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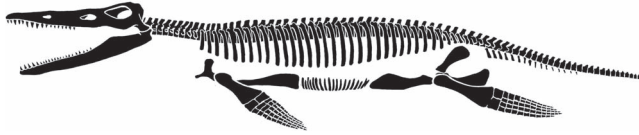
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# B R E V I O R A

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## A NEW SPECIES OF *OSTEOCEPHALUS* STEINDACHNER, 1862 (ANURA, HYLIDAE), FROM BRAZILIAN AMAZONIA

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**ABSTRACT.** Treefrogs of the genus *Osteocephalus* have been the focus of several taxonomic and phylogenetic studies, especially in the last two decades. These recent studies have shown that the diversity of this charismatic Amazonian genus is still largely underestimated. Through the evidence of morphological and molecular data, we describe a new species of the *Osteocephalus alboguttatus* species group from the Purus-Madeira Interfluve, southwestern Brazilian Amazonia. The new species differs from other *Osteocephalus* by having a small body size (snout–vent length 32.1–44.1 mm), skin texture non-sexually dimorphic, dorsum smooth with a few scattered small tubercles, vocal sac single and subgular, frontoparietal ridges not externally visible, and a dark tan brown iris with lighter vermiculation. The rapid increase in the number of new frog species described from the Purus-Madeira Interfluve highlight the importance of sampling poorly explored and remote areas in Amazonia, as well as the value of supporting taxonomic research to accelerate species documentation in face of the biodiversity crisis.

**KEY WORDS:** Amazonas; Arapixi Extractive Reserve; bamboo-dominated forests; *Osteocephalus alboguttatus* species group; Purus-Madeira Interfluve; taxonomy

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## INTRODUCTION

The genus *Osteocephalus* Steindachner, 1862 (Amphibia, Anura, Hylidae), harbors 28 treefrog species distributed across Amazonian lowland rainforests and Andean foothills (Jungfer et al., 2013; AmphibiaWeb, 2021). The first attempt to recognize the diversity in this genus was made by Goin (1961), who provided a key to the genera of hylid frogs, with diagnosis to *Osteocephalus*, including skin texture, shape of vocal sac in males, and cranial osteology. Under this

combination were recognized five species: *Osteocephalus britti* (Melin, 1941), *Osteocephalus buckleyi* (Boulenger, 1882), *Osteocephalus leprieurii* (Duméril and Bibron, 1841), *Osteocephalus pearsoni* (Gauge, 1929), and *Osteocephalus taurinus* Steindachner, 1862. This author also included *Osteocephalus planiceps* Cope, 1874, in the synonymy of *O. leprieurii*.

Trueb and Duellman (1971) were the first to review the taxonomy of this genus extensively and provided relevant baselines for the advancement of associated knowledge. However, the “conservative approach” in which their taxonomic decisions were based also led to a series of misinterpretations. For example, they transferred *O. planiceps* (that was in the synonymy of *O. leprieurii sensu* Goin, 1961) and *Osteocephalus vilarsi* (Melin, 1941) to the synonymy of *O. taurinus*. They also synonymized *Osteocephalus festae* (Peracca, 1904), *Osteocephalus cabrerai* (Cochran and Goin, 1970), and *Osteocephalus carri* (Cochran and Goin, 1970) with *O. buckleyi*, resulting in a considerable reduction of the species content of *Osteocephalus* at that time. Subsequent advances in taxonomy of the genus included the description of *Osteocephalus subtilis* by Martins and Cardoso (1987), the expansion of generic concept by Duellman and Hoogmoed (1992), the description of *Osteocephalus oophagus* by Jungfer and Schiesari (1995), and the resurrection of *O. cabrerai* and *O. planiceps* from the synonymy of *O. buckleyi* and *O. taurinus*, respectively (Duellman and Mendelson, 1995). In the last few decades, the aggregation of new morphological data and advances in analytical capacity led to a massive increase in the taxonomic knowledge of *Osteocephalus*, with the description of half of its currently known species diversity (e.g., Ron and Pramuk, 1999; Jungfer et al., 2000; Jungfer and Lehr, 2001; Jungfer and Hödl, 2002; Lynch, 2002; Moravec et al., 2009;

Jungfer, 2010, 2011; Ron et al., 2012; Jungfer et al., 2013, 2016; Duellman, 2019; Chasiluisa et al., 2020).

Following the advances in taxonomic knowledge, recent studies addressed the phylogenetic relationships within *Osteocephalus* (Moravec et al., 2009; Ron et al., 2010, 2012; Salerno et al., 2012; Jungfer et al., 2013; Ferrão et al., 2019; Chasiluisa et al., 2020). The most comprehensive phylogenetic revision revealed that *Osteocephalus* was in fact paraphyletic (Jungfer et al., 2013). To solve this issue, some species formerly attributed to *Osteocephalus* were transferred to the genus *Tepuihyla* Ayarzagüena, Señaris, and Gorzula, 1993, and to the newly erected genus *Dryaderces* (Jungfer et al., 2013). Furthermore, five species groups were defined by Jungfer et al. (2013) on the basis of molecular data: *Osteocephalus alboguttatus* (Boulenger, 1882), *O. buckleyi*, *O. leprieurii*, *O. planiceps* and *O. taurinus* species groups.

The *O. alboguttatus* species group was recognized to accommodate three species from western Amazonia (Jungfer et al., 2013): *O. alboguttatus*, *Osteocephalus heyeri* Lynch, 2002, and *O. subtilis* Martins and Cardoso, 1987. Despite the absence of morphological synapomorphies supporting this species group, its members share a small body size (snout–vent length < 50 mm), dark irises, and the absence of keratinized spicules on dorsum. *Osteocephalus alboguttatus* was firstly described as *Hyla alboguttata* by Boulenger (1882) and transferred to *Osteocephalus* more than a century later by Wiens et al. (2006). On the other hand, *O. subtilis* and *O. heyeri* were described without arguments supporting its generic allocation. Although the associated information on *O. heyeri* and *O. subtilis* is sparse, the geographic ranges of these species seem narrowly restricted (Jungfer et al., 2013). Data on reproductive behavior are also scarce for

members of this species group, but the smooth dorsum and subgular vocal sacs led Jungfer et al. (2013) to hypothesize that they are phytotelm-breeders. However, this hypothesis is still treated as uncertain (Blotto et al., 2021).

During fieldwork in southwestern Brazilian Amazonia, the first author (P.R.M.-S.) collected some specimens of *Osteocephalus* with morphological affinities with members of the *O. alboguttatus* species group. Nevertheless, we cannot attribute these specimens to any nominal species on the basis of their morphological distinctiveness, which supports them as representatives of a new species. In this study, we combined both morphological and molecular data to describe this new species and allocate it into the *O. alboguttatus* species group.

## MATERIAL AND METHODS

Three adult specimens were collected in the municipality of Sena Madureira (state of Acre) in November 2011 and Arapixi Extractive Reserve (municipality of Boca do Acre, state of Amazonas) in January 2016, both localities in southwestern Brazilian Amazonia. Specimens were euthanatized with peritoneal injection of 2% lidocaine, fixed with 10% neutral buffered formalin, and stored in 70% ethanol. Liver tissue samples were obtained before fixation and preserved in 100% ethanol. We analyzed the external morphology of the newly collected specimens and additional *Osteocephalus* deposited at the following zoological collections: Museu Nacional (MNRJ), Rio de Janeiro, Brazil; Universidade Federal do Acre (UFAC-RB), Rio Branco, Brazil; Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil; Instituto Nacional de Pesquisas da Amazônia (INPA-H), Manaus, Brazil; Coleção Zoológica Paulo Burhein, Universidade Federal do

Amazonas (CZPB), Manaus, Brazil; Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts, U.S.A., Biodiversity Museum University of Kansas (KU), Lawrence, Kansas, U.S.A. (specimens listed in the Appendix 1).

We used a digital caliper under a stereomicroscope to the nearest 0.1 mm to take 10 morphometric measurements following Waters et al. (2016): horizontal eye diameter (ED), eye–nostril distance (EN), foot length (FL), head length (HL), head width (HW), interorbital distance (IOD), snout–vent length (SVL), horizontal tympanum diameter (TD), tibia length (TL), and upper eyelid width (UEW). Color in life was analyzed and described from field notes and digital photographs. Sex was determined by the presence of vocal slits and nuptial excrescences. Webbing formulae followed the standardization of Savage and Heyer (1967) as adapted by Myers and Duellman (1982). Morphological terminology followed Waters et al. (2016).

For molecular analyses, we sequenced a 547–base pair (bp) fragment of the 16S ribosomal RNA, considered a universal barcode for Neotropical amphibians (Vences et al., 2005). Genomic DNA was extracted by standard protocols for a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Wisconsin, U.S.A.), with the target fragment amplified by polymerase chain reaction (PCR) with the primers 16Sar and 16Sbr and standard protocols (Palumbi et al., 1991). The PCR product was purified with PEG (polyethyleneglycol) 8000 and sequenced by standard protocols of the Big Dye Terminator Kit (Applied Biosystems, Waltham, Massachusetts, U.S.A.) in an ABI PRISM 3500 (Applied Biosystems) automated sequencer. We used Geneious (Biomatters, Auckland, New Zealand; Kearse et al., 2012) to check and edit the raw sequence.

The newly generated 16S sequence was included in a data set of homologous sequences downloaded from GenBank representing 48 individuals, 22 species, and all species groups of *Osteocephalus*. Sequences representing the closely related genera *Dryaderces*, *Tepuihyla*, *Itapotihyla*, and *Phyllomedusa* were used as outgroups. Information related to sequences used in molecular analyses are presented in Appendix 2.

The molecular dataset was aligned by the MAFFT (multiple alignment using fast Fourier transform) online server through the E-INS-i strategy with default parameters (Kato and Standley, 2013). With the use of the aligned dataset with 550 bp and 55 terminals, we performed phylogenetic inferences under Bayesian (BI) and maximum likelihood (ML) optimality criteria. Best adjusted model of nucleotide evolution for these inferences was determined under the Bayesian information criterion (BIC) with PartitionFinder v2.1.1 (Lanfear et al., 2017). The BI inference was conducted in MrBayes v3.2.6 (Ronquist et al., 2012) with two parallel runs of the Markov chain Monte Carlo (MCMC) algorithm with 10 million iterations. Convergence of parameters (split frequencies  $SD < 0.01$  and estimated sample size  $> 200$ ) was assessed with Tracer v1.7 (Rambaut et al., 2018), and the maximum clade credibility tree was extracted after 25% burn-in. The ML inference was conducted in the IQ-TREE (Nguyen et al., 2015) online server (Trifinopoulos et al., 2016). Branch support was estimated with 10,000 ultrafast bootstrap replications, 1,000 maximum iterations, and a minimum correlation coefficient of 0.99. Following Huelsenbeck and Ronquist (2005) and Minh et al. (2013), we consider as highly supported branches those with posterior probability values  $> 0.95$  (BI) and ultrafast bootstrap values greater than 95 (ML). We used MEGA v7 (Kumar et al., 2016) to compute uncorrected pairwise

genetic distances between samples of the *O. alboguttatus* species group, with gaps removed with a pairwise deletion option.

## RESULTS

Both phylogenetic inferences yielded identical topologies, with some well-supported major clades and lower supported relationships among them (Fig. 1). Most of these major clades represent the species groups proposed for *Osteocephalus*, corroborating the monophyletic nature of the *O. alboguttatus*, *O. leprieurii*, *O. taurinus*, and *O. planiceps* species groups. Only the *O. buckleyi* species group was recovered as paraphyletic. The tree topology retrieved the *O. alboguttatus* species group as a highly supported clade, in both BI and ML analyses. Our results also show distinct lineages attributed to *O. subtilis*. Two poorly supported subclades were recovered: one containing *O. heyeri* and the upland *O. subtilis* from Serra do Divisor, state of Acre, Brazil, and other containing *O. alboguttatus*, the new species, and lowland *O. subtilis* from its type locality (Cruzeiro do Sul, state of Acre, Brazil). The new species is recovered as sister to *O. alboguttatus* with low (BI) to moderate support (ML) (Fig. 1). The lowest interspecific pairwise genetic distance within the *O. alboguttatus* species group (1.5%) were obtained between *O. heyeri* from Leticia in Colombian Amazonia and lowland *O. subtilis* (Table 1). This result indicates that they can even be conspecific, and their identities should be carefully reviewed and properly assessed. Regarding the new species, genetic distances range from 2.7% (compared with lowland *O. subtilis*) to 5.1% (compared with upland *O. subtilis*). The latter comparison also represented the highest genetic distance between lineages within the *O. alboguttatus* species group included in our analysis. In light of this

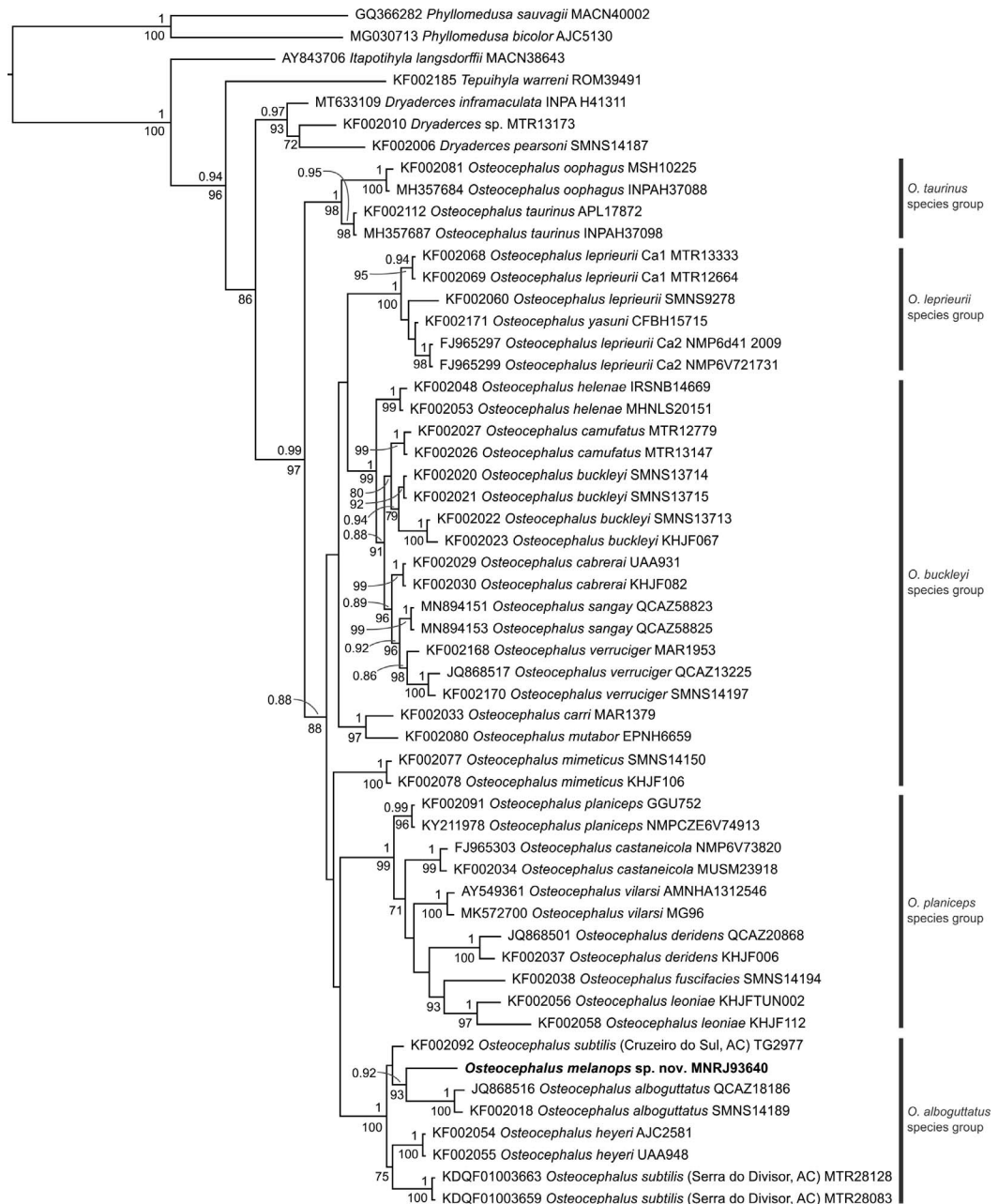


Figure 1. Bayesian (BI) phylogenetic tree of *Osteocephalus* and closely related genera, inferred from 547-bp fragment of the mitochondrial gene 16S. Results of a maximum likelihood (ML) analysis are included, and branch support values for both inferences are shown above and below branches (posterior probabilities for BI and ultrafast bootstraps for ML, respectively). Support values below 0.8 (BI) and 70 (ML) were omitted. The new species described in this study is highlighted in bold.

TABLE 1. Uncorrected pairwise genetic distances between *Osteocephalus melanops* and the most closely related taxa, from the *Osteocephalus alboguttatus* species group. Distances are calculated from a fragment of the mitochondrial gene 16S. Values are presented in percentages (%). The new species is highlighted in bold.

Sample	1	2	3	4	5	6	7	8
<b>1. <i>Osteocephalus melanops</i> MNRJ 93640</b>								
2. <i>Osteocephalus subtilis</i> TG 2977	<b>2.7</b>							
3. <i>Osteocephalus heyeri</i> AJC 2581	<b>3.1</b>	1.5						
4. <i>Osteocephalus heyeri</i> UAA 948	<b>3.1</b>	1.5	0					
5. <i>Osteocephalus alboguttatus</i> SMNS 14189	<b>4.0</b>	2.7	3.5	3.5				
6. <i>Osteocephalus alboguttatus</i> QCAZ 18186	<b>4.0</b>	3.1	3.8	3.8	0.6			
7. <i>Osteocephalus subtilis</i> MTR 28128	<b>5.1</b>	2.4	2.9	2.9	4.1	4.4		
8. <i>Osteocephalus subtilis</i> MTR 28083	<b>5.1</b>	2.4	2.9	2.9	4.1	4.4	0	

molecular distinctiveness and evidence of morphological diagnosis, we describe below the new species of *Osteocephalus*.

#### Taxonomic account

##### *Osteocephalus melanops*, sp. nov.

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Figures 2–5

*Holotype*. MNRJ 93639 (field no. PRMS 350, Figs. 2–5), an adult male collected on 23 January 2016 in the Extractive Reserve Arapixi, municipality of Boca do Acre, state of Amazonas, Brazil (08°58'29"S, 67°51'38"W; 135 m above sea level [a.s.l.]) by Paulo R. Melo-Sampaio and Josimar C. da Silva.

*Paratopotype*. MNRJ 93640 (field no. PRMS 377, Figs. 2–4), an adult female collected on 25 January 2016 by Paulo R. Melo-Sampaio and Josimar C. da Silva.

*Paratype*. UFAC-RB 5553 (Fig. 4), an adult female collected on 26 November 2011 in Ramal do Cassiriã, in front of Colônia Matão, municipality of Sena Madureira, state of Acre, Brazil (09°09'05"S, 68°32'35"W; 166 m a.s.l.), by Paulo R. Melo-Sampaio, Júnior M. L. Maciel, and Evandro Silva.

*Etymology*. The specific epithet *melanops* is derived from Greek “melanos-” meaning black and “ops-” meaning eye. The name is a reference to intense dark coloration of the species' irises.

*Generic Placement*. The new species is assigned to the genus *Osteocephalus* on the basis of molecular phylogenetic relationships and morphological similarities with other species of the genus.

*Diagnosis*. A small-sized species of *Osteocephalus* characterized by: 1) SVL of adults 32.1–44.1 mm; 2) skin texture of dorsum non-sexually dimorphic, smooth with a few scattered small tubercles; 3) skin texture of flanks shagreened; 4) canthus rostralis rounded; 5) frontoparietal ridges not externally visible; 6) supratympanic fold thin, from the posterior edge of the eye, sloping in an arch toward the arm insertion, not reaching tympanum posteroventrally; 7) webbing on inner edge of third finger slightly extending beyond penultimate subarticular tubercle; 8) distal subarticular tubercle on finger IV bifid; 9) dorsum light tan with irregular blotches; 10) throat, chest, and abdomen uniformly cream to white; 11) large cream subocular spot; 12) flanks creamy white, with variable amount of dark vermiculation; 13) vocal sac single, subgular; 14) tibiofibular bones green in preservative; 15) in life, iris dark tan brown, with variable amount of lighter vermiculation.

*Description of Holotype*. Adult male with SVL of 32.1 mm. Head wider than body and slightly wider than long (HW/HL = 1.06). Head slightly concave dorsally. Canthus



Figure 2. Right hand of the male holotype MNRJ 93639 (A) and female paratopotype MNRJ 93640 (B) of *Osteocephalus melanops*. Scale bars = 5 mm.

rostralis straight in dorsal view, slightly elevated. Loreal region concave. Snout protruding in lateral view and truncate in dorsal view. Dentigerous processes of vomers at level of choanae, separated from each other, straight, with 16 vomerine teeth (eight in each side). Supratympanic fold present, running from the eye-tympanum area to above the arm-body insertion. Tympanum conspicuous, rounded, with diameter about 72% of the eye diameter. Axillary membrane present but poorly developed. Dorsum with scattered flat tubercles, laterally smooth, shagreened anteriorly to insertion of the arm. Throat finely

granular; chest shagreened; belly granular. Posterior surface of thighs finely granular. Vent opening with a very small anal flap, located at upper level of thighs. Subcloacal and proximal thigh area covered with several small, flat tubercles.

Arms granular with a longitudinal row of small tubercles throughout the ventrolateral edge of the forearm and reaching the fourth finger. Dark nuptial excrescences covering the prepollex. Subpalmar tubercle large and elliptical. A bifid palmar tubercle and four rounded outer metacarpal tubercles. Subarticular tubercles single and rounded, except the distal tubercle on finger IV (bifid).





Figure 3. Left foot of the male holotype MNRJ 93639 (A) and female paratopotype MNRJ 93640 (B) of *Osteocephalus melanops*. Scale bars = 5 mm.

Relative length of fingers  $3 > 4 > 2 > 1$ . Finger webbing formula: I absent II  $2-3^+$  III  $3^-2^{1/2}$  IV (Fig. 2). Hind limbs smooth except on proximal half of the ventral surface of the thigh (granular) and a few inconspicuous tubercles on the posterior tarsus. A large elliptical inner metatarsal tubercle and a small rounded outer one. Subarticular tubercles present, single, and conical. Toe webbing formula: I  $1^+-2^-$  II  $1^+-1$  III  $2^-2^-$  IV  $2^-1^{1/3}$  V; relative length of adpressed toes:  $4 > 5 \geq 3 > 2 > 1$  (Fig. 3).

*Morphometric Measurements of Holotype.* EN, 3.6; ED, 3.9; FL, 21.0; HL, 11.2; HW, 11.9; IOD, 3.1; SVL, 32.1; TD, 2.8; TL, 17.6; UEW, 4.1.

*Color of Holotype.* In life, dorsum light brown with large cream blotches posteriorly; a well-defined dark brown interorbital transversal bar (Fig. 4) and small dark brown spots in the sacral region; dorsal surfaces of forearms light brown with dark brown marks, dorsal surfaces of thighs and shanks light brown with dark brown transversal bands. Sides of head light brown with a wide

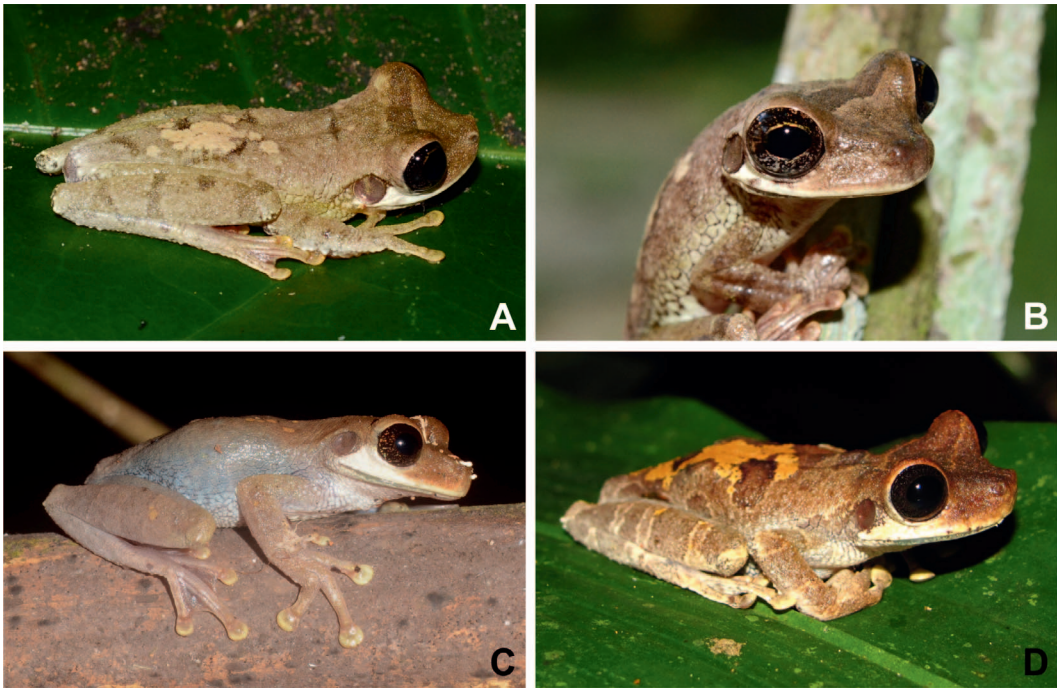


Figure 4. Color in life of *Osteocephalus melanops*. (A, B) Dorsal view and close-up of the iris of the male holotype MNRJ 93639; (C) lateral view of the female paratopotype MNRJ 93640; (D) lateral view of the female paratype UFAC-RB 5553. Photographs: Paulo R. Melo-Sampaio.

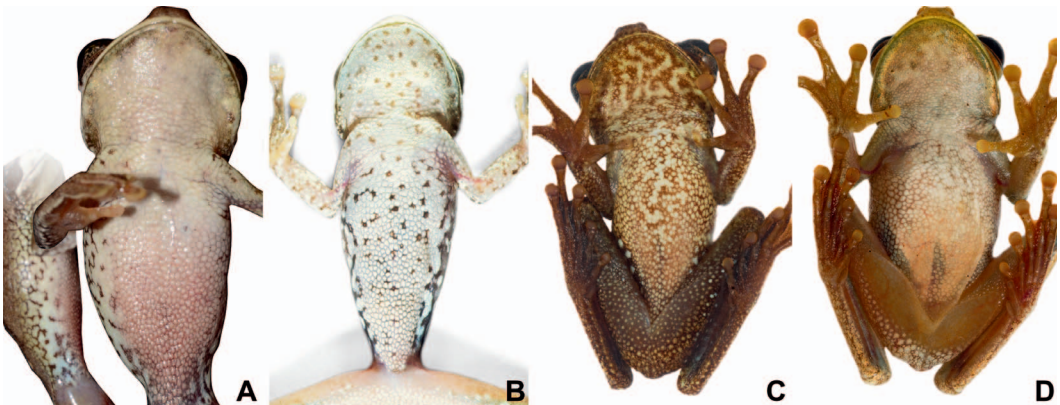


Figure 5. Ventral view of species from *Osteocephalus alboguttatus* species group. (A) *Osteocephalus melanops* holotype MNRJ 93639. (B) *Osteocephalus alboguttatus*, QCAZ 15972. (C) *Osteocephalus heyeri*, unvouchered. (D) *Osteocephalus subtilis*, unvouchered. Photographs: Paulo R. Melo-Sampaio (A); Santiago Ron—www.bioweb.bio (B); Germán Chávez (C, D).

subocular cream spot from midposteroventral border of orbit to periphery of tympanum and border of jaw. Flank cream, areolate, with darker thin reticulation (Fig. 5). Iris dark tan brown with golden vermiculation in upper half and silvery vermiculation in lower half (Fig. 4).

In preservative, dorsal and lateral surfaces light brown, posteriorly with cream blotches. Tympanum light tan brown bordered by a dark brown ring. Wide cream subocular spot, extending posteriorly to the middle border of tympanum. The well-defined brown interorbital bar is evident. Dark brown transversal bar absent in the urostyle. Ventral surface creamy white, ventrolateral surfaces of the body white. Melanophores are evident around the lower lip, fewer on the chin. Forelimb tan laterally and dorsally, with a line of creamy white tubercles ventrally. Posterior surfaces of thighs light tan to orange. Cloacal region tan. Cloacal tubercles creamy white. Green bones, visible through the skin of tibia.

*Variation.* The female paratopotype has a darker iris and a more uniform cream color at dorsal surface of finger and toe discs (Fig. 4). The snout of the paratopotype is also more rounded than the holotype in lateral view. Morphometric measurements of the paratopotype and paratype females are as follow. *MNRJ 93640*: EN, 4.4; ED, 4.5; FL, 27.2; HL, 12.6; HW, 14.4; IOD, 3.8; SVL, 40.6; TD, 2.8; TL, 21.6; UEW, 4.7. *UFAC-RB 5553*: EN, 4.9; ED, 4.7; FL, 27.5; HL, 14.4; HW, 15.2; IOD, 3.9; SVL, 44.1; TD, 3.0; TL, 23.5; UEW, 4.8.

*Comparisons.* Morphological characters of the previously described species are presented in parenthesis unless otherwise stated. *Osteocephalus melanops* can be distinguished from members of the *O. taurinus* species group by having a smaller body size, with a maximum SVL = 44.1 mm (minimum SVL = 64.2 mm in *O. taurinus* and 46.2 mm in *O.*

*oophagus*), a dark brown iris with black reticulation (iris greenish gold with bold dark brown regular radiation), frontoparietal ridges absent (present), keratinized tubercles absent in male dorsum (present) (Jungfer and Schiesari, 1995; Jungfer, 2010; Jungfer et al., 2013).

From members of the *O. lepriurii* species group, *O. melanops* can be distinguished by having males with single and subgular vocal sac (paired and distensible ventrolaterally to laterally in *O. lepriurii* and laterally in *Osteocephalus yasuni* Ron and Pramuk, 1999), keratinized tubercles absent in male dorsum (present), subdigital excrescences absent in male (present in *O. lepriurii* and *O. yasuni*), rounded canthus rostralis (distinct and angular in *O. lepriurii* and *O. yasuni*), green bones (white) and dark brown horizontal bar on iris absent (present in *O. lepriurii* and *O. yasuni*) (Ron and Pramuk, 1999; Jungfer and Hödl, 2002; Jungfer et al., 2013).

From members of the *O. planiceps* species group, *O. melanops* can be distinguished from *Osteocephalus castaneicola* Moravec, Aparicio, Guerrero-Reinhard, Calderón, Jungfer, and Gvoždík, 2009, *O. planiceps*, and *O. vilarsi* by having a maximum SVL = 44.1 mm (minimum SVL = 47.7 mm in *O. castaneicola*, 48.0 mm in *O. planiceps*, and 47.5 mm in *O. vilarsi*), a dark tan brown iris (bronze with black lines radiating from the pupil), and a smooth dorsum (conspicuously tuberculated in adult males of *O. vilarsi* and *O. planiceps*), and frontoparietals not visible through skin (frontoparietals ridges well developed in *O. vilarsi* and *O. planiceps*). From species of this group with similar body sizes (*Osteocephalus deridens* Jungfer, Ron, Seipp, and Almendáriz, 2000, *Osteocephalus fuscifacies* Jungfer, Ron, Seipp, and Almendáriz, 2000, *Osteocephalus leoniae* Jungfer and Lehr, 2001), *O. melanops* can be distinguished by a smaller

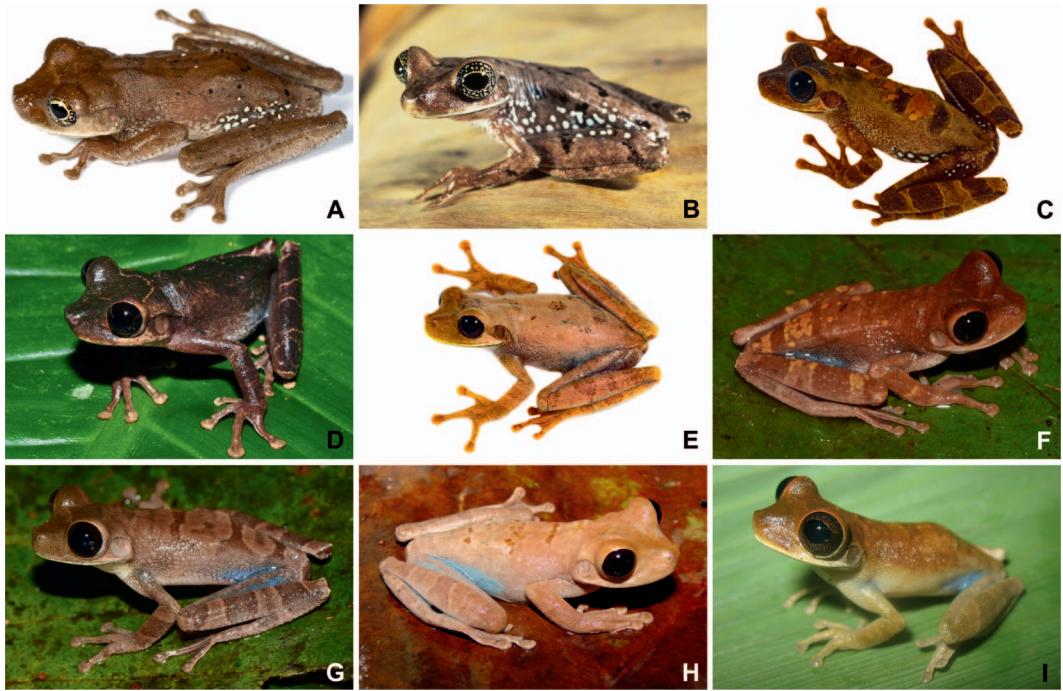


Figure 6. Dorsal view of species from *Osteocephalus alboguttatus* species group. (A, B) *Osteocephalus alboguttatus*, unvouchered specimen and QCAZ 15972, respectively. (C, D) *Osteocephalus heyeri*, SINCHI 0727 and CZPB 1625, respectively. (E–I) *Osteocephalus subtilis*, unvouchered specimen (D), SINCHI 00485 (E), MTR 28225 (F), MTR 28100 (G), and CORBIDI 6176 (H), respectively; *Osteocephalus cf. subtilis*, an unvouchered specimen (I). Photographs: Morley Read—www.bioweb.bio (A); Santiago Ron—www.bioweb.bio (B); Germán Chávez (C, E); Alexandre Almeida (D); Pedro Peloso (F, G); Pablo Venegas (H); Jhon Jairo López Rojas (I).

female SVL, up to 44.1 mm (minimum SVL = 53.2 mm in females of *O. fuscifacies* and *O. leoniae*), and by having a dark tan brown iris (bicolored or golden yellow) (Jungfer et al., 2000; Jungfer and Lehr, 2001; Chávez et al., 2008; Moravec et al., 2009; Ferrão et al., 2019).

From members of the *O. buckleyi* species group, *O. melanops* can be distinguished by having a single vocal sac (paired vocal sac), keratinized tubercles absent in male dorsum (present), posterior surface of thigh immaculate (with large dark brown spots), and canthus rostralis smooth (strongly tuberculate) (Ron et al., 2012; Jungfer et al., 2013, 2016). *Osteocephalus melanops* also has bifid sub-articular tubercle on finger IV (barely bifid or

single in *Osteocephalus duellmani* Jungfer, 2011 and single in *Osteocephalus sangay* Chasiluisa, Caminer, Varela-Jaramillo, and Ron, 2020) (Jungfer, 2011; Chasiluisa et al., 2020).

From the closest related species of the *O. alboguttatus* species group (Figs. 5, 6), *O. melanops* can be distinguished by having a tan brown dorsum with cream blotches (light brown dorsum with small blackish dots in *O. alboguttatus*, brown dorsum with transversal bars in *O. subtilis*), a large cream subocular spot (subocular spot dirty tan in *O. heyeri*), a uniform whitish-cream venter (venter whitish with brown spots in *O. alboguttatus*, cream with dark brown reticulation in *O. heyeri*), flanks and hidden dorsal surfaces of thighs cream with darker reticulation, without

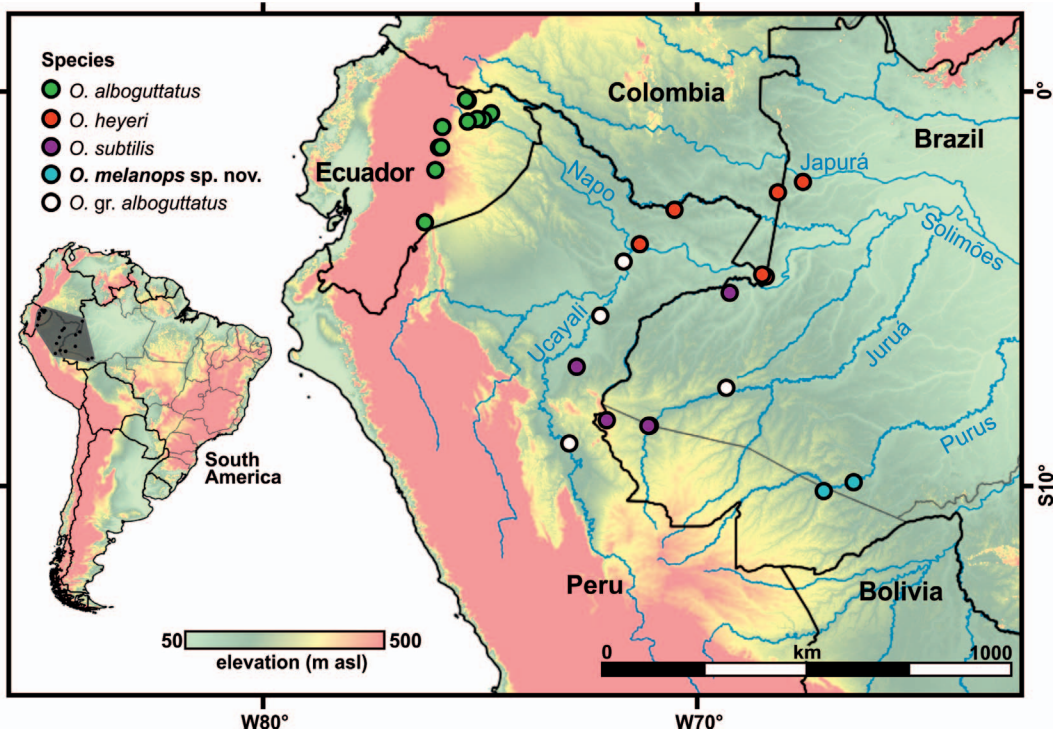


Figure 7. Geographical distribution of the *Osteocephalus alboguttatus* species group. Data compiled from literature (summarized by Frost, 2021) and museum specimens (GBIF online database; GBIF.org, 2021).

small rounded white spots (cream with numerous small rounded white spots in *O. alboguttatus*, brownish and usually with black-edged white spots in *O. heyeri*, uniformly bluish in *O. subtilis*), thin supratympanic folds (well-developed in *O. subtilis*), a reticulated iris (uniformly black iris in *O. subtilis*), and a smaller body size, with maximum SVL = 32.1 mm in male (minimum SVL = 35 mm in *O. subtilis* males) (Boulenger, 1882; Martins and Cardoso, 1987; Lynch, 2002).

**Geographic Distribution and Habitat.** *Osteocephalus melanops* is only known from the type locality and a second nearby site, both at the east bank of the Purus River, southwestern Brazilian Amazonia (Fig. 7). In this region, *O. melanops* is sympatric with other four species of *Osteocephalus*: *O.*

*castaneicola*, *Osteocephalus helenae* (Ruthven, 1919), *O. lepreurii*, and *O. taurinus* (Fig. 8). Individuals of the new species were found from 0.5 to 2 m height on the vegetation of a bamboo-dominated forest both in *terra firme* (nonflooded) and *várzea* (seasonally flooded) habitats always close to small streams. Type locality is a site with low anthropogenic disturbance, since it is a conservation unit for sustainable use, where activities such as hunting and plant extractivism have little effect on frog fauna, and small clearing and controlled burning for subsistence plantations play minor roles.

## DISCUSSION

Twenty-nine species of *Osteocephalus* are currently recognized (AmphibiaWeb, 2021;

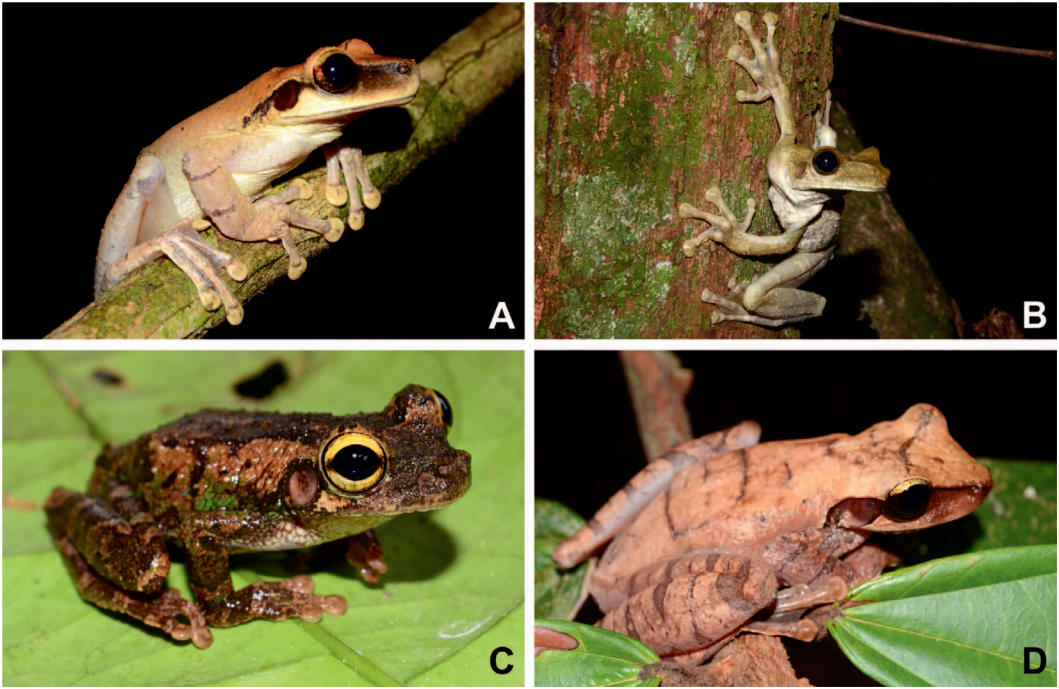


Figure 8. Sympatric species of *Osteocephalus* at Extractive Reserve Arapixi, municipality of Boca do Acre, state of Amazonas, Brazil. (A) *Osteocephalus lepreurii* (field no. PRMS 0312). (B) *Osteocephalus taurinus* (field no. PRMS 0155). (C) *Osteocephalus helenae* (field no. PRMS 0261). (D) *Osteocephalus castaneicola* (field no. PRMS 0082).

present study, but see Frost, 2021), of which nine are known to occur in southwestern Brazilian Amazonia: *O. buckleyi*, *O. cabrerai*, *O. castaneicola*, *O. deridens*, *O. lepreurii*, *O. melanops*, *O. planiceps*, *O. subtilis*, and *O. taurinus* (Souza, 2009; Jungfer et al., 2013; Ferrão et al., 2019; Melo-Sampaio et al., 2021a; this study). Nevertheless, the description of *O. melanops* and the existence of distinct lineages of *Osteocephalus* in this region (Jungfer et al., 2013; Vacher et al., 2020; present study) demonstrates that the diversity of the genus is still underestimated. Although Jungfer et al. (2013) stated that the discovery of candidate new species within the *O. alboguttatus* species group was unlikely, the description of *O. melanops* and the existence of two highly distinct lineages associated with *O. subtilis* evidence that the

knowledge on real diversity of this species group is still incipient.

Increasing the resolution of *Osteocephalus* diversity also depends on the constant review of historically published data (e.g., Ferrão et al., 2019; Ortiz et al., 2020). While conducting this research, we came across with a mistake published in the original description of *O. heyeri* (Lynch, 2002) that, despite having already been briefly corrected by Jungfer (2010), deserves to be highlighted again to prevent future misinterpretations. On the basis of external morphology of specimens depicted in Lynch (2002, fig. 1), the lower right photograph clearly depicts the *O. cabrerai* specimen (ICN jdl22733), and not an *O. heyeri* as stated (Jungfer, 2010). Such a mistake raises doubts about the actual voucher correspondence for the

other three depicted specimens of *O. heyeri*. However, we suggest that a simple inversion occurred between two photographs (upper left vs. lower right), and the specimen depicted on the upper left should correspond to the *O. heyeri* female holotype (ICN 46916), and the remaining two specimens should be correctly referenced. Moreover, on the basis of morphological affinities such as uniform dark iris and flanks lacking well-defined spots, we hypothesize that the specimen depicted in the upper right of this figure most likely represents an *O. subtilis* instead of an *O. heyeri*. The recent records of *O. subtilis* occurring close to the known distribution of *O. heyeri* in the Javari River basin (López and Ortega, 2013; Chávez et al., 2016) reinforce that *O. heyeri* and *O. subtilis* must be sympatric at least in part of their distributions (see Fig. 7), and errors in their taxonomic attributions may be occurring more widely.

In fact, misidentifications appear to be even more pervasive in the taxonomic history of the *O. alboguttatus* species group. We analyzed the preserved paratype of *O. heyeri* (KU 220885), and on the basis of external morphology, we also suggest that it should not be conspecific with this species because of the presence of conspicuous dark blotches on its dorsum. This hypothesis is supported by the red-striped iris this specimen had in life, according to the photograph depicted in Duellman (2019) (in which the voucher number is incorrectly referenced as KU 220285). Such a characteristic is not known for members of the *O. alboguttatus* species group, and we suggest that specimen KU 220885 should be in fact a member of the *O. planiceps* species group. Therefore, all these arguments point to a composite type series of *O. heyeri* and that this may also be occurring for other members of the *O. alboguttatus* species group, but such hypoth-

eses still need to be tested after broader analyses of their type material.

New hypotheses and increased resolution on systematics and taxonomy of the *O. alboguttatus* species group are also largely hampered by sampling biases. Methods usually applied in anuran inventories are known to underestimate the diversity of species with natural low abundances and low-frequency calls, which seems to be the case for members of this species group (Cardoso and Vielliard, 1990). Another hampering factor is the pervasive misidentification led by phenotypic conservatism among species in this group (Jungfer et al., 2013; this study), especially when collection and posterior examination of specimens are not feasible (López and Ortega, 2013). Additionally, the lack of diagnostic characteristics in preserved specimens, especially the coloration patterns, also prevents the unveiling of the species diversity in this group. Therefore, we highlight that the documentation of coloration in life is crucial for the advancement of the taxonomic knowledge related to this species group.

The description of *O. melanops* expands the distribution of the *O. alboguttatus* species group to the Purus-Madeira Interfluve, where recent samplings also have unveiled several new species of amphibians (e.g., Melo-Sampaio et al., 2013, 2018; Ferrão et al., 2016, 2017, 2018a, b, 2020a, b; Lima et al., 2020). The increasing number of species descriptions in this interfluve is a result of two main factors: the crescent (but still low) representativeness of Amazon-based taxonomists, and the increase of inventories in poorly sampled areas (Ferrão et al., 2016; Melo-Sampaio et al., 2020, 2021b). Alarmingly, conservation of this highly biodiverse region is severely threatened by habitat loss led by illegal deforestation and wildfires (Barni et al., 2015; Brando et al., 2020; Silveira et al., 2020; Souza et al., 2020; Melo-

Sampaio et al., 2021c). Although several protected areas exist in southwestern Amazonia (e.g., Arapixi Extractive Reserve and National Forest of Iquiri), the increase in small-patch deforestation of protected areas in this region limits the effective conservation of its biodiversity (Kalamandeen et al., 2018). A number of conservation strategies are necessary to improve the effectiveness of protected areas and support the delimitation of new protected areas in this region. We highlight the need for a massive investment in training new taxonomists and funding taxonomic studies in poorly sampled areas to accelerate both the documentation and description of Amazonian biodiversity.

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## Appendix 1. Examined specimens. Symbols: \* = photograph.

*Osteocephalus castaneicola* ( $n = 37$ ). BRAZIL: ACRE: Feijó (UFAC-RB 4437, 4443, 4493); Mâncio Lima (UFAC-RB 1662, 1864, 1966); Manoel Urbano (UFAC-RB 4517, 4518); Marechal Thaumaturgo (UFAC-RB 2197, 2198, 2468, 2652, 2654); Sena Madureira (UFAC-RB 4706, 4726); Senador Guiomard (MNRJ 91672, UFAC-RB 4586, 4587, 4588, 4589 4590, 4591, 5197, 5652, 5653, 5753); Porto Acre (UFAC-RB 5756, 5757). AMAZONAS: Boca do Acre (UFAC-RB 4149, PRMS 0082, 0087, 0101, 0122, 0358). PERU: LORETO: Contamana (UFAC-RB 3598, 3599, 3605). *Osteocephalus helenae* ( $n = 33$ ). BRAZIL: ACRE: Senador Guiomard (UFAC-RB 4709, 4710, 4743–4750, 5748). AMAZONAS: Boca do Acre (PRMS 0105, 0165–0184, 0261, 0308). *Osteocephalus heyeri* ( $n = 1$ ). BRAZIL: AMAZONAS: Rio Japurá (CZPB 1625). *Osteocephalus lepieurii* ( $n = 9$ ). BRAZIL: ACRE: Bujari (UFAC-RB 5730–5734). AMAZONAS: Boca do Acre (PRMS 0143, 0158, 0312, 0355). *Osteocephalus*

*oophagus* ( $n = 5$ ). BRAZIL. AMAZONAS: Manaus, Reserva Ducke (INPA-H 36007, 36902, 37088, 37095, 37168). *Osteocephalus planiceps* ( $n = 28$ ). ECUADOR. NAPO: Puerto Misahulli at La Cruz Blanca (MCZ-A 111188, 111190), Limoncocha (MCZ-A 98000, 98019, QCAZ 63543), Laguna Taracoa (MCZ-A 97755). ORELLANA: Yasuni National Park (QCAZ 14844\*, 20797\*, 40987\*, 51085\*, 55257\*, 55297–55298\*, 55364\*, 55378\*, 55380\*, 64119–64121\*, 64125–64131\*), Napo River (QCAZ 43891\*, 44420\*). *Osteocephalus subtilis* ( $n = 10$ ). BRAZIL: ACRE: Cruzeiro do Sul (MZUSP 60561, holotype); Mâncio Lima (UFAC-RB 1457–1463, 2741). PERU: UCAYALI: Ojo de Contaya (UFAC-RB 3565). *Osteocephalus gr. alboguttatus* ( $n = 4$ ). BRAZIL: AMAZONAS: Ipixuna, rio Juruá (INPA-H 2681, 2688, 2690, 2826). *Osteocephalus taurinus* ( $n = 11$ ). BRAZIL. ACRE: Sena Madureira (UFAC-RB 4728, 5559, 5735, 5736); Rio Branco (UFAC-RB 5752). AMAZONAS: Boca do Acre (PRMS 0155, 0236, 238); Careiro Castanho (INPA-H 25874, 25875, 25881). *Osteocephalus vilarsi* ( $n = 19$ ). BRAZIL. AMAZONAS: São Gabriel da Cachoeira, Missão Taracua (INPA-H 40458, 40461, 40470, GNM 488\* [holotype]); Novo Airão, Rio Negro Sustainable Development Reserve (INPA-H 40452, 40454, 40456, 40459, 40460, 40462, 40465, 40468), Jaú National Park (INPA-H 40455, 40463, 40464, 40467, 40466, 40472, 40473). *Osteocephalus yasuni* ( $n = 4$ ). BRAZIL. ACRE: Manuel Urbano (UFAC-RB 4519). ECUADOR. NAPO: Yasuni Scientific Research Station (QCAZ 11336\* [holotype], QCAZ 10879\* [paratopotype], QCAZ 11329\* [paratopotype]).

**Appendix 2.** Samples included in molecular analyses, with respective data on voucher/field numbers, localities of occurrence and GenBank accession numbers.

Species	Species Group	Voucher/ Field number	Locality	GenBank accession number
<i>Osteocephalus alboguttatus</i>	<i>O. alboguttatus</i>	QCAZ18186	Ecuador, Napo, Jatun Sacha	JQ868516
<i>Osteocephalus alboguttatus</i>	<i>O. alboguttatus</i>	SMNS14189	Ecuador, Napo, Jatun Sacha	KF002018
<i>Osteocephalus heyeri</i>	<i>O. alboguttatus</i>	AJC2581	Colombia, Amazonas, Leticia	KF002054
<i>Osteocephalus heyeri</i>	<i>O. alboguttatus</i>	UAA948	Colombia, Amazonas, Leticia	KF002055
<i>Osteocephalus melanops</i> sp. nov.	<i>O. alboguttatus</i>	MNRJ93640	Brazil, Amazonas, Boca do Acre	OL439063
<i>Osteocephalus subtilis</i>	<i>O. alboguttatus</i>	TG2977	Brazil, Acre, Cruzeiro do Sul, Mata do BIS	KF002092
<i>Osteocephalus subtilis</i>	<i>O. alboguttatus</i>	MTR28128	Brazil, Acre, Serra do Divisor	KDQF01003663
<i>Osteocephalus subtilis</i>	<i>O. alboguttatus</i>	MTR28083	Brazil, Acre, Serra do Divisor	KDQF01003659
<i>Osteocephalus camufatus</i>	<i>O. buckleyi</i>	MTR12779	Brazil, Amazonas, Igarapé-Açu, Rio Abacaxis	KF002027
<i>Osteocephalus camufatus</i>	<i>O. buckleyi</i>	MTR13147	Brazil, Amazonas, Igarapé-Açu, Rio Abacaxis	KF002026
<i>Osteocephalus buckleyi</i>	<i>O. buckleyi</i>	SMNS13714	Ecuador, Napo, Jatun Sacha	KF002020
<i>Osteocephalus buckleyi</i>	<i>O. buckleyi</i>	SMNS13715	Ecuador, Napo, Jatun Sacha	KF002021
<i>Osteocephalus buckleyi</i>	<i>O. buckleyi</i>	SMNS13713	Ecuador, Orellana, Yasuní	KF002022
<i>Osteocephalus buckleyi</i>	<i>O. buckleyi</i>	KHJF067	Peru, Loreto, 28 km S Iquitos	KF002023
<i>Osteocephalus cabrerai</i>	<i>O. buckleyi</i>	UAA931	Colombia, Amazonas, Leticia	KF002029
<i>Osteocephalus cabrerai</i>	<i>O. buckleyi</i>	KHJF082	Peru, Loreto, Río Iauasiyacu	KF002030
<i>Osteocephalus carri</i>	<i>O. buckleyi</i>	MAR1379	Colombia, Boyacá, Pajarito	KF002033
<i>Osteocephalus helenae</i>	<i>O. buckleyi</i>	IRSNB14669	Guyana, Potaro-Siparuni, Kaieteur NP	KF002048
<i>Osteocephalus helenae</i>	<i>O. buckleyi</i>	MHNLS20151	Venezuela, Delta Amacuro, Reserva Forestal Río Grande	KF002053
<i>Osteocephalus mimeticus</i>	<i>O. buckleyi</i>	SMNS14150	Peru, Huánuco, Fundo Flor, Río Pachitea	KF002077
<i>Osteocephalus mimeticus</i>	<i>O. buckleyi</i>	KHJF106	Peru, Huánuco, Boquerón del Padre Abad	KF002078
<i>Osteocephalus mutabor</i>	<i>O. buckleyi</i>	EPNH6659	Ecuador, Napo, Carretera Jondachi-Coca km 44	KF002080
<i>Osteocephalus sangay</i>	<i>O. buckleyi</i>	QCAZ58823	Ecuador, Morona Santiago, Sardinayacu	MN894151
<i>Osteocephalus sangay</i>	<i>O. buckleyi</i>	QCAZ58825	Ecuador, Morona Santiago, Sardinayacu	MN894153
<i>Osteocephalus verruciger</i>	<i>O. buckleyi</i>	MAR1953	Colombia, Caquetá, Municipio de Florencia, Vereda Sucre	KF002168
<i>Osteocephalus verruciger</i>	<i>O. buckleyi</i>	QCAZ13225	Ecuador, Cascada San Rafael, Napo	JQ868517
<i>Osteocephalus verruciger</i>	<i>O. buckleyi</i>	SMNS14197	Ecuador, Sucumbíos, Río Azuela	KF002170
<i>Osteocephalus leprieurii</i>	<i>O. leprieurii</i>	MTR13333	Brazil, Amazonas, Palhalzinho, Rio Abacaxis	KF002068
<i>Osteocephalus leprieurii</i>	<i>O. leprieurii</i>	MTR12664	Brazil, Amazonas, Ponta do Apinari, Rio Abacaxis	KF002069
<i>Osteocephalus leprieurii</i>	<i>O. leprieurii</i>	SMNS9278	French Guiana, Cayenne, Arataí	KF002060
<i>Osteocephalus leprieurii</i>	<i>O. leprieurii</i>	NMP6V73820	Bolivia, Pando, San Antonio del Matti	FJ965303
<i>Osteocephalus leprieurii</i>	<i>O. leprieurii</i>	MUSM23918	Peru, Cusco, Miaria	KF002034
<i>Osteocephalus yasuni</i>	<i>O. leprieurii</i>	CFBH15715	Brazil, Acre, Tarauaca	KF002171

## Appendix 2. Continued.

Species	Species Group	Voucher/ Field number	Locality	GenBank accession number
<i>Osteocephalus castaneicola</i>	<i>O. planiceps</i>	NMP6d412009	Bolivia, Pando, Palmira	FJ965297
<i>Osteocephalus castaneicola</i>	<i>O. planiceps</i>	NMP6V72173	Bolivia, Pando, Nacebe	FJ965299
<i>Osteocephalus deridens</i>	<i>O. planiceps</i>	QCAZ20868	Ecuador, Yasuni, Orellana	JQ868501
<i>Osteocephalus deridens</i>	<i>O. planiceps</i>	KHJF006	Peru, Loreto, 28 km S Iquitos	KF002037
<i>Osteocephalus fuscifacies</i>	<i>O. planiceps</i>	SMNS14194	Ecuador, Napo, Jatun Sacha	KF002038
<i>Osteocephalus leoniae</i>	<i>O. planiceps</i>	KHJFTUN002	Peru, Amazonas, Santa María de Nieva	KF002056
<i>Osteocephalus leoniae</i>	<i>O. planiceps</i>	KHJF112	Peru, San Martín, 14 km NE Tarapoto	KF002058
<i>Osteocephalus planiceps</i>	<i>O. planiceps</i>	GGU752	Peru, Ucayali, Imiria	KF002091
<i>Osteocephalus planiceps</i>	<i>O. planiceps</i>	NMPCZE6V74913	Peru, Ucayali, Imiria	KY211978
<i>Osteocephalus vilarsi</i>	<i>O. planiceps</i>	AMNHA1312546	Venezuela, Amazonas, Neblina Base Camp on Rio Mawarinuma (=Rio Baria)	AY549361
<i>Osteocephalus vilarsi</i>	<i>O. planiceps</i>	MG96	Brazil, Amazonas, Missao Taracua	MK572700
<i>Osteocephalus oophagus</i>	<i>O. taurinus</i>	MSH10225	Brazil, Amazonas, E. E. Anavilhanas	KF002081
<i>Osteocephalus oophagus</i>	<i>O. taurinus</i>	INPA-H37088	Brazil, Amazonas, Reserva Ducke	MH357684
<i>Osteocephalus taurinus</i>	<i>O. taurinus</i>	APL17872	Brazil, Amazonas, Manaus, Conjunto Petros	KF002112
<i>Osteocephalus taurinus</i>	<i>O. taurinus</i>	INPA-H37098	Brazil, Amazonas, Reserva Ducke	MH357687
<i>Dryaderces inframaculata</i>	outgroup	INPA-H41311	Brazil, Pará, middle Tapajós River	MT633109
<i>Dryaderces pearsoni</i>	outgroup	SMNS14187	Bolivia, Beni, Rurrenabaque	KF002006
<i>Dryaderces</i> sp.	outgroup	MTR13173	Brazil, Amazonas, Areal, Rio Abacaxis	KF002010
<i>Tepuihyla warreni</i>	outgroup	ROM39491	Guyana, District 7, Mt. Ayanganna	KF002185
<i>Itapotihyla langsdorffii</i>	outgroup	MACN38643	Argentina, Misiones, General Belgrano, 10 km N Bernardo de Irigoyen, Salto Andresito	AY843706
<i>Phyllomedusa bicolor</i>	outgroup	AJC5130	Colombia, Amazonas, Amazonas, Leticia, Reserva Natural Park	MG030713
<i>Phyllomedusa sauvagii</i>	outgroup	MACN40002	Argentina, Salta, Oran, Pichanal, Ruta Prov. 5 y Rio San Francisco	GQ366282