

Five new country records of Amazonian anurans for Brazil, with notes on morphology, advertisement calls, and natural history

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Abstract. We report for the first time the occurrences in Brazil of *Boana hobbsi*, *Osteocephalus deridens* and *Tepuihyla shushupe* (Hylidae), and confirm the occurrences of *Ranitomeya defleri* and *Ranitomeya variabilis* (Dendrobatidae) referring voucher specimens obtained from an expedition to Estação Ecológica Juami-Japurá, state of Amazonas, Brazil, in February 2017. Additionally, we provide data on morphology of voucher specimens, advertisement calls, field observations and geographic distribution of these species. We also describe the external morphology of the female *R. defleri*, which was previously unknown to science.

Keywords. Amazon, Amphibia, conservation units, Dendrobatidae, Hylidae, Japurá River

Introduction

Brazil houses the greatest diversity of amphibian species in the world, with current estimates surpassing a thousand species (Segalla et al., 2016). However, early assessments in the 21th century correctly assumed amphibian species richness in Brazilian Amazonia to be severely underestimated, as the number of species known to occur in the region at that time ranged between ca. 160 and 230 species (Azevedo-Ramos and Galatti, 2002; Ávila-Pires et al., 2007). Roughly a decade later, we know that at least 370 species inhabit Amazonian ecosystems in Brazil (Segalla et al., 2016; Frost, 2018), a number that will likely increase as a result of revisionary work on geographically widespread species

complexes (e.g., Gehara et al., 2014; Ferrão et al., 2016; Rojas et al., 2018). A second source of discoveries of Amazonian species are expeditions to poorly sampled areas. Recent fieldwork in relict mountain landscapes in the northern reaches of Roraima and Amazonas, for example, uncovered at least seven amphibian species unknown to science, additional species with taxonomic uncertainties and new geographic records (Moraes et al., 2017; Jung, 2018).

The Estação Ecológica Juami-Japurá (ESEC Juami-Japurá, hereafter) is an 8,315 km² conservation unit located in northwestern state of Amazonas, in the municipality of Japurá. It encompasses the whole drainage of the Juami River, a southern blackwater tributary of the larger Japurá River, and covers areas of seasonally flooded and *terra-firme* forest environments. This conservation unit was established in 1983 for the purpose of scientific research and biodiversity conservation, and no human settlements are currently found within the limits of the reserve (ICMBio, 2007). In a recent field expedition to ESEC Juami-Japurá, we discovered two species new to science (*Allobates juami* Simões et al., 2018a and *Phyzelaphryne nimio* Simões et al., 2018b) and recorded for the first time the occurrence of five anuran species in Brazil. Below, we describe these noteworthy geographic records, providing additional information on species identification, field observations and geographic distribution.

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Materials and Methods

Fieldwork was carried out between 02 and 16 February 2017, during the rainy season (November to May – ICMBio, 2007), within ESEC Juami-Japurá, in a sampling site approximately 45 km upstream the mouth of the Juami River, along a southern small tributary (Igarapé da Fartura). Both Juami and Igarapé da Fartura are fast-running, blackwater courses. Streamside vegetation is composed of *terra-firme* and seasonally flooded (*igapó*) rainforests, with frequent patches of white-sand vegetation (*campinaranas*). Surveys were conducted along trails radiating from an abandoned hunting site (1.9606°S, 67.9369°W). We explored the streamside environments (*igapó* and *campinarana* vegetation physiognomies) and established narrow trails across *terra-firme* forests heading south and west of the camping site. Trails were approximately 0.5 m wide, and ca. 500–800 m long. All records were georeferenced with a GPS receiver Garmin 60CSX with datum WGS 84.

We conducted nocturnal and diurnal visual and acoustic surveys along trails. When possible, call recordings were conducted with Marantz PMD620 MKII or Tascam digital recorders using built-in microphones, at a sampling rate of 44.1 kHz. Air temperature at time of recording was measured with a digital thermometer. Spectral analyses of calls were conducted with frequency resolution of 82 Hz and 2048 points, using Blackman window type. Acoustic analyses were conducted in Raven Pro 1.4 (Bioacoustics Research Program, 2011). All specimens were captured manually, transported to an improvised laboratory in the field, anesthetized and killed with topical benzocaine solution (50 mg/g), fixed in 10% formalin solution and preserved in 70% ethanol approximately 15 days after fixation. Snout-vent length (SVL) of preserved specimens was measured with a digital caliper to the nearest 0.1 mm. All voucher specimens were housed in the amphibian collection of Museu de Ciências e Tecnologia of the Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Brazil.

The females of one of the species recorded for the first time in Brazil, *Ranitomeya defleri* Twomey and Brown, 2009, were previously unknown to science. Hence, we provide morphometric measurements and description of morphometric traits of the single female voucher specimen. Measurements and morphological terminology followed those of the original description (Twomey and Brown, 2009).

Results

Ranitomeya defleri Twomey and Brown, 2009

Voucher specimens. One male (MCP 13916, SVL = 14.2 mm) and one female (MCP 13912; SVL = 15.5 mm) collected on 09 February 2017 (1.9620°S, 67.9346°W, 81 m a.s.l.). One juvenile (MCP 13911; SVL = 9.5 mm) and one male (MCP 13915; SVL = 13.8 mm) collected on 08 and 12 February 2017, respectively (1.9606°S, 67.9369°W, 71 m a.s.l.).

General description. A brightly coloured species of *Ranitomeya*, distinguished from other congeneric species by its small size and colour pattern (Fig. 1A). In life, dorsal background colour of head and body is solid black, with irregular iridescent yellow markings. A greenish yellow interrupted vertebral stripe is present on the posterior two-thirds of dorsum (Figs. 1 and 2). A yellow hourglass-shaped mark is present ventrally, from throat to chest, on black background (Fig. 1B). Ventral surfaces of body and limbs with iridescent blue reticulations on black background, forming round black spots. Finger I distinctly shorter than Finger II. Disc of Finger III more than 2.0 times wider than finger width.

Female description. Specimen MCP 13912 is the first female *R. defleri* known to science. To contribute to the knowledge on the variation of the species, we report morphometric values and character states that differ from previous accounts based only on males, as well as the range of morphometric measurements for the two adult males (in parentheses). Measurements are in mm: Femur length = 7.5 (6.0–7.0), tibia length = 7.1 (5.1–6.5); distance from knee to knee when legs are positioned orthogonally to body axis = 13.3 (10.6–11.2); foot length 6.2 (5.4–6.0); lengths of fingers I, II, III and IV, respectively = 2.5 (2.0), 3.2 (2.5–2.7), 4.4 (3.7–3.8) and 3.5 (2.0–3.0); width of disc on tip of Finger III = 1.1 (1.0); width of Finger III below disc = 0.5 (0.4–0.5); head length = 6.5 (4.5); head width = 5.9 (4.6–5.0); body width measured at the level of axillae = 6.9 (5.4–5.8); width of upper eyelid = 1.5 (1.4–1.6); interorbital distance = 1.8 (1.5); horizontal tympanum diameter = 2.0 (1.0–1.1); horizontal eye diameter = 2.0 (1.8–2.0); distance from the posterior corner of the eye to tympanum = 1.2 (0.4–0.3). Dorsal skin smooth, weakly granular only on limbs. Skin smooth ventrally. Body shape ovoid in dorsal and ventral views, head width evidently narrower than body width. Head longer than wide, with a distinctively wider gap between posterior corner of the eye and tympanum than that of male specimens. Snout round to truncate in dorsal view, short and round in lateral view. Nares open



Figure 1. New species records for Brazil obtained in Estação Ecológica Juami-Japurá, state of Amazonas, in February 2017. (A) Male *Ranitomeya defleri* (MCP 13916) photographed while carrying two tadpoles. (B) Ventral view of the same specimen. (C) Male *Ranitomeya variabilis* (MCP 13914). (D) Nocturnal colouration of a male *Boana hobbsi* (MCP 13361). (E) Male *Osteocephalus deridens* (MCP 13770). (F) Male *Tepuihyala shushupe* (MCP 13432).

laterally, visible in ventral view, not visible in dorsal view. Tympanum round, distinguishable only under magnification. Forearm length 56% of tibia length. Inner and outer metatarsal tubercles larger and more protruding than those of male specimens. Female larger than male specimens, with no overlap observed between morphometric measurements except eye diameter, width of eyelid and width of the distal phalanx of Finger III. The remaining morphological traits agree with the description of *R. defleri* (Twomey and Brown, 2009).

Field notes. All specimens were found in *terra-firme* forest, always perched at least 1.2 m above ground on broadleaf understory vegetation (herbs from families Maranthaceae and Cyclanthaceae). The male MCP 13916 was found at night, perched 1.2 m high on broadleaf understory vegetation, carrying two tadpoles on its lower back (Fig. 1A). The female MCP 13912 was found less than 5.0 cm from the male, sheltered between the same two overlaying leaves.

Geographic distribution. Although the species was assumed to occur in Brazil (Twomey and Brown, 2009; Segalla *et al.*, 2016) no confirmatory records were published or made available in GBIF (GBIF Secretariat, 2017a). Our new record extends the species distribution to Brazil, and 195 km (distances in straight line throughout the paper) southeast of the nearest record, on the Apaporis River, Colombia (Brown *et al.*, 2011; Fig. 4).

***Ranitomeya variabilis* (Zimmermann and Zimmermann, 1988)**

Voucher specimens. One adult male (MCP 13914, SVL = 14.8 mm) collected on 07 February 2017 (1.9646°S, 67.9358°W, 86 m a.s.l.). Two adult males (MCP 13910 and 13913, SVL = 14.5 and 15.3, respectively) collected on 09 February 2017 (1.9638°S, 67.9352°W).

General description. Colour pattern of specimens collected in our sampling site match that of the Lowland-Vaupés morphotype of Brown *et al.* (2011), which is characterized by the presence of a black spot on tip of snout, and the mid dorsal yellow stripe terminating at about the level of arm insertion, forming a black “Y” mark on dorsum (Fig. 1C). Throat is bright yellow, and remaining ventral surfaces are finely reticulated in light blue, on a black background.

Field notes. Male MCP 13914 was found at night in between the leaves of a tank bromeliad, approximately 2.5 m above the forest floor. The male guarded an egg clutch containing five large, completely transparent eggs, with well-developed embryos (MCP 13803). The other two males were also collected at night, between



Figure 2. Dorsal (top) and ventral (bottom) views of a preserved female (MCP 13912, left) and male (MCP 13915, right) *Ranitomeya defleri* collected at Estação Ecológica Juami-Japurá, Amazonas, Brazil.

leaves of tank-bromeliads which grew on fallen logs, overhanging a small forest stream in an area of *terra-firme*.

Geographic distribution. Although the species was assumed to occur in Brazil (Brown *et al.*, 2011; Segalla *et al.*, 2016), the species distribution was in fact extrapolated from Colombian geographic records close to the border with Brazil (J.L. Brown and E. Twomey, pers. comm.), with no actual voucher specimens from Brazil. Also, as in *R. defleri*, no confirmatory records were published or made available in GBIF (GBIF Secretariat, 2017b). Our new record extends the species distribution to Brazil, and approximately 195 km southeast of the nearest record, along the Caquetá River, in Colombia (Brown *et al.*, 2011; Fig. 4).

***Boana hobbsi* (Cochran and Goin, 1970)**

Voucher specimens. Two males (MCP 13360, SVL = 40.7 mm; MCP 13361, SVL = 37.7 mm), collected on 11 February 2017 (1.9648°S, 67.9422°W, 65 m a.s.l.).

General description. At night, specimens have brown dorsum with scattered, irregularly spaced dark-brown spots (Fig. 1D). A cream dorsolateral stripe extends from tip of snout, over the upper eyelid and tympanum, and posteriorly, reaching the urostyle region. Iris is yellow, light brown around the pupil. Lateral surfaces of head and body same colour as dorsum, fading to light cream

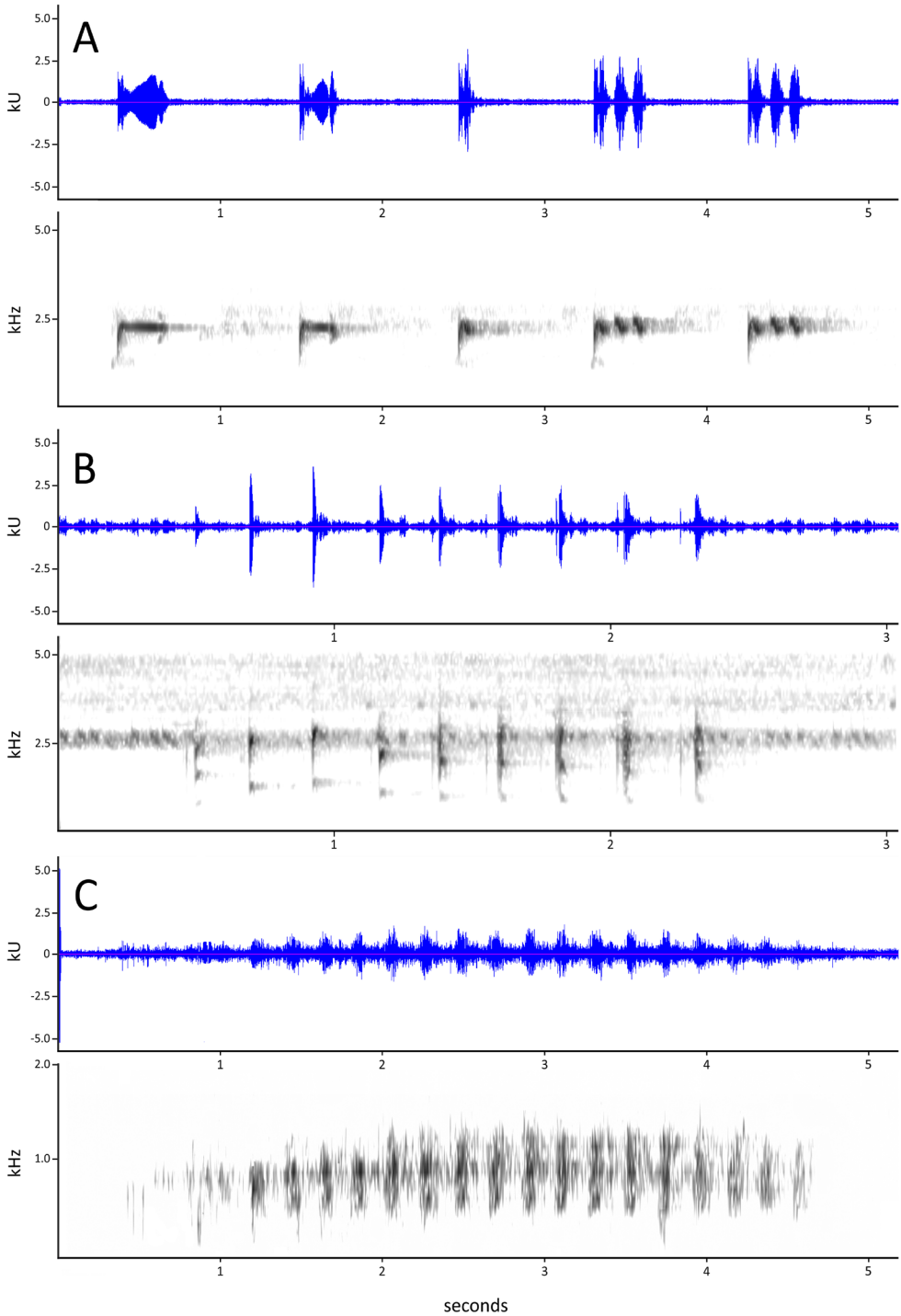


Figure 3. Oscillograms (upper graphs) and spectrograms (lower graphs) of advertisement calls of anuran species recorded in Estação Ecológica Juami-Japurá, state of Amazonas, Brazil. (A) *Boana hobbsi* (MCP 13361), recorded at 26.5 °C. (B) *Osteocephalus deridens*, an unvouchered male recorded at 25.8 °C. (C) *Tepuihyla shushupe*, an unvouchered male recorded at 25.6 °C. Spectrograms produced in Raven Pro 1.4 with window size = 512, brightness = 65–70, and contrast = 60–75; a band filter was applied to remove background noise below 100 Hz in all graphs.

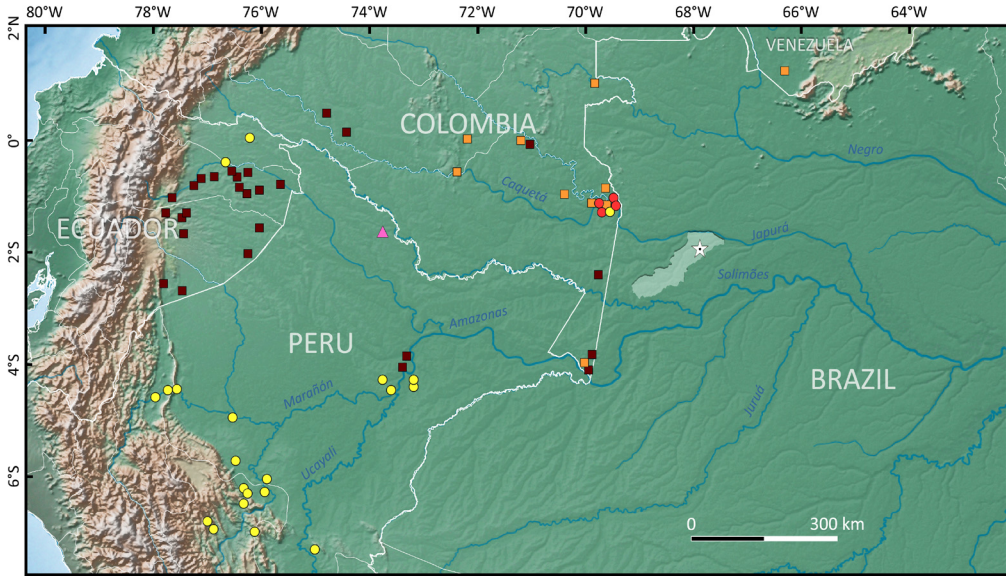


Figure 4. Geographic location of our new species records in Estação Ecológica Juami-Japurá (highlighted area), in the state of Amazonas, Brazil, and additional records of the same species reported outside Brazil. White star: location of new records, all in Igarapé da Fartura, a small eastern tributary of the Juami River. The following locations were extracted from the literature (see references in text): Red dots: *Ranitomeya defleri*. Yellow dots: *Ranitomeya variabilis*. Orange squares: *Boana hobbsi*. Brown squares: *Osteocephalus deridens*. Pink triangle: *Tepuihyia shushupe*.

towards the ventral region. Ventral surfaces of body and head greenish white to translucent. Limbs are the same colour as dorsum in dorsal view, dark yellow in ventral view. During the day, colour of dorsal surfaces changes from brown to greyish green, with scattered, irregularly spaced black spots.

Field notes. Both specimens were found at night while calling perched on dense flooded-forest vegetation (*igapó*) alongside the course of Igarapé da Fartura, approximately 20 cm above the surface of the creek. Calls were emitted sporadically, but those emitted by a single male triggered calls from other males inhabiting the same area for a few minutes, followed by long (> 30 s) silent intervals. One specimen (MCP 13361) was recorded at 21:38h under 26.5 °C. Advertisement calls were formed by the emission of long sequences of single, highly tonal notes, sporadically followed by a pair of trills consisting of three short notes (Fig. 3A). Average dominant frequency of tonal notes ($n = 5$) was 2.25 ± 0.01 kHz (range 2.24–2.26 kHz). Average lower and upper frequencies of tonal notes were 2.03 and 2.37 kHz, respectively. Average duration of tonal notes was 0.28 ± 0.03 s (range 0.24–0.31 s). Trills ($n =$

2) had a duration of 0.32 and 0.34 s. Average dominant frequency of short notes in trills ($n = 6$) was 2.21 ± 0.01 kHz (range 2.20–2.22 kHz). Average lower and upper frequencies of short notes were 1.92 and 2.50 kHz, respectively. Average duration of short notes was 0.07 ± 0.02 s (range 0.05–0.09 s).

Geographic distribution. *Boana hobbsi* was previously recorded in eastern Colombia, in the departments of Amazonas, Caquetá and Vaupés (Cochran and Goin, 1970; Pyburn, 1978; Ruiz-Carranza *et al.*, 1996; Acosta-Galvis, 2000; Lynch, 2005). The species was also reported for the Baría River, at the extreme south of state of Amazonas, Venezuela (McDiarmid and Paolillo, 1988; Barrio-Amorós, 1999). Our new record extends the species distribution to Brazil, and 197 km southeast of the nearest record, on the Apaporis River (Fig. 4).

***Osteocephalus deridens* Jungfer *et al.*, 2000**

Voucher specimens. One male (MCP 13770, SVL = 32.8 mm) collected on 06 February 2017 (1.9605°S, 67.9357°W, 76 m a.s.l.). Two males (MCP 13768, SVL = 31.6 mm; MCP 13769, SVL = 30.4 mm) collected on

11 February 2017, (1.9705°S, 67.9503°W, 87 m a.s.l.).

General description. A relatively small species of *Osteocephalus*. Skin on dorsum shagreened, with sparsely scattered tubercles, lacking spiny keratinized tips (Fig. 1E). Tubercle rows absent on tarsus and on the supra-orbital region. Sub-ocular spot present, white to cream in life. Iris cream, with no dark reticulations. Bones conspicuously green, visible through ventral skin of thigh. Ventral surfaces uniformly white.

Field notes. Specimens were found on (MCP 13768) or concealed inside (MCP 13769, 13770) tank bromeliads of the same species (*Aechmea* cf. *corymbosa*). Two advertisement calls of one unvouchered male were recorded at 23:28h at 25.8 °C (Fig. 3B). The male called from a tank bromeliad on the forest canopy. Calls ($n = 2$) were formed by nine short notes, and call duration ranged between 1.35–1.63 s. Average duration of notes ($n = 18$) within a call was 0.019 ± 0.002 s (range 0.016–0.024 s). Average duration of silent intervals between notes ($n = 16$) was 0.206 ± 0.013 s (range 0.185–0.233 s). Average dominant frequency of notes was 2.56 ± 0.23 kHz (range 2.04–2.88 kHz). The fundamental frequency of notes was visible on spectrograms between 0.90–1.3 kHz.

Geographic distribution. *Osteocephalus deridens* is found in rainforests located within the departments of Caquetá and Vaupés in Colombia (Lynch, 2005; Jungfer, 2011), the provinces of Napo, Francisco de Orellana, Sucumbíos and Pastaza in Ecuador (Read and Ron, 2018) and the department of Loreto, in Peru (Jungfer et al., 2013). Our new record extends the species distribution to Brazil, and 195 km southeast of the nearest record in the department of Amazonas, eastern Colombia (Fig. 4).

***Tepuihyla shushupe* Ron et al., 2016**

Voucher specimen. One adult male (MCP 13432; SVL = 83.0 mm), collected on 08 February 2017 (1.9606°S, 67.9369°W, 71 m a.s.l.).

Description. Dorsal skin with abundant large tubercles. Colour of dorsum and flanks light olive-green. Posterior margins of dorsal skin tubercles dark brown. Iris centrally cream, marginally reddish-orange (Fig. 1F). Extensive webbing between fingers, but not reaching the proximal edge of finger discs. Extensive webbing on toes, reaching the proximal edge of toes I, II, III and V. Serrated fringe present ventrolaterally along the outer edge of forearm and Finger IV.

Field notes. The male called from the canopy during the night and was found while moving along the stem of a tall tree, perched approximately 8 m high. In the same

sampling site, calls were emitted by other males during all night. However, each male emitted a single call every 15–30 minutes. Calls consisted in a trill of notes with very low dominant frequency. A single call was recorded from a large distance on 07 February 2017, at 22:45h and 25.6 °C (Fig. 3C). It was formed by 18 notes with a total duration of 3.59 s, emitted with a peak frequency of 0.88 kHz. Lower and upper frequency boundaries of the call (estimated -10 dB below the peak frequency) were 0.563 and 1.269 kHz. Due to sound degradation, we were not able to estimate duration or spectral properties of individual notes.

Geographic distribution. The species was known only from its type locality in the headwaters of rivers Ere and Campuya, in the Putumayo River basin in Loreto, Peru (Ron et al., 2016). The occurrence of the species in Brazil has been suggested based on a geographic record of *Tepuihyla tuberculosa* (Boulenger, 1882) at the Uaupés River basin, in Amazonas, but the referred specimen has not been examined (Ron et al., 2016). Our record confirms the occurrence of *T. shushupe* in Brazil and extends the eastern boundary of its range in approximately 640 km (Fig. 4).

Discussion

Among the five new records reported here, the dendrobatid frogs *Ranitomeya defleri* and *R. variabilis*, were previously assumed to occur in Brazil based on the proximity of their easternmost geographic records in Colombia with the Brazilian boundary on Caquetá and Apaporis rivers (Brown et al., 2011; Segalla et al., 2016), but no voucher specimens obtained from field work in Brazil were ever identified. All *R. defleri* collected matched the diagnostic morphological and colour pattern of type specimens (Twomey and Brown, 2009), except for the pale vertebral line, which is unbroken in specimens in the type series and fragmented, appearing as a series of small spots in specimens from ESEC Juami-Japurá. The single female specimen collected resembled males in overall external morphology, except for its bigger size, a much more ovoid body plan and for the larger and more conspicuous metatarsal tubercles. *Ranitomeya variabilis* is a polymorphic species, with extensive variation in colour pattern across its geographic range, with different colour patterns roughly grouped into highland and lowland colour morphotypes (Brown et al., 2011). Specimens from ESEC Juami-Japurá more closely resemble the colour morphotype found in the department of Vaupés, Colombia, which is also the nearest species record to our sampling area.

Boana hobbsi produces one of the most distinctive calls among Amazonian treefrogs, with two tonal introductory notes followed by two or three pulsed notes in a predictable succession, and is also distinguished from congeneric species by its unique colour pattern. The species was reported from similar blackwater streamside environments in Colombia, and these habitats are taken as the species' reproductive sites (Pyburn, 1978), although its reproductive mode and tadpoles are still unknown. *Osteocephalus deridens* is a common inhabitant of tank bromeliads in white sand forests in Peruvian and Ecuadorian Amazonia (Jungfer *et al.*, 2000, 2013). Colour in life of specimens collected in ESEC Juami-Japurá differ from those of typical specimens from Napo, Ecuador, in presenting dark-green shades instead of dark tan with light tan markings on dorsum (Jungfer *et al.*, 2000). The remaining diagnostic characters, including the laugh-like advertisement calls, match those of the original species description.

Tepuihyla shushupe is the second species in the genus to be recorded in Brazil. Although a relatively large treefrog, this species is a canopy dweller and its dorsal skin texture and colour pattern provide very efficient camouflage against tree stems (Ron *et al.*, 2016), impairing its detection in species surveys that rely primarily on visual encounters. Its characteristically loud calls can be detected from a distance of at least 500 m (authors' pers. obs.), but silent intervals elapsed from a call emission to the next are long (ca. 10–30 minutes) and the presence of the species may be bypassed by researchers if walking at a fast pace during acoustic surveys. The detection of *T. shushupe* and species with similar habits in future surveys would potentially benefit from auxiliary sampling methods deploying automated sound-recording units, as those applied in passive acoustic monitoring programs (Ribeiro Jr. *et al.*, 2017). Females, eggs and tadpoles of this species are still unknown.

The five species reported here for the first time in Brazil were found within a geographically remote conservation unit, which is only accessible by waterways and destined to strict protection of biodiversity and to scientific research. Access to the reserve is granted only to staff and visiting researchers. The demography of any of these species and their potential vulnerability to environmental alterations induced by climate change or land use by human populations remain unknown. Thus, we are currently unable to evaluate their conservation status (*sensu* IUCN Red List categories). However, due to the geographic remoteness of ESEC Juami-Japurá and its large remnant of non-fragmented, well-preserved

primary rainforest, we consider that these species currently benefit from adequate protection within the Brazilian territory.

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