LRRC8D_3: HKY+G	LRRC8D_2, LIG4_1, MED13: HKY	LRRC8D_2, MED13_1, LIG4_2: HKY
7		PPL_1: HKY	SALL1_3, MED13_2, LIG4_3: HKY	MED13_2, LIG4_3, SALL1_2: HKY
8		PPL_2: HKY	LRRC8D: JC+I	LRRC8D_3: HKY+I
9		PPL_3: TRN+I+G	LRRC8D_3: HKY+I	PPL_1: HKY
10		SALL1_1: HKY	PPL_1: HKY	PPL_2: HKY
11			PPL_2: HKY	PPL_3: GTR+G+I
12			PPL_3: GTR+G+I	SALL1_1: HKY
13			SALL1_1: HKY	
Alignment length (bp)	601	2204	3043	3048
Likelihood	-8,858.6	-3,591.4	-5,332.5	-5,885.8
AICc	18,864.5	7,443.5	10,973.9	12,202.1

APPENDIX 5

MORPHOMETRIC PRINCIPAL COMPONENTS ANALYSIS LOADINGS

Loadings of the first two components of a Principal Components Analysis for raw morphometrics (left) and body size effect removed (right), with the eigenvalues, variance, and accumulative variance (in %).
The variables with highest loadings are in bold.

Raw Morphometrics			Residual Morphometrics		
	PC1	PC2		PC1	PC2
SVL	0.67	0.30	FL	0.64	0.47
SaL	0.31	-0.07	TL	0.44	-0.07
TL	0.29	-0.29	HW	0.37	-0.40
FL	0.27	-0.60	WTIVD	0.18	-0.10
HL	0.26	0.19	SaL	0.16	0.35
HW	0.21	-0.35	WTIIID	0.15	-0.09
Tal	0.19	0.14	UaL	0.12	0.13
FaL	0.15	0.09	WFIID	0.12	-0.05
UaL	0.15	-0.04	WTIID	0.11	-0.09
HAND.III	0.15	0.24	IN	0.11	-0.10
HAND.I	0.12	0.18	WTV D	0.10	-0.05
SL	0.11	0.06	WFIVD	0.09	-0.03
HAND.II	0.11	0.16	DPT	0.09	0.00
HAND.IV	0.10	0.07	WFIID	0.08	-0.03
ED	0.09	0.03	WTIIIP	0.07	-0.04
IN	0.09	-0.10	WTIP	0.06	-0.04
IO	0.08	0.04	WTV P	0.06	-0.05
EN	0.06	0.00	WTIVP	0.06	-0.02
EW	0.06	0.00	WPFII	0.05	-0.04
TYM	0.04	-0.04	WPFIV	0.05	-0.04
WTIVD	0.02	-0.17	TYM	0.05	0.04
DPT	0.02	-0.08	WTID	0.05	-0.03
WTIIID	0.02	-0.14	WPFIII	0.05	-0.03
WFIID	0.02	-0.12	WTIIP	0.04	-0.02
DTT	0.02	0.09	WPF I	0.04	-0.03
WFID	0.02	-0.02	WFID	0.03	0.00
WFIID	0.02	-0.07	EN	0.01	-0.10
WFIVD	0.02	-0.09	EW	0.01	0.04
WTIID	0.02	-0.11	ED	0.00	0.08
WTV D	0.02	-0.09	IO	0.00	-0.02
WTID	0.01	-0.05	SL	-0.01	0.05
WTIVP	0.01	-0.05	HAND.IV	-0.01	0.17
WPF I	0.01	-0.03	FaL	-0.04	0.16

APPENDIX 5 *continued*

Raw Morphometrics			Residual Morphometrics		
	PC1	PC2		PC1	PC2
WPFII	0.01	-0.05	Tal	-0.05	0.44
WPFIII	0.01	-0.05	DTT	-0.06	0.04
WTIP	0.01	-0.06	HAND.II	-0.08	0.21
WTIIP	0.01	-0.07	HL	-0.08	-0.01
WTVP	0.01	-0.05	HAND.I	-0.09	0.24
WPFIV	0.01	-0.05	HAND.III	-0.15	0.25
WTIIP	0.01	-0.04	Eigenvalue	0.73	0.47
Eigenvalue	24.81	0.68	Variance (%)	19.69	12.73
Variance (%)	86.64	2.37	Accumulative variance (%)	19.69	32.42
Accumulative variance (%)	86.64	89.01			

APPENDIX 6

ACOUSTIC PRINCIPAL COMPONENTS ANALYSIS LOADINGS

Loadings of the first two components of a Principal Components Analysis for acoustic data (trill and notes data, respectively), with the eigenvalues, variance, and accumulative variance (in%).
The variables with highest loadings are in bold.

Raw trills			Raw note		
	PC1	PC2		PC1	PC2
High frequency	-0.63	-0.34	High frequency	-0.62	-0.31
Peak frequency	-0.58	0.14	Peak frequency	-0.58	0.04
Low frequency	-0.51	0.46	Low frequency	-0.52	0.49
Band frequency	-0.12	-0.81	Band frequency	-0.10	-0.80
Notes number	0.00	-0.02	Silent/note duration	0.00	0.12
Note emission rate	0.00	0.00	Note emission rate	0.00	-0.02
Trill duration	0.00	0.00	Note duration	0.00	0.00
Silent/note duration	0.00	0.00	Eigenvalue	9,794.84	399.11
Note silence	0.00	0.00	Variance (%)	94.34	3.84
Eigenvalue	9,613.71	167.80	Accumulative variance (%)	94.34	98.18
Variance (%)	97.21	1.70			
Accumulative variance (%)	97.21	98.91			

