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A new species of poison-dart frog (Anura: Dendrobatidae) from Manu province, Amazon region of southeastern Peru, with notes on its natural history, bioacoustics, phylogenetics, and recommended conservation status

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Abstract

We describe and name a new species of poison-dart frog from the Amazonian slopes of the Andes in Manu Province, Madre de Dios Department, Peru; specifically within the Amarakaeri Communal Reserve and the buffer zone of Manu National Park. *Ameerega shihuemoy* sp. nov. is supported by a unique combination of characters: black dorsum with cream to light orange dorsolateral lines, blue belly reticulated with black, and the lack of axillary, thigh and calf flash marks. Within *Ameerega*, it shares the general appearance of *A. altamazonica*, *A. boliviana*, *A. hahneli*, *A. ignipedis*, *A. petersi*, *A. picta*, *A. pongoensis*, *A. pulchripecta*, *A. simulans*, *A. smaragdina*, and *A. yungicola*; each possessing a granular black to brown dorsum, a light labial bar, a conspicuous dorsolateral line running from the snout to the groin, and a metallic blue belly and underside of arms and hind limbs. From most of these species it can be distinguished by lacking flash marks on the axillae, thighs, and calves (absent in only *A. boliviana* and *A. smaragdina*, most *A. petersi*, and some *A. pongoensis*), by having bright cream to orange dorsolateral stripes (white, intense yellow, or green in all other species, with the exception of *A. picta*), and by its blue belly reticulated with black (bluish white and black in *A. boliviana*, green and blue with black marbling in *A. petersi*, and green and blue lacking black marbling in *A. smaragdina*). Its mating call also shows clear differences to morphologically similar species, with a lower note repetition rate, longer space between calls, and higher fundamental and dominant frequencies. Phylogenetic analyses based on the 16S mitochondrial rRNA fragment also support the distinctiveness of the new species and suggest that *A. shihuemoy* is most closely related to *Ameerega macero*, *A. altamazonica*, *A. rubriventris*, and two undescribed species (*Ameerega* sp. from Porto Walter, Acre, Brazil, and *Ameerega* sp. from Ivochote, Cusco, Peru). Genetically, the new species is most similar to the sympatric *A. macero*, from which it clearly differs in characteristics of its advertisement call and coloration. The new species is found near rocky streams during the dry season and near temporary water bodies during the rainy season. Tadpoles are found in lentic water along streams, or in shallow, slow-moving streams. Given its small geographic range, we recommend that *A. shihuemoy* should be considered 'Near threatened' (NT) according to IUCN Red List criteria.

Key words: Advertisement call, Amarakaeri Communal Reserve, *Ameerega*, habitat, Manu Biosphere Reserve, premontane forest, rainforest, taxonomy

Introduction

The upper Amazon basin of Peru is home to an incredible diversity of poison frogs (family Dendrobatidae), with both species richness and endemism being extraordinarily high in this area (Brown *et al.* 2006). The number of named species has rapidly increased in the past two decades as a result of advances in molecular taxonomy and intensive field exploration (Brown & Twomey 2009). Currently there are 63 described species of Dendrobatidae in Peru, and the genus *Ameerega* Bauer, 1986 contains over 33% (21 species) of these (Frost 2016).

Species in the genus *Ameerega* are subdivided into two groups, the *Ameerega picta* group and the *Ameerega trivittata* group (Lötters *et al.* 2007). Cryptic species in the *Ameerega picta* group have been discovered using integrative taxonomic approaches combining adult coloration, advertisement call features, tadpole morphology, and DNA barcoding (e.g. Haddad & Martins 1994; Lötters *et al.* 1997, 2005, 2009; Twomey & Brown 2008; Vaz-Silva & Medeiros 2011).

During fieldwork in the buffer zone of the Manu Biosphere Reserve (BZ-MBR) and the Amarakaeri Communal Reserve (ACR), we found a population of poison frogs that resembles *Ameerega macero* (Rodriguez & Myers 1993) but differs substantially in coloration and advertisement call. Comparisons of other species, analyses of mtDNA, and assessments of advertisement calls suggest that this population represents a species new to science.

Here, we use an integrative approach to describe this new species, assigned to the *Ameerega picta* group. Additionally, we studied the habitat selection of this species, examined which environmental variables may explain its distribution, and provided a preliminary assessment of the conservation status following the IUCN Red List criteria (IUCN 2001).

Material and methods

Sampling. Specimens were collected from nine localities along the foothills of the eastern slopes of the Peruvian Andes in the Madre de Dios Department of southeastern Peru. Coordinates were obtained using a Garmin PSMAP 62sc Handheld Navigator GPS and Google Earth. Specimens were taken and euthanized using nembutal and lidocaine. A piece of tissue was removed (from the feet for most specimens) and preserved in 96% ethanol; tissues and specimens were deposited at the Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC), the Museo de Historia Natural de la Universidad Nacional San Agustín de Arequipa (MUSA), and the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM). Whole individuals were fixed in 10% formalin and later transferred to 70% ethanol for permanent storage.

Morphology. Coloration pattern was described from both living specimens and digital photographs. Sex and maturity were determined by dissection and direct examination of gonads with the aid of a stereomicroscope. Comparisons of external features are based on both original descriptions and examination of museum specimens (see Appendix I for materials examined). Terminology for morphological characters and diagnostic characteristics follow Myers *et al.* (1998) and Bernal *et al.* (2007). For morphometric measurements we used a digital calliper to the nearest 0.01 mm but in order to avoid pseudo-precision, measurements were rounded to 0.1 mm. Abbreviations are as follows: SVL, snout-vent length; TL, tibia length measured between heel and outer surface of flexed knee; GBW, greatest body width taken just under the axillae; HW, head width between angles of jaws; HL, head length, sagittal distance from tip of snout to angle of jaw; ED, eye diameter; IOD, interorbital distance; TSCN, tip of snout to center of nares; NED, distance from center of nares to anterior edge of eye; IND, distance between centers of nares; EL, eye length; HDT, horizontal diameter of tympanum; MTD, distance from corner of mouth to lower edge of tympanic ring; HaL, hand length measured from the proximal edge of large medial palmar tubercle to tip of longest third finger; W3FD, width of third finger disc; W3F, width of third finger below disc; W3TD, width of third toe disc; W3T, width of third toe below disc; W4TD, width of fourth toe disc; W4T, width of fourth toe below disc. A detailed description of morphological characters of one back-riding tadpole (stage 25; Gosner 1960) is provided following the terminology used by Altig & McDiarmid (1999). The oral disc (stage 41; Gosner 1960) was photographed under a stereomicroscope (Stereo Discovery.V20; camera Axiocam ERc 5s), and described following the terminology of McDiarmid & Altig (1999).

Bioacoustics. Calls were recorded with a Zoom H2N digital recorder and an Olympus Compact Zoom ME 32 microphone. Eighty advertisement calls of three males were analysed. Calls were characterized and analyzed

(temporal and spectral variables) in Raven pro 1.4 (Bioacoustics Research Program 2011) and compared to vocalizations of morphologically similar species. For comparative purposes, we also present data on the advertisement calls of six other *Ameerega* species that also belong to the *Ameerega picta* group.

Phylogenetic analysis. The phylogenetic position of the new species was determined comparing the 16S rRNA mitochondrial fragment of the new species with sequences from other *Ameerega* species, downloaded from Genbank (Appendix II). All taxa selected for comparisons belong to the main clades identified by Twomey & Brown (2008) and Brown & Twomey (2009), and *Colostethus fugax* Morales and Schulte 1993 was used as an outgroup. DNA extraction, amplification, sequence alignment, and sequence analysis of the new species followed the methods of Roberts *et al.* (2006). Newly obtained sequences were deposited in GenBank (Appendix II). Prior to conducting phylogenetic analysis, PartitionFinder, version 1.1.1 (Lanfear *et al.* 2012) was used to select the appropriate models of nucleotide evolution, and used the Bayesian information criterion (BIC) to determine the best substitution model. Phylogenetic analysis was done using Maximum Likelihood (ML) approach using RaxML version 8.2.4 (Stamatakis 2006), where the “f-a” function was employed to conduct a bootstrap analysis and search for the optimal likelihood tree. The analysis included 77 terminals and a 509 bp alignment. The GTR + I + Γ model of nucleotide substitution (as suggested by PartitionFinder) was used to perform 200 trees searches, and assessed node support using 1000 bootstrap replicates.

Habitat selection. The study of habitat selection in the new species was carried out at the Manu Learning Centre (MLC) research station, a regenerating forest in the buffer zone of the Manu Biosphere reserve (71°23'28"W 12°47'21"S). This site has two distinct known human disturbance histories (see Whitworth *et al.* 2016a for a detailed description of the site); low disturbance forest (identified with the acronym SLR—selectively logged regenerating forest) and complete clearance due to conversion to intensive agriculture (acronym CCR—completely cleared regenerating forest). The new species was found only along streams within the SLR forest; therefore, a study to identify the structural variables related to frog presence/absence along streams was carried out. Eight structural habitat variables were assessed along streams at 177 sampling locations, both along streams where frogs were found and along streams where frogs were not found. Each site corresponds to an area of 25 m² and was surveyed between August and September 2013. Structural habitat variables estimated were: canopy cover, percentage of wood debris, amount of ground cover by leaf litter, leaf litter depth, number of large rocks with a diameter greater than 50 cm, number of potential refuges (cavities where a hand made into a fist can fit), stream velocity (using a flowmeter) and the presence of a still body of water. In order to determine which explanatory variables were significantly associated with frog presence/absence, we used generalized linear models (GLM) with binomial error distribution and a log link function (Brotons *et al.* 2004). The response term was binomially distributed (0 denoted species absence and 1 denoted species presence). The second-order Akaike's information criterion corrected for small sample size (AICc) was used to rank models according to the lowest AICc (Burnham & Anderson 2002; Symonds & Moussalli 2011). We used model averaging across all models with an AICc $\Delta < 2$ to calculate the average parameter estimates based on all models in which the parameter appeared, weighted by their Akaike weights (Anderson *et al.* 2000). All statistical analyses were performed in R (R Core Team 2012). In order to check habitat variables for variation inflation using VIF values, we used the R package *car* (Fox & Weisberg 2011). There was no effect of multicollinearity among variables (all VIF values were well below the threshold indicative of multicollinearity; therefore no variable elimination was necessary—see Appendix III). For generalized linear modelling, AICc values, and top-model averaging, we used the R package *MuMIn* (Bartoń 2014). In order to assess the goodness-of-fit of the best supported models we implemented the McFadden's R² index using the R package *pscl* (Jackman 2015).

Ameerega shihuemoy sp. nov.

(Figures 1–3, 6)

Cryptophyllobates sp: Chaparro & Ochoa 2005 p.7 (MHNC 4779 collected on 07 December 2004 by J. C. Chaparro & J. A. Ochoa at Erika Lodge, Departamento Madre de Dios).

Ameerega gr. *pictus*: Chaparro *et al.* 2016 p. 2 (from Amarakaeri Communal Reserve, Departamento Madre de Dios).

Ameerega sp1: Whitworth & Villacampa 2014 p. 3 (from Manu Learning Centre, Departamento Madre de Dios).

Holotype. MHNC 15488 (Fig. 1), an adult female, from near Cupudnoe river, 440 m elevation, Peru (Coordinates:

12°47'26.70" S, 70°58'21.30" W), Distrito Madre de Dios, Provincia Manu, Departamento Madre de Dios, collected on 25 April 2010 by R. C. Gutiérrez.

Paratopotypes. Seven specimens: Five adult females (MUSA 3178, MUSA 3180–3182, MHNC 12988), and two immature females (MUSA 3177, MUSA 3179), collected with the holotype by R. C. Gutiérrez.

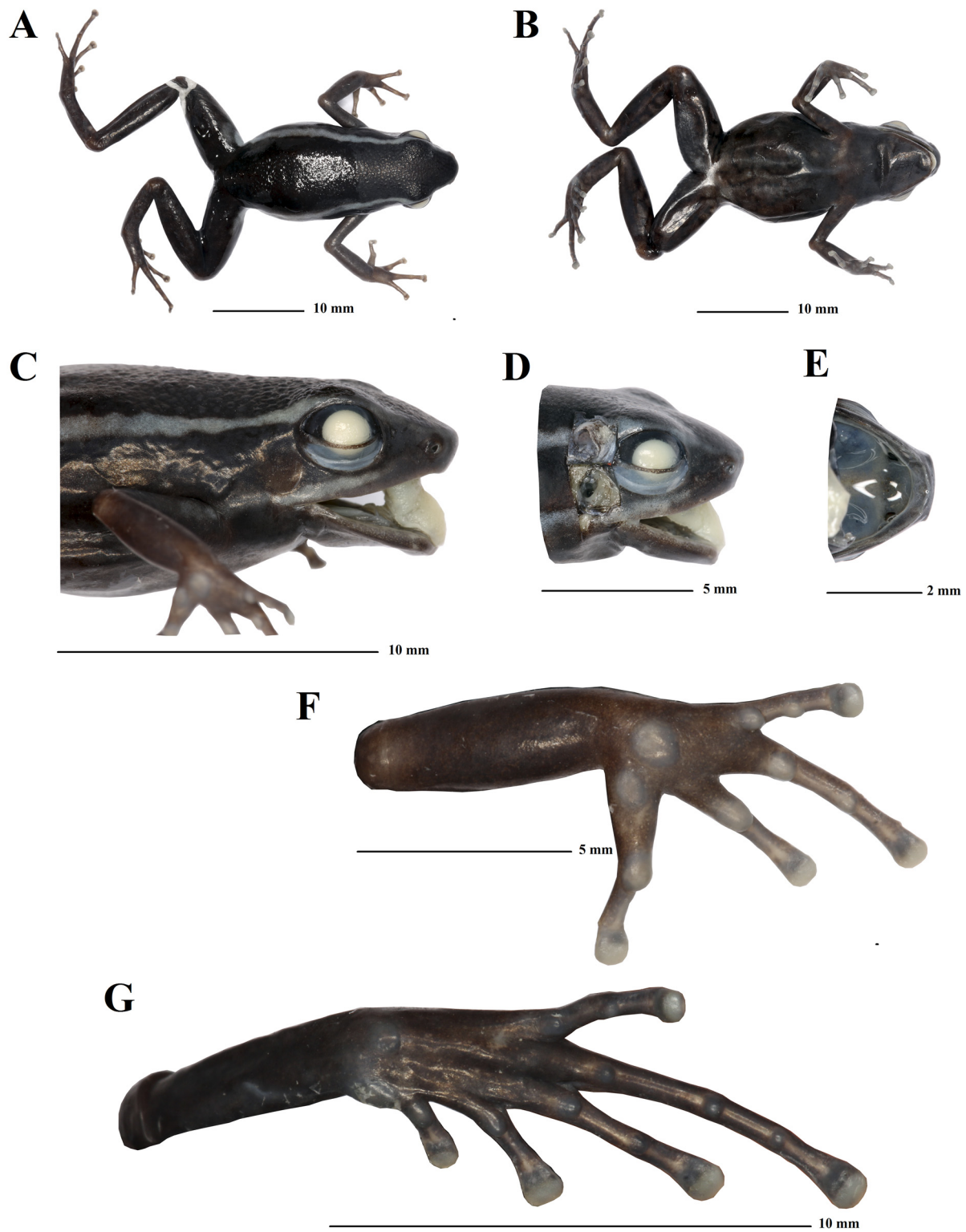


FIGURE 1. A, dorsal, and B, ventral view of the body; C, lateral view of head; D, and tympanum under skin; E, mouth showing choanae details; F, ventral view of the hand; G, and foot, of the adult female holotype MHNC 15488 (SVL = 25.7 mm) of *Ameerega shihuemoy* sp. nov. Scale on every picture. Photos by J.C. Chaparro.

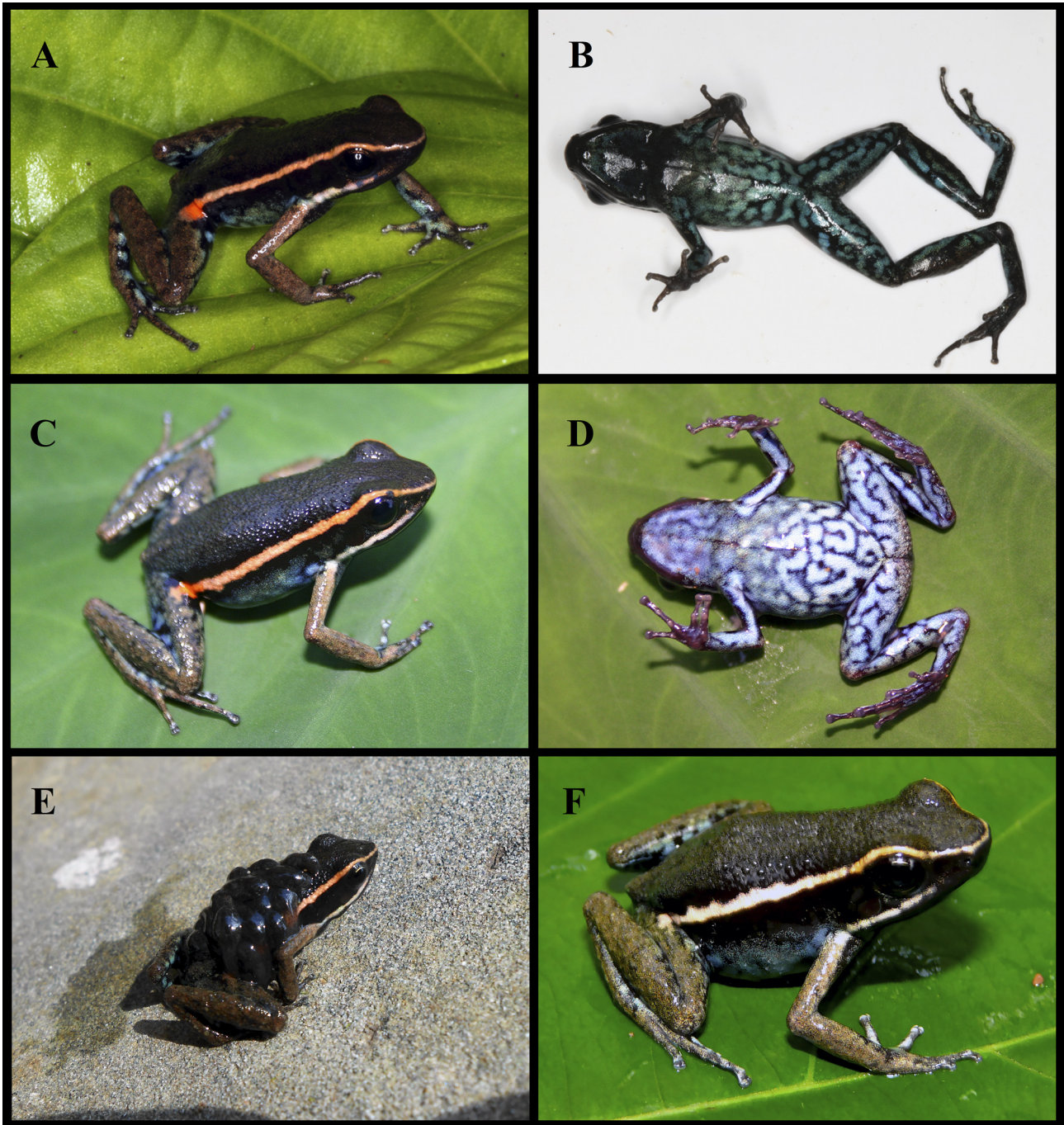


FIGURE 2. A, dorsal; B, and ventral views of the body of the subadult female paratype MHNC 14561 (SVL = 20.7 mm); C, dorsal; D, and ventral views of the body of the subadult male paratype MHNC 4779 (SVL = 17.4 mm); E, adult male carrying tadpoles; F, adult male paratype MHNC 15863. Photos by J.C. Chaparro (A-D), R. Coronel (E), R. Santa Cruz (F).

Paratypes. Fifteen specimens, all from Provincia Manu, Departamento Madre de Dios, Peru. Two adult females (MHNC 10406–10407), and one adult male (MHNC 10408), from the Reserva Comunal Amaraakaeri, 32 km east of Huepetuhe, near the headwaters of Colorado river, 846 m elevation, (Coordinates: 12°59'59.25" S, 70°50'31.32" W), collected on 07 November 2010 by J. Delgado and J. G. Estrada. Two adult females (MHNC 10525–10526), and two adult males (MHNC 10536, MHNC 10542), from the Reserva Comunal Amaraakaeri, 21.5 km southwest of Shintuya, near Azul river, 480 m elevation, (Coordinates: 12°48'50.8" S, 71°06'07.8" W), collected on 23 May 2011 by J. Delgado. An adult male (MHNC 15863), from the Reserva Comunal Amaraakaeri, Pad A, Lote 76, near Dahuene river, 530 m elevation, (Coordinates: 12°58'38.47" S, 71°01'30.73" W), collected on 20 July 2016 by R. Coronel and G. Valencia. Two immature specimens (MHNC 10806, MHNC 10811), from 3.5

km south of Shintuya, near Serjali river, 450 m elevation, (Coordinates: 12°42'49.02" S, 71°15'17.51" W), collected on 20 January 2011 by J. G. Estrada. Two immature specimens (MHNC 10816, MHNC 10826), from 10 km southwest of Itahuania, near Shilive river, 450 m elevation, (Coordinates: 12°43'02.93" S, 71°09'07.56" W), collected on 26 January 2011 by J. G. Estrada. An immature female (MHNC 14561; Figs. 2A, B), from the Reserva Comunal Amarakaeri, Pad A, Lote 76, near Colorado river, 825 m elevation, (Coordinates: 12°59'17.97" S, 71°0'52.67" W), collected on 04 February 2015 by T. Gregory. An adult specimen (MHNC 5012), from Aguas Calientes, 2.75 km east (downstream) from Shintuya, (Coordinates: 12°40'7.27" S, 71°16'12.20" W), collected on 09 July 2014 by S. J. Serrano. An immature specimen (MHNC 4779; Figs. 2C, D), from Erika Lodge, 500 m elevation, (Coordinates: 12°45'23.53" S, 71°22'48.68" W), collected on 07 December 2004 by J. C. Chaparro and J. A. Ochoa.

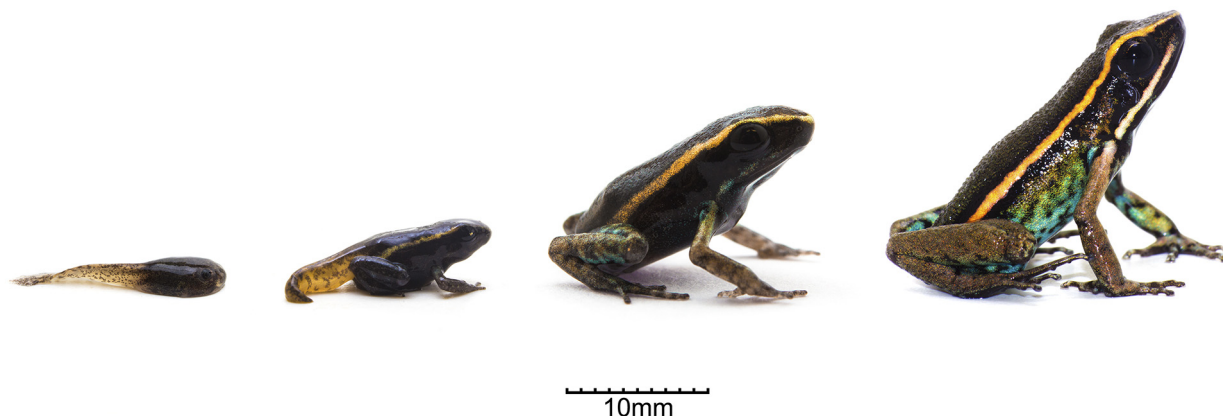


FIGURE 3. Color patterns of *Ameerega shihuemoy* from tadpole to adult MUSM 31692. Photos by Marcus Brent-Smith.

Referred specimens. Five specimens: MUSM31611, MUSM31664, MUSM31691, MUSM31692 and MUSM31730, from Manu Learning Centre Research Station, 460 m elevation, Peru, (Coordinates: 12°47'21.849" S, 71°23'28.06" W), Distrito Manu, Provincia Manu, Departamento Madre de Dios, Peru, collected on 12 February, 05 July, 21 July, and 25 July 2012, and on 03 February 2013, respectively, by A. Whitworth.

Etymology. The specific name *shihuemoy* (English pronunciation: shee-way-moy) corresponds to the Harakmbut word for "poison dart frog". The Amarakaeri are aboriginals from Amazonian Peru and their language belongs to the Harakmbut linguistic group. They coexist with the new species.

Diagnosis and comparisons with other species. Following Grant *et al.* (2006), the new species is assigned to the genus *Ameerega* on the basis of the following characters: dorsal skin finely granular; Finger I > Finger II when adpressed; toe and finger webbing absent; presence of bright flash marks and absence of ventrolateral line. This is a medium species of *Ameerega* with an adult SVL of 23.3 ± 2.7 (19.3–30.4 mm, N = 25). Vocal slits present; snout subacuminated, short and protruding. The head and dorsal surfaces are blackish with bronze-brown shades. The flanks are black with metallic bluish-green blotches, with a distinct bright orange or coral stripe extending from groin to above the eye and forward along the canthus rostralis to join around the snout. A flash spot is observed on the hip and upper surface of thighs, and a pale orange spot is located on the anterodorsal base of the thighs. It lacks concealed axillary and calf spots. The ventral surfaces have black reticulations with blue. The maxillary arch has teeth and the dentigerous process of vomers is lacking. The choanae are partly concealed by the palatal shelf of maxilla. The finger discs are weakly to moderately expanded, and when adpressed the first finger is slightly longer than second, and the fourth is shorter than first and second fingers, while the third is longer than all others. The finger discs are weakly to moderately expanded. The finger disc of the third finger of adults is about 1.2 times wider than the immediately adjacent part of the finger. The third finger shows no sexual dimorphism. Basal webbing is present on toes II–IV.

Morphologically, divergence of *A. shihuemoy* from other species is supported by a unique combination of characters: the lack of the conspicuous axillary, thigh and calf flash marks, characteristics of many other species of *Ameerega*; black granular dorsum with cream to light orange dorsolateral lines, metallic blue venter and underside

of extremities with black reticulations and spots. Within *Ameerega*, it shows the general appearance of *A. altamazonica* Twomey & Brown 2008, *A. boliviana* (Boulenger 1902), *A. hahneli* (Boulenger 1883), *A. ignipedis* Brown & Twomey 2009, *A. petersi* (Silverstone 1976), *A. picta* (Bibron 1838), *A. pongoensis* (Schulte 1999), *A. pulchripecta* (Silverstone 1976), *A. simulans* (Myers, Rodriguez & Icochea 1998), *A. smaragdina* (Silverstone 1976), and *A. yungicola* (Lötters, Schmitz & Reichle 2005), with which it shares a granular black to brown dorsum, a light labial bar, a conspicuous dorsolateral line running from the snout to the groin, and a metallic blue belly and underside of arms and hind limbs. From all these species it can be distinguished by lacking flash marks on the axillae, thighs, and calves (absent only in *A. boliviana*, *A. simulans*, *A. smaragdina*, most *A. petersi*, and some *A. pongoensis*), by having bright cream to orange dorsolateral stripes (white, pale yellowish-green, intense yellow, or green in all other species but *A. picta*), and by its blue belly reticulated with black (bluish white and black in *A. boliviana*, green and blue with black marbling in *A. petersi*, and green and blue lacking black marbling in *A. smaragdina*).

Measurements (in mm) of the holotype. The female holotype (Fig. 1) has SVL, 25.7; TL, 12.3; GBW, 8.2; HW, 7.9; HL, 8.0; ED, 5.2; IOD, 3.0; TSCN, 2.0; NED, 2.6; IND, 2.9; EL, 3.3; HDT, 2.0; MTD, 0.8; HaL, 7.0; WTFD, 0.7; WTF, 0.5; WTTD, 0.8; WTT, 0.4; WFTD, 0.9; WFT, 0.4.

Description of the type series. External morphology: A small to medium-size *Ameerega*, with adult males attaining approximately 19.2–21.8 mm SVL and adult females approximately 21.5–25.7 mm SVL (measurements and proportions summarized in Table 1). Dorsal skin of head, body, shank, thigh, and hind limbs coarsely and conspicuously granular; skin smooth or nearly smooth on forelimbs, and smooth on sides of head, body and on ventral surfaces. Head slightly wider between jaw articulations than between outer edges of upper eyelids; head usually narrower than body or about as wide. Head width between jaws 29–33% of SVL. Snout sloping, bluntly pointed or rounded in profile, truncate to rounded (usually) or bluntly pointed in dorsal and ventral view. Nares situated near tip of snout, directed slightly posterolaterally; nares visible from front and from below but not from above. Canthus rostralis short, protruding; loreal region nearly vertical, slightly concave to flat. Interorbital distance wider than upper eyelid. Eye shorter than snout length; distance from center of naris to eye 60–87% of eye length. Tympanum 35–65% of eye size. Hand (Fig. 1F) moderately large, its length 23–28% of SVL and 74–90% of head width between angles of jaws. Relative lengths of adpressed fingers IV > I > II > III; fingers I, II, and IV approximately similar in size when adpressed, with overlapping discs; adpressed first finger varies from slightly shorter to slightly longer than second. Finger discs weakly to moderately expanded; third finger disc 1.2–1.8 times wider than distal end of adjacent phalanx. A large outer metacarpal (= palmar) tubercle on the base of the palm and a smaller inner metacarpal tubercle on the base of first finger, these being relatively small, with rounded surfaces. One subarticular tubercle on fingers I & II, and two subarticular tubercles on fingers III & IV; subarticular tubercles well developed and prominently raised, although distal one on finger III and both in IV sometimes weaker; supernumerary tubercles, finger keels, and outer metacarpal fold absent; tibia length 47–54% of SVL; relative lengths of adpressed toes, IV > III > V > II > I; first toe usually reaching base of subarticular tubercle of second toe; basal webbing on toes II–IV; toe fringes absent; outer metatarsal fold absent; a large inner metatarsal fold located on the distal half of tarsus and extended near the inner metatarsal tubercle (Fig. 1G); ventrolateral side of tarsus relatively smooth, not especially rugose or tubercular; toes with moderately expanded discs, wider than finger discs (Fig. 1G); one to three moderately raised subarticular tubercles (one each on toes I & II, two each on III & V, and three on IV); two large metatarsal tubercles with low, rounded surfaces; inner metatarsal tubercle slightly larger than outer metatarsal tubercle; supernumerary tubercles absent.

Color pattern in life. (Based on living specimens and photographs; Figs. 2, 3). The noticeably granular body is blackish with bronze shades middorsally, turning black and blue dorsolaterally. The flanks are mostly black with metallic bluish green, with a distinct bright orange, coral, or cream stripe extending obliquely from groin to above the eye, and forward along the canthus rostralis to join around the snout. A pale whitish-bronze labial stripe commences between naris and eye, and extends posteriorly under the eye and tympanum to the base of the upper arm. A bright orange to coral spot is present on the hip and upper thigh; there is no calf spot and no pale markings in axilla or groin. The limbs are bronze-brown with shades of green and black. The ventral surfaces are overall blue with variable black reticulation, while in some individuals the ventral surfaces are overall black with blue reticulation.

Color in preservative (Fig. 1). The bright orange and coral colors fade to pale grey; the blue ventral surfaces fade to grey in a dark reticulum of variable distinctiveness.

TABLE 1. Measurements (in mm) of *Ameerega shihuenoy* type series.

SEX	MHNC 15448	MUSA 3181	MUSA 3178	MHNC 10525	MHNC 12988	MHNC 10526	MHNC 10406	MHNC 10407	MUSA 3180	MUSA 3182	MHNC 10408	MHNC 10536	MHNC 10542
	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Male	Male	Male
SVL	25.7	25.2	25.1	24.4	24.0	23.5	23.2	23.1	22.8	21.5	21.8	21.6	19.2
TL	12.3	12.9	11.4	11.8	12.2	11.2	11.5	11.2	11.6	11.7	11.0	10.9	9.0
HaL	7.0	6.8	6.3	6.3	6.5	6.0	6.2	6.0	6.1	5.5	6.1	5.8	4.5
HL	8.0	7.2	7.9	7.8	7.5	7.7	8.2	8.3	6.7	6.9	6.8	7.9	7.4
HW	7.9	7.6	7.8	7.4	7.6	6.8	7.3	7.4	6.8	7.2	6.8	6.7	6.0
GBW	8.2	8.4	7.9	8.5	7.2	7.6	8.3	7.4	6.8	6.7	7.9	7.6	7.6
IOD	3.0	3.1	3.0	2.3	2.7	2.1	2.3	2.3	2.8	2.3	2.1	2.3	2.1
ED	5.2	5.2	5.1	5.0	5.1	5.1	4.7	5.1	4.9	4.8	4.5	4.7	4.3
HDT	2.0	1.9	1.5	1.4	1.5	1.3	1.5	1.4	1.5	1.1	1.4	1.1	1.0
EL	3.3	3.0	3.3	3.1	3.1	3.1	2.9	2.9	3.1	3.0	2.7	2.7	2.9
TSCN	2.0	1.6	1.8	1.3	1.6	1.5	1.2	1.5	1.8	1.8	1.0	1.1	1.1
NED	2.6	2.6	2.3	2.2	2.1	2.0	1.9	2.1	2.1	1.8	2.3	1.8	2.0
IND	2.9	2.7	2.9	2.9	2.9	2.5	2.3	2.8	2.6	2.8	2.7	2.6	2.2
MTD	0.8	0.8	0.9	0.7	0.9	0.7	0.7	0.5	0.7	0.6	0.8	0.6	0.7
W3FD	0.7	0.8	0.6	0.7	0.8	0.7	0.6	0.7	0.7	0.5	0.6	0.5	0.7
W3F	0.5	0.5	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.4	0.4
W4TD	0.9	0.9	0.8	0.7	0.8	0.7	0.5	0.7	0.7	0.5	0.6	0.7	0.6
W4T	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.5	0.4
W3TD	0.8	0.8	0.7	0.7	0.7	0.5	0.7	0.7	0.7	0.6	0.4	0.6	0.6
W3T	0.4	0.5	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.3

Vocalization. Recordings of three *A. shihuemoy* males were taken at the Manu Learning Centre reserve. The advertisement call of this new species can be characterized as a series of chirp-like, pulsed notes. Notes are repeated at a rate of 0.8–1.0 notes per second (0.9 ± 0.1), duration of individual notes range from 84–109 ms (98.4 ± 6.8 ms), spaced 969–1196 ms apart (1042.5 ± 186.6 ms), with eight pulses (8.2 ± 0.8) per note. Dominant frequency ranges from 4478.9–4909.6 Hz (4672.72 ± 251.0 Hz) and is not frequency-modulated. Calling activity happens most frequently in the early mornings between 05:00 to 09:00 and late afternoon between 16:00 to 18:00. We also recorded a second call in *A. shihuemoy* consisting of three notes in quick succession (within 174.9 ± 16.6 ms of each other), repeated once every four to five seconds. The three notes in this call have different duration; the first note (87.2 ± 2.5 ms) typically has a longer duration than the second (69.4 ± 2.6 ms) and the third note (72.8 ± 2.8 ms). This three-note call seems to function as an aggressive or territorial call.

We compared the call of the following species with *Ameerega shihuemoy*: two recordings of *A. simulans* (74 advertisement calls) by J. M. Padial from Marcapata, Quispicanchis Province, Department Cusco, Peru, were obtained from Fonoteca Zoológica (Museo Nacional de Ciencias Naturales 2016); two recordings of *A. boliviana* (45 advertisement calls) by J. Bosch & I. De la Riva from Correo-Apolo, La Paz, Bolivia; one recording of *A. yungicola* (30 advertisement calls) by M. Pacheco-Suarez from Caranavi, Yungas, Bolivia obtained from “Guía fotográfica de los anfibios de la region de las yungas Bolivia” (Pacheco-Suarez 2015); two recordings of *A. hahneli* (46 advertisement calls) by J. Serrano-Rojas from Shintuya, Madre de Dios, Peru; two recordings of *A. picta* (63 advertisement calls) by J. M. Padial from Madidi National Park, Northern Bolivia and from Paractito, Cochabamba Department, Central West Bolivia deposited at Fonoteca Zoológica (Museo Nacional de Ciencias Naturales 2016); two recordings of *A. macero* (55 advertisement calls) by J. Serrano-Rojas from Manu Learning Centre, Madre de Dios, Peru. The advertisement call of *A. shihuemoy* is easily distinguished (Fig. 4) from the morphologically similar *Ameerega* species of the *Ameerega picta* group (for measurable call parameters and intra- and interspecific variation see Table 2).

TABLE 2. Summary of numerical parameters of vocalizations of *Ameerega shihuemoy* and morphologically similar *Ameerega* species.

Parameters	<i>A. shihuemoy</i>		<i>A. simulans</i>		<i>A. picta</i>		<i>A. hahneli</i>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calls/N° of individuals	80/3	-	74/2	-	63/2	-	46/2	-
Notes/min	52.8	5.4	79.2	3.6	130.2	6.0	511.8	4.2
Notes/s	0.9	0.1	1.3	0.1	2.2	0.1	8.5	0.1
Duration of the note (ms)	98.4	6.8	104.9	10.2	45.8	2.4	13.4	2.8
Break between calls (ms)	1042.5	186.6	691.4	162.7	429.9	44.6	107.4	12.6
Fundamental frequency (Hz)	4237.0	281.9	4060.9	74.6	3770.7	76.7	2516.8	83.7
Dominant frequency (Hz)	4672.7	251.0	4460.3	157.7	4044.2	94.7	4550.0	49.1

continued.

Parameters	<i>A. boliviana</i>		<i>A. yungicola</i>		<i>A. macero</i>	
	Mean	SD	Mean	SD	Mean	SD
Calls/N° of individuals	45/2	-	30	-	55/2	-
Notes/min	72.0	4.2	313.2	0.0	519.4	2.2
Notes/s	1.2	0.1	5.2	0.0	8.7	0.0
Duration of the note (ms)	80.9	8.6	47.8	4.7	37.6	1.3
Break between calls (ms)	783.2	88.6	148.1	7.3	76.1	2.7
Fundamental frequency (Hz)	3416.1	68.2	3475.7	43.5	3353.7	38.1
Dominant frequency (Hz)	3846.0	46.3	3703.7	0.0	3617.6	0.0

The note duration of the new species (84–109 ms) is longer than *A. hahneli* (11–18 ms), *A. macero* (36–40 ms), *A. picta* (41–52 ms) and *A. yungicola* (40–57 ms), but similar to *A. boliviana* (75–94 ms) and *A. simulans* (91–118

ms). The space between calls in *A. shihuemoy* is longer than in the other six species. The number of notes per second in the advertisement call of *A. shihuemoy* is lower than the other six species. The call of the new species has a higher fundamental frequency than all other species but similar to *A. simulans*, and a higher dominant frequency than all other species but similar to *A. hahneli* and *A. simulans*, except the latter species has pronounced upward frequency-modulated notes by about 950 Hz (Fig. 5). It is important to take into consideration that this comparison was performed on recordings from three individuals of *A. shihuemoy* from a single location.

Tadpoles. Ontogenetic variation of 11 characteristics measured for 16 tadpoles in stages 24–46 (Gosner 1960) are summarized in Table 3. The tadpole of *A. shihuemoy* sp. nov. belongs to the exotrophic ecomorphological guild, benthic type as defined by Altig & Johnson (1989). This description is based on a tadpole (MHNC lot 12987) at developmental Stage 25 (Gosner 1960; Figs. 3A, C) and measuring a total length 17.2 mm; body length 6.2 mm; maximum width 4.1 mm; depth 3.0 mm; internarial distance 0.6 mm; eye to nares distance 0.5 mm; eye diameter 0.6 mm; interorbital distance 1.0 mm, oral disc width 1.2 mm; tail length 11.0 mm. The body is globular, compressed in lateral view, and ovoid in dorsal view. The snout is rounded in dorsal view (Figs. 6A and C). The mouth is located anteroventrally and is surrounded by a small oral disc (Figs. 6B and D). Papillae are laterally emarginated, simple and conical. Marginal papillae absent on anterior labium, present in one complete row on posterior labium. Anterior jaw sheath has a medial indentation with reduced serration, posterior jaw sheath V-shaped and has serration throughout. Lateral processes long, extending well past lower jaw. Labial Tooth Row Formula (LTRF) is 2(2)/3(1). A-1 complete, A-2 with medial gap, same width as A-1. P-1, P-2, and P-3 complete; P-1 and P-2 equal width, P-3 shorter. Nares are oval, small, without projections and inflexions, and are located dorsolaterally. Eyes are small and oriented dorsolaterally. The spiracle is single, sinistral, and located just before mid-body. Fins are concave and the posterior end is rounded, reaching their maximum height at the last third of the tail; the dorsal fin does not extend onto the body. The maximum tail height is reached at about the end length and is as high as body height. In preservative, the body is dark grey, the belly is translucent with intestines slightly visible; caudal musculature creamy white with small, irregular grey flecks and slightly translucent fins. In life, the body is dark brown with black spots, the belly is transparent but slightly pigmented posteriorly, intestines well visible through skin, caudal musculature poorly pigmented and transparent tail fin with melanophores in small and irregular clusters along the tail.

TABLE 3. Morphometric measurements (mean \pm SD, in millimeters) of developmental stages of tadpoles of *Ameerega shihuemoy*. Total length (TL), body length (BL), body height (BH), body width (BW), tail length (TAL), tail muscle height at the base of the tail (TMH), tail muscle width at the base of tail (TMW), eye-nares distance (END), internarial distance (IND), eye diameter (ED), interorbital distance (IOD), and oral disc width (OD).

Character	Gosner stages										
	24 (N=1)	25 (N=3)	26 (N=2)	27 (N=1)	40 (N=2)	41 (N=2)	42 (N=1)	43 (N=1)	44 (N=1)	45 (N=1)	46 (N=1)
TL	17.8	16.9 \pm 1.9	17 \pm 2.8	18.3	22.6 \pm 0.2	23.1 \pm 0.6	16.6	14.5	12.1	10.5	10.4
BL	6.4	6 \pm 0.9	6.5 \pm 0.8	9.5	9.4 \pm 0.1	9.4 \pm 0.4	8.5	9.2	11.9	9.5	10.4
BW	3.9	3.6 \pm 0.5	3.9 \pm 0.4	4.7	5.3 \pm 0	6.8 \pm 1.1	4.3	4.6	3.5	4.8	4.6
BH	2.2	2.6 \pm 0.4	3 \pm 0.2	3.5	3.8 \pm 0.4	4.5 \pm 0.1	3.2	4.2	3.3	2.4	2.4
TAL	11.4	10.9 \pm 1.1	10.5 \pm 2	11.8	13.2 \pm 0.1	13.7 \pm 0.1	8.1	5.3	0.2	1.0	–
TMW	1.1	1.5 \pm 0.2	1.3 \pm 0.1	2.0	2.2 \pm 0.2	2.8 \pm 0.1	2.2	2.7	1.4	1.9	–
TMH	1.7	1.6 \pm 0.2	2.1 \pm 0.2	2.0	3.1 \pm 0.2	3.5 \pm 0.1	2.3	2.7	1.1	1.7	–
END	1.0	0.6 \pm 0.3	0.6 \pm 0.2	1.1	0.9 \pm 0.1	1 \pm 0.3	0.6	1.6	1.0	0.8	1.8
IND	0.6	0.6 \pm 0.1	0.9 \pm 0.2	1.0	0.9 \pm 0.2	1.2 \pm 0.2	1.1	1.1	0.9	0.9	0.8
ED	0.6	0.6 \pm 0.2	0.7 \pm 0.1	1.0	1.3 \pm 0	1.3 \pm 0.1	1.4	1.3	1.5	1.4	1.5
IOD	0.8	0.9 \pm 0.2	1.2 \pm 0	1.7	1.7 \pm 0.4	2 \pm 0.1	2.0	2.5	2.1	2.3	2.4
OD	1.1	1.1 \pm 0.4	1.2 \pm 0	1.2	2.15 \pm 0.1	2.2 \pm 0	2.3	1.8	2.6	3.2	3.9

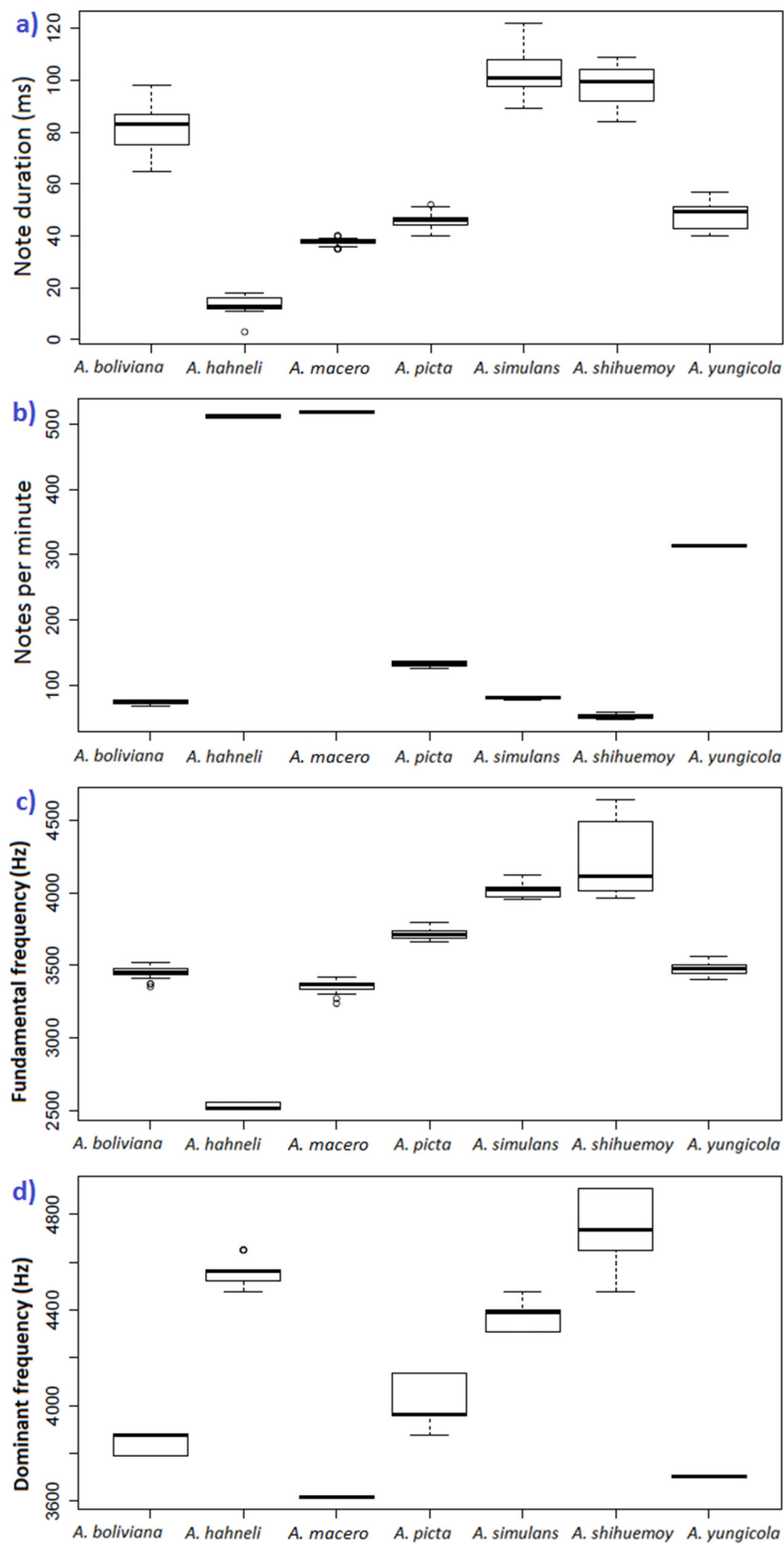


FIGURE 4. Box plots representing the median (black horizontal line), interquartile range (box), range (whiskers) and outside values (circles) of call parameters comparison among *Ameerega shihuemoy*, *A. boliviana*, *A. hahneli*, *A. picta*, *A. simulans* and *A. yungicola* where: a) note duration (ms), b) calling rate, c) fundamental frequency (Hz) and d) dominant frequency (Hz).

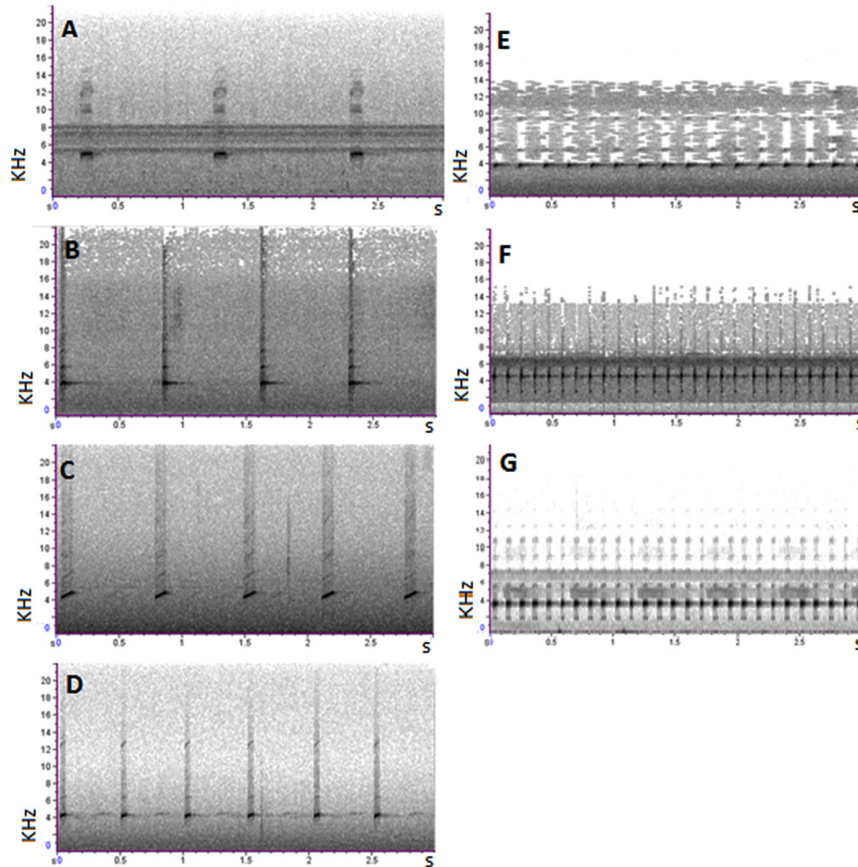


FIGURE 5. Spectrograms of advertisement calls of morphologically similar species of *Ameerega*. **A)** *A. shihuemoy*, recorded at Manu Learning Centre, Madre de Dios, Peru 12 June 2014 (temperature not noted). **B)** *A. boliviana*, recorded from Correo-Apolo, La Paz, Bolivia. **C)** *A. simulans* recorded from Marcapata, Cusco, Peru. **D)** *A. picta* recorded from Madidi National Park, Bolivia. **E)** *A. yungicola* recorded from Caranavi, Yungas, Bolivia. **F)** *A. hahneli*, recorded from Shintuya, Madre de Dios, Peru. **G)** *A. macero*, recorded at Manu Learning Centre, Madre de Dios, Peru.

In addition, we described observations in the field following Gosner (1960). We found two clutches of eggs in small rocky cavities; they were monitored every two days in order to take notes about the development of eggs. The first set was found on 25 June 2013, with 22 eggs at stage 13; a stage in which the neural plate develops on the dorsal surface, and eggs were covered by a transparent orange mucilage. After nine days, eggs become tadpoles, where they reach stage 17 (tail bud). The same embryos were abandoned by the father after two days on 05 July 2013. The embryos were getting dirty and dry, and inside we could observe the larvae of an insect, which could likely prey upon the embryos.

The second set was found on 10 July 2013, containing 25 eggs in stage 13 covered by a transparent orange mucilage. After ten days, eggs were observed at stage 19 (heart beat), which is represented mainly by the development of the gills and tail. Seven days later tadpoles were at stage 21 (cornea transparent), in which the tail becomes increasingly transparent. Finally, three days later (30 July 2013) only five tadpoles were found at stage 22 (tail fin circulation); we assumed that the father was carrying the rest of tadpoles.

One year later we found two other tadpoles at stage 26 (limb bud), on 11 June 2014. After four days, these reached stage 38 (toe development XIII). Another group of four tadpoles were found at stage 40 (cloacal tail piece XV–XVII), which changed to stage 42 (nose development XXI) after four days. Two more tadpoles were found at stage 42 (nose development XXI) on 15 June 2014; the stage 46 (metamorphosis complete) was reached after three days. In summary, we suggest that the development between stages 13–22 takes twenty days, stages 26–38, four days, and stages 40–46, seven days. However, the time of development of stages 22–26, and stages 36–40 remains unknown.

Phylogenetics. Our Maximum Likelihood (ML) tree was generally congruent with previous molecular phylogenies based on mitochondrial DNA (Twomey & Brown 2008, Brown & Twomey 2009) and supported the

distinctiveness of the *Ameerega shihuemoy* from other closely related taxa. The new species was most closely related to *Ameerega macero*, *A. altamazonica*, *A. rubriventris* (Lötters *et al.* 1997), and two undescribed species—*Ameerega* sp. from Porto Walter, Acre, Brazil, and *Ameerega* sp. from Ivochote, Cusco, Peru (Fig. 7). In general, the topology we recovered is similar to the ones obtained by Twomey & Brown (2008) and Brown & Twomey (2009), although there were some differences. These differences were likely a result of using only one mitochondrial gene (our study) versus three mitochondrial genes. Nevertheless, in addition to determining the relationship between the new species and other closely related taxa, our analysis inferred three main clades—*bassleri*, *hahneli* and *petersi*—identified in these previous studies (Twomey & Brown 2008, Brown & Twomey 2009). Our analysis also inferred several species pairs that were inferred to be closely related according to the previous analyses (e.g., *A. picta* and *A. yungicola*; *A. rubriventris* and *A. altamazonica*).

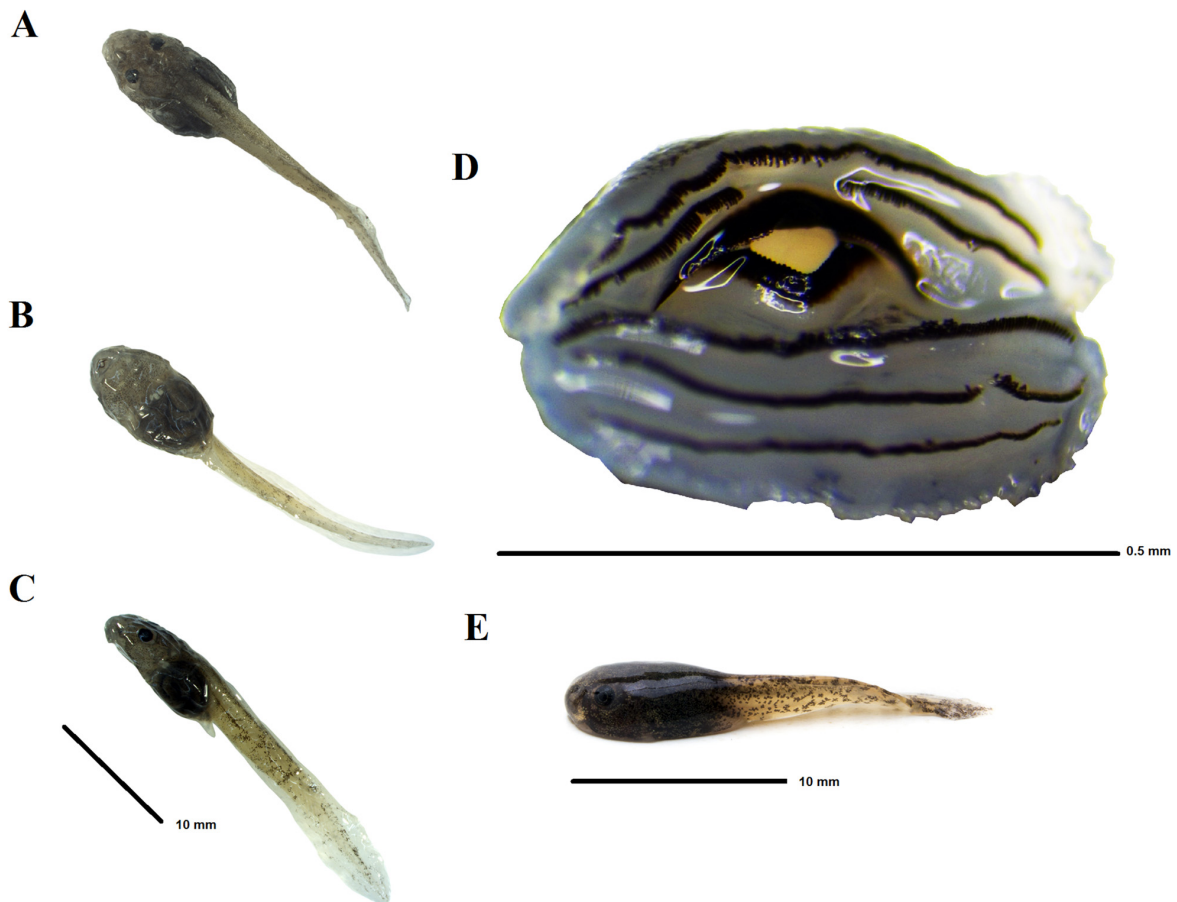


FIGURE 6. Tadpole of *Ameerega shihuemoy* at Gosner stage 25: (A) dorsal, (B) ventral, (C) lateral, (D) Oral disc at Gosner stage 41, (E) Free-living tadpole at Gosner stage 25. Photos by S. J. Serrano.

Distribution and natural history. *Ameerega shihuemoy* is distributed in southeastern Peru at the transition between the montane forest and the lowlands. Its altitudinal range spans from 340 to 850 m above sea level. This species is known from nine localities; three in the buffer zone of the Manu Biosphere Reserve (The Manu Learning Centre, Erika lodge, and Aguas Calientes, Shintuya) and another six locations in the Amaraeri Communal Reserve. The area is humid and hot: during the wet season, the average daily temperature is 24.78°C, the average humidity is 90.58% and the rainfall is 3098 mm; during the dry season the average temperature is 23.74°C, the average humidity is 84.89% and the average monthly rainfall is 1557 mm (climate information from Whitworth *et al.* 2016b).

Ameerega shihuemoy is most commonly found in low disturbance forest. During the dry season it is frequently found amongst boulders along or near forest streams (Fig. 8). During the rainy season it moves away from the streams into the forest interior. The activity patterns appear to be distinctly crepuscular, being most active in the early morning (05:30 to 09:00) or evening (16:00 to 18:00) when males call vigorously. Males typically call from exposed positions of rocks, leaf litter, or woody debris. Calling has been heard throughout the year. Crevices or

holes made of boulders or roots are used as refuges. During the night, individuals rest on low vegetation between 0.1 to 0.5 m above the ground.

Reproduction takes place near both permanent and seasonal streams. On 25 June 2013 and 10 July 2013 two clutches of eggs were found inside of small shelters next to a stream. These clutches contained 22 and 25 eggs respectively, and were guarded by males. On 30 July 2013, one uncollected male was observed transporting ten tadpoles along a slow moving stream. We detected free-living *A. shihuemoy* tadpoles and metamorphs co-occurring with metamorphs of *A. macero* in the same streams; with shallow, slow moving, clear water and bottoms of sand and dead leaves.

Results of the GLM analysis in both forest types (CCR and SLR) showed that six environmental variables were the most important habitat features to explain the species presence near streams (frog presence ~ canopy cover + leaf litter cover + number of potential refuges + presence of large rocks + presence of a still body of water + stream flow; Table 4, Appendix IV). The best supported model explained 89.05% of the variation in the data. When we ran the same analysis just in streams where the frogs were found (SLR forest), the GLM analysis presents two top models with $\Delta AICc < 2$ (Table 5, Appendix V). The best supported model explained 83.41% of the variation in the data. Model averaging was carried out in these two models. The relative importance for leaf litter cover, number of potential refuges, presence of large rocks, presence of a still body of water and stream flow were 1.0, signalling that they were important predictors. Canopy cover had some support (0.69), suggesting lower importance when comparing just within the SLR forest, and was therefore excluded from the top-preferred, and more parsimonious model (Appendix V).

This top model suggests that a greater number of potential refuges (often created by the presence of a high quantity of large rocks), a great amount of leaf litter cover, the presence of still bodies of water, and a low stream flow (likely to benefit breeding strategy) are the most important predictors of the presence of *A. shihuemoy* near streams.

Conservation status. Applying IUCN Red List criteria (IUCN Red List of Threatened Species, 2001 Criteria & Categories (version 3.1.); http://www.iucnredlist.org/info/categories_criteria2001, accessed 20 February 2016), which indicates that if a species occurs in less than ten threat-defined locations and the extent of occurrence is less than 20,000 km², it should be classified as Vulnerable or Endangered. *Ameerega shihuemoy* is known from nine localities distributed in the buffer zone of the Manu Biosphere Reserve and the Amarakaeri Communal Reserve (Fig. 9), with an estimated extent of occurrence of ca. 1,124 km², as such, we suggest that this new species might be classified as Vulnerable. However, due to the lack of intensive sampling effort in the Amarakaeri Communal Reserve, which may host a greater number of locations, we propose that *A. shihuemoy* should likely be categorized as Near Threatened (NT).

TABLE 4. Model selection for environmental variables potentially explaining habitat selection of *Ameerega shihuemoy* based on Akaike's Information Criterion corrected for small sample size (AICc) showing the ten first candidates models and the null model: log likelihood (logLik), k (number of parameters), AICc values, AICc differences between the best model and each candidate model ($\Delta AICc$) and Akaike weights (ω_i).

Model	K	AICc	$\Delta AICc$	ω_i
frog presence~ A + B + C + D + E + F	6	39.015	0.000	0.395
frog presence~ A + B + C + D + E + F + G	7	41.169	2.154	0.135
frog presence~ A + B + C + D + E + F + H	7	41.210	2.194	0.132
frog presence~ B + C + D + E + F	5	41.567	2.552	0.110
frog presence~ B + C + D + E + F + G	6	43.303	4.288	0.046
frog presence~ A + B + C + D + E + F + G + H	8	43.388	4.372	0.044
frog presence~ B + C + D + E + F + H	6	43.593	4.578	0.040
frog presence~ A + B + C + D + F	5	45.232	6.217	0.018
frog presence~ B + C + D + E + F + G + H	7	45.414	6.399	0.016
frog presence~ B + C + D + F	4	46.134	7.118	0.011
frog presence~1	1	224.474	185.459	0.000

Predictor variables are canopy cover (A), leaf litter cover (B), number of potential refuges (C), percentage of large rocks (D), presence of a still body of water (E), stream flow (F), leaf litter depth (G) and Wood debris percent (H).

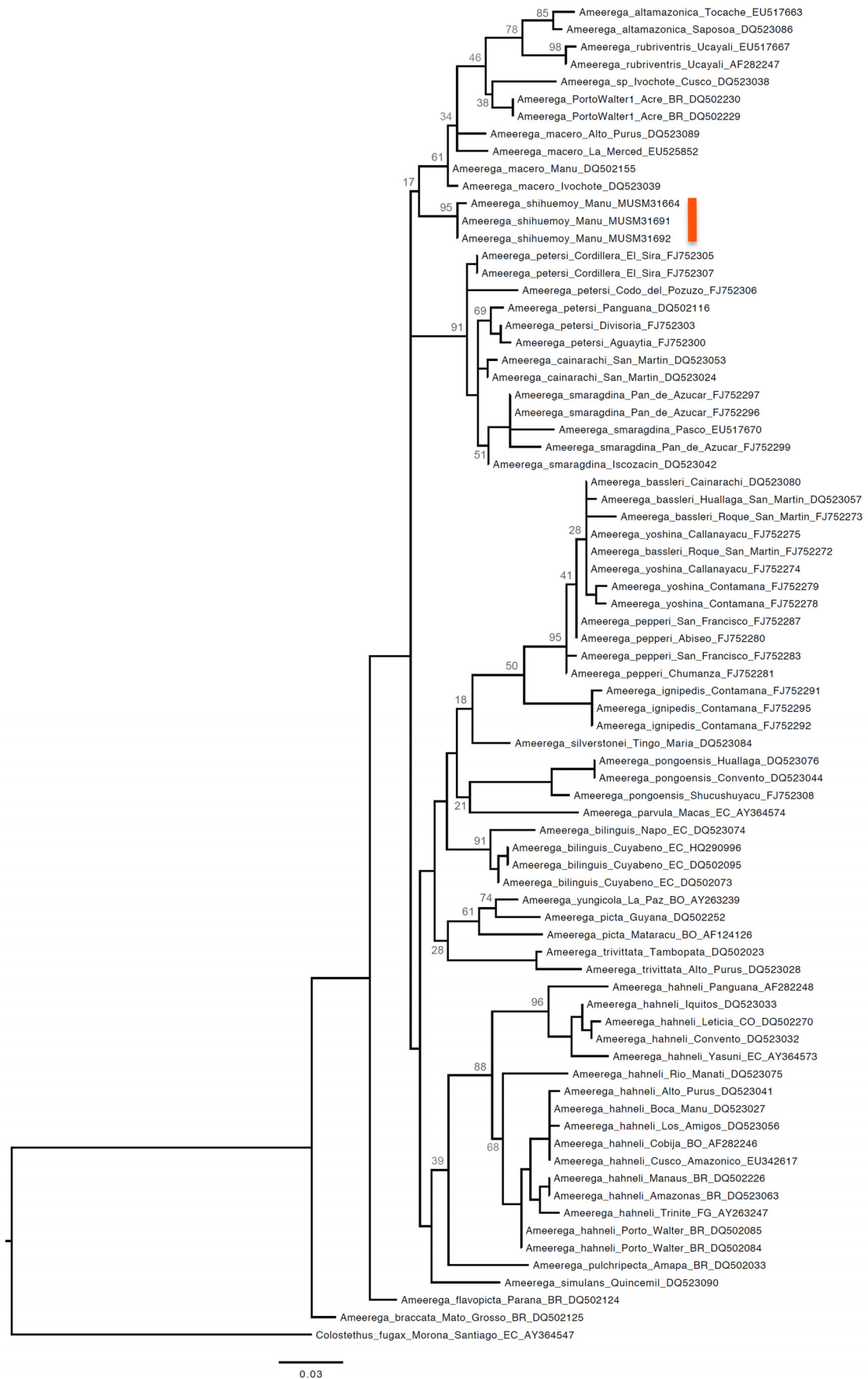


FIGURE 7. Maximum Likelihood (ML) phylogeny of *Ameerega* based on 16S ribosomal RNA gene. Numbers above nodes are bootstrap values.



FIGURE 8. Habitat at Manu Learning Centre, Manu, Madre de Dios, Peru, where several individuals of *Ameerega shihuemoy* were observed calling on July 2015. Photos by Katie Lin.

TABLE 5. Model selection for environmental variables potentially explaining habitat selection of *Ameerega shihuemoy*, on streams within the SLR forest, based on Akaike's Information Criterion corrected for small sample size (AICc), showing the ten first candidates models and the null model: log likelihood (logLik), k (number of parameters), AICc values, AICc differences between the best model and each candidate model (Δ AICc) and Akaike weights (ω_i).

Model	K	AICc	Δ AICc	ω_i
frog presence~ A + B + C + D + E + F	6	38.109	0.000	0.284
frog presence~ B + C + D + E + F	5	39.667	1.558	0.130
frog presence~ A + B + C + D + E + F + H	7	40.385	2.276	0.091
frog presence~ A + B + C + D + E + F + G	7	40.400	2.292	0.090
frog presence~ A + B + C + D + F	5	41.082	2.973	0.064
frog presence~ B + C + D + F	4	41.728	3.619	0.046
frog presence~ B + C + D + E + F + G	6	41.733	3.625	0.046
frog presence~ B + C + D + E + F + H	6	41.914	3.805	0.042
frog presence~ A + B + C + D + E + F + G + H	8	42.712	4.603	0.028
frog presence~ B + D + E + F	4	42.793	4.684	0.027
frog presence~1	1	164.154	126.045	0.000

Predictor variables are canopy cover (A), leaf litter cover (B), number of potential refuges (C), percentage of large rocks (D), presence of a still body of water (E), stream flow (F), leaf litter depth (G) and Wood debris percent (H).

Remarks. The area that *Ameerega shihuemoy* inhabits is threatened by human disturbance associated with activities such as logging, agriculture, and gold mining, especially within the Biosphere Reserve (Finer *et al.* 2015). Currently, these threats are increasing rapidly due to a draft law in favor of the construction of The Nuevo Eden–

Boca Manu–Boca Colorado road that cuts through the buffer zones of the Amarakaeri Communal Reserve and Manu National Park (Finer *et al.* 2016). This road has already begun to be illegally constructed with the purpose of fuel transport for illegal mining and logging. The approval of this road will trigger increased human disturbance within the buffer zone, resulting in a potential new deforestation hotspot in the Madre de Dios region. Changes affecting the habitat surrounding the reserve could ultimately lead to degradation of habitat within the nearby protected areas (Laurance *et al.* 2012). This could therefore result in both a reduction of the number of known populations of *A. shihuemoy* and reduce the overall area of occupancy of the species. The discovery of a new species in Manu Province underscores the need of continued habitat protection in Madre de Dios region (Jarvis *et al.* 2015), which is home to more than 114 amphibian species, and is one of the most biodiverse regions on the planet for herpetofauna (von May *et al.* 2009; Catenazzi *et al.* 2013) and a variety of other taxa.

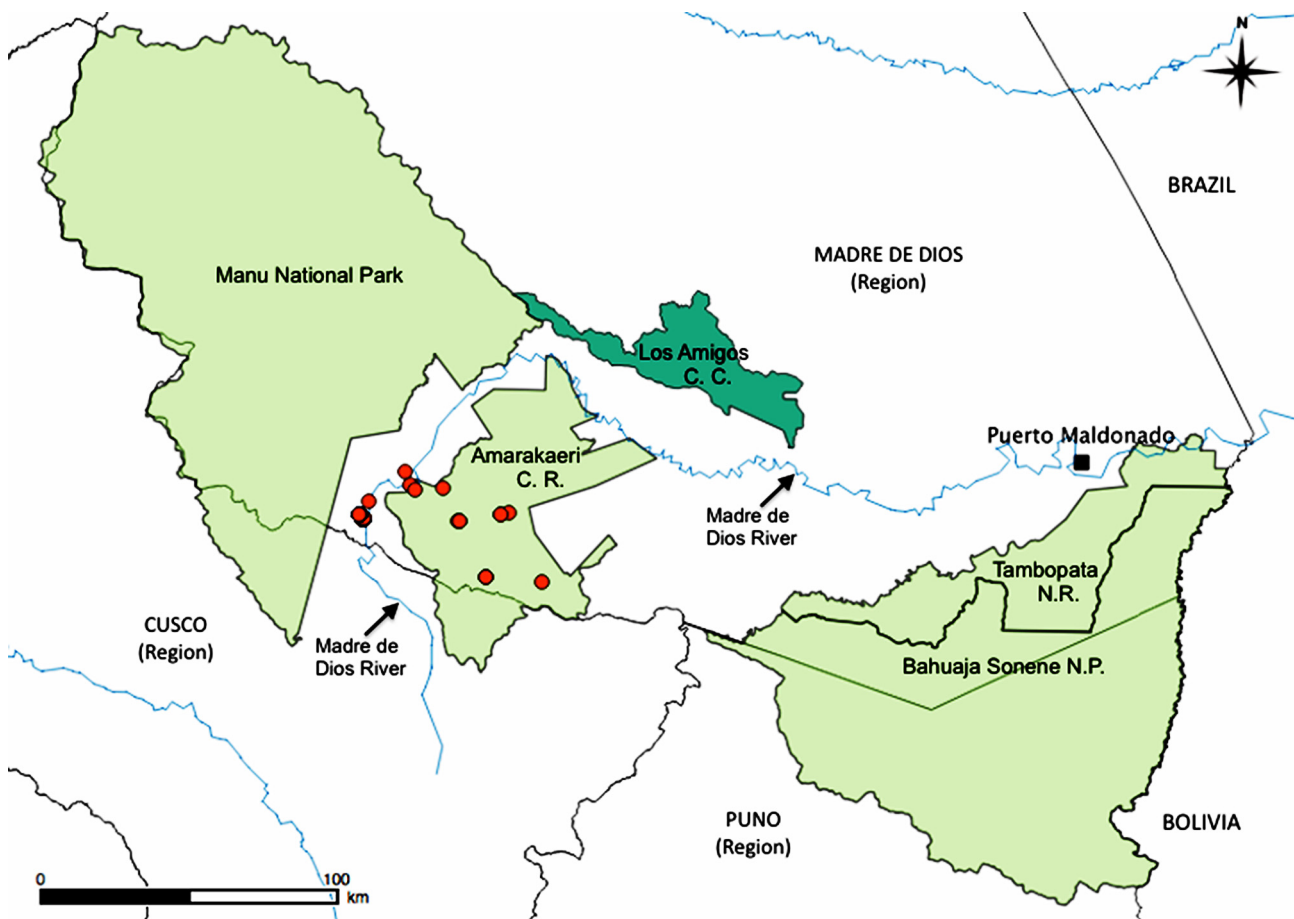


FIGURE 9. Currently known distribution of *Ameerega shihuemoy* (orange circles). The main natural protected areas in the Madre de Dios region are shown in green. Amarakaeri Communal Reserve covers an area of 402,335,62 ha; *A. shihuemoy* has been found at six localities inside this reserve.

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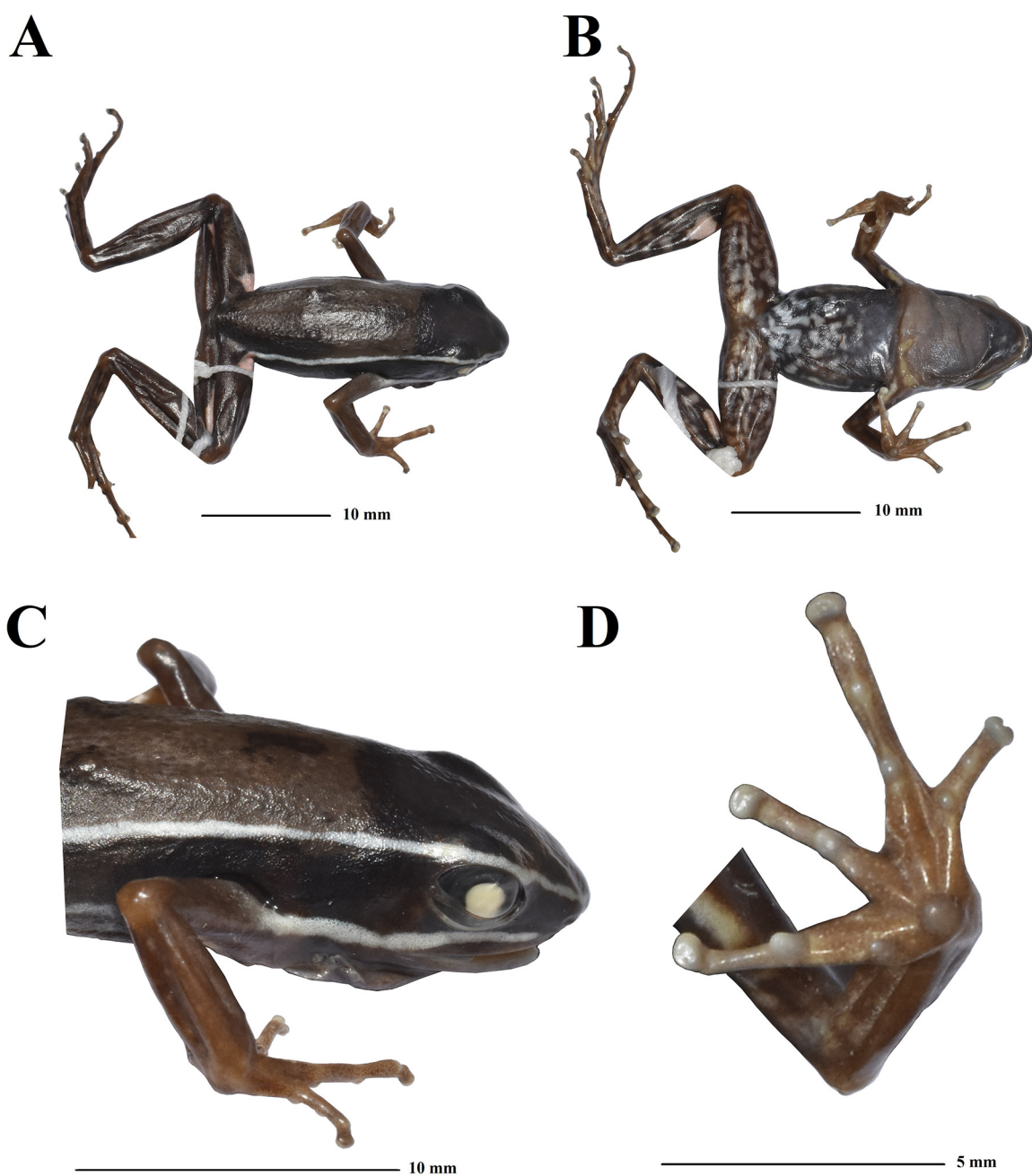


FIGURE 10. A, dorsal, and B, ventral view of the body; C, lateral view of head; and D, ventral view of the hand, of the adult holotype CBF 3900 of *Ameerega yungicola*. Scale on every picture. Photos by Daniela Rössler.

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APPENDIX I. Type material of *Ameerega* examined for this study.

- Ameerega bassleri*, PERU: Departamento San Martín: Roque, 1097 m.
- Ameerega boehmei*, BOLIVIA: Departamento Santa Cruz: Serranía de Chochís, near El Portón (1860' S, 6040' W, ca. 720 m above sea level), Chiquitanía region, Provincia San José de Chiquitos, CM 36155 (paratype).
- Ameerega boliviana*, BOLIVIA: Departamento La Paz: San Carlos, BMNH 1947.2.13.89–90 (syntypes); San Ernesto, 1947.2.13.91 (syntypes).
- Ameerega braccata*, BRAZIL: Mato Grosso: São Vicente, Chapada dos Guimarães, NHMW 3818.1–2, NHMW 3818.1 (syntypes).
- Ameerega cainarachi*, PERU: Departamento San Martín: Catarata Guacamaillo, MHNC 6732–33, MHNC 4789.
- Ameerega erythromos*, ECUADOR: Provincia Pichincha: Centro Científico, Río Palenque, 47 km S of Santo Domingo de los Colorados, 170 m, MCZ 96384 (holotype).
- Ameerega hahneli*, PERU: Departamento Loreto: Yurimaguas, Huallaga River, BMNH 1947.2.15.17 (lectotype).
- Ameerega macero*, PERU: Departamento Madre de Dios: Pantiacolla, MHCN 4428–29; Erika Lodge, MHNC 4769, MHNC 4787; Departamento Cusco: Provincia La Convención: Pozo Samanio, MHNC 6092; Malvinas, MHNC 6176–77.
- Ameerega parvula*, PERU: Departamento Loreto: Provincia Datem del Marañón: Teniente López, MHNC 7683, MHNC 7702.
- Ameerega picta*, BOLIVIA: Departamento Santa Cruz: Santa Cruz, MNHNP 4910 (syntypes)
- Ameerega simulans*, PERU: Departamento Cusco: Provincia Paucartambo: Atalaya, 600 m, near junction of Río Carbón with Río Alto Madre de Dios (12°59'S, 71°10'W), AMNH 155165–6 (paratypes), MHNC 5044, MHNC 5120; San Pedro, MHNC 4110, MHNC 4117, MHNC 4120, MHNC 4130; Provincia Quispicanchi: Km 6.2 on road from Puente Fortaleza to Quincemil, MHNC 5414; San Lorenzo, MHNC 6886, MHNC 6902, MHNC 6903; Nusinuscato, MHNC 7321, MHNC 7408, MHNC 7425, MHNC 7457; Quincemil, MHNC 7460; Departamento Puno: on ridge along Río Távora just below confluence of Río Candamo and Río Huacamayo, 550 m (13°31'S, 69°41'W), AMNH 155164 (paratype).
- Ameerega yungicola*, BOLIVIA: Departamento La Paz: km 10 on road from Caranavi to Yolosa (15° 53' 17" S, 67° 33' 09" W, ca. 600 m above sea level), CBF 3900 (Photographs of the holotype; Fig. 10).

APPENDIX II

GenBank accession numbers for taxa sampled in this study. Genbank accession codes of the new sequences are highlighted in bold font.

Species	Locality	Genbank Nbr.	Reference
<i>Ameerega altamazonica</i>	Peru: San Martin, Saposoa	DQ523086	Roberts <i>et al.</i> 2006
<i>Ameerega altamazonica</i>	Peru: San Martin, Tocache	EU517663	Twomey & Brown 2008
<i>Ameerega bassleri</i>	Peru: San Martin, Cainarachi	DQ523080	Brown & Twomey 2009
<i>Ameerega bassleri</i>	Peru: San Martin, Roque	FJ752272	Brown & Twomey 2009
<i>Ameerega bassleri</i>	Peru: San Martin, Roque	FJ752273	Brown & Twomey 2009
<i>Ameerega bassleri</i>	Peru: San Martin, Huallaga Canyon	DQ523057	Roberts <i>et al.</i> 2006
<i>Ameerega bilinguis</i>	Ecuador: Napo, Primavera	DQ523074	Roberts <i>et al.</i> 2006
<i>Ameerega bilinguis</i>	Ecuador: Sucumbios, Cuyabeno	DQ502095	Grant <i>et al.</i> 2006
<i>Ameerega bilinguis</i>	Ecuador: Sucumbios, Cuyabeno	DQ502073	Grant <i>et al.</i> 2006
<i>Ameerega bilinguis</i>	Ecuador: Sucumbios, Laguna Grande	HQ290996	Santos <i>et al.</i> 2009
<i>Ameerega braccata</i>	Brazil: Matto Grosso, Manso	DQ502125	Grant <i>et al.</i> 2006
<i>Ameerega cainarachi</i>	Peru: San Martin, Valle de Cainarachi	DQ523024	Roberts <i>et al.</i> 2006
<i>Ameerega cainarachi</i>	Peru: San Martin, Valle de Cainarachi	DQ523053	Roberts <i>et al.</i> 2006
<i>Ameerega flavopicta</i>	Brazil: Parana, Rio Tocantins	DQ502124	Grant <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Ucayali, Alto Purus	DQ523041	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Brazil: Amazonas	DQ523063	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Madre de Dios, Boca Manu	DQ523027	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Bolivia: Pando, Cobija	AF282246	Lötters & Vences 2000
<i>Ameerega hahneli</i>	Peru: San Martin, Convento	DQ523032	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Madre de Dios, Cusco Amazonico	EU342617	Santos <i>et al.</i> 2009
<i>Ameerega hahneli</i>	Peru: Loreto, Iquitos, Puente Itaya	DQ523033	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Colombia: Amazonas, Leticia	DQ502270	Grant <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Madre de Dios, Rio Los Amigos	DQ523056	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Brazil: Amazonas, near Manaus	DQ502226	Grant <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Huanuco, Panguana Biological Station	AF282248	Lötters & Vences 2000
<i>Ameerega hahneli</i>	Brazil: Acre, Porto Walter	DQ502084	Grant <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Brazil: Acre, Porto Walter	DQ502085	Grant <i>et al.</i> 2006
<i>Ameerega hahneli</i>	Peru: Loreto, Rio Manati	DQ523075	Roberts <i>et al.</i> 2006
<i>Ameerega hahneli</i>	French Guiana: Reserve Trinite	AY263247	Vences <i>et al.</i> 2003
<i>Ameerega hahneli</i>	Ecuador: Orellana, Yasuni	AY364573	Santos <i>et al.</i> 2003
<i>Ameerega ignipedis</i>	Peru: Loreto, Contamana	FJ752291	Brown & Twomey 2009
<i>Ameerega ignipedis</i>	Peru: Loreto, Contamana	FJ752292	Brown & Twomey 2009
<i>Ameerega ignipedis</i>	Peru: Loreto, Contamana	FJ752295	Brown & Twomey 2009
<i>Ameerega macero</i>	Peru: Ucayali, Alto Purus	DQ523089	Roberts <i>et al.</i> 2006
<i>Ameerega macero</i>	Peru: Cusco, Ivochote	DQ523039	Roberts <i>et al.</i> 2006
<i>Ameerega macero</i>	Peru: Junin, near La Merced	EU525852	Twomey & Brown 2008
<i>Ameerega macero</i>	Peru: Madre de Dios, Manu	DQ502155	Grant <i>et al.</i> 2006
<i>Ameerega parvula</i>	Ecuador: Macas	AY364574	Santos <i>et al.</i> 2003
<i>Ameerega pepperi</i>	Peru: San Martin, San Francisco	FJ752283	Brown & Twomey 2009
<i>Ameerega pepperi</i>	Peru: San Martin, San Francisco	FJ752287	Brown & Twomey 2009

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APPENDIX II. (Continued)

Species	Locality	Genbank Nbr.	Reference
<i>Ameerega pepperi</i>	Peru: San Martin, Abiseo	FJ752280	Brown & Twomey 2009
<i>Ameerega pepperi</i>	Peru: San Martin, Chumanza	FJ752281	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Huanuco, Panguana Biological Station	DQ502116	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Ucayali, Aguaytia	FJ752300	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Ucayali, Divisoria	FJ752303	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Huanuco, Cordillera El Sira	FJ752305	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Huanuco, Cordillera El Sira	FJ752307	Brown & Twomey 2009
<i>Ameerega petersi</i>	Peru: Huanuco, Codo del Pozuzo	FJ752306	Brown & Twomey 2009
<i>Ameerega picta</i>	Guyana: Mazaruni-Potaro, Kartabo Pt.	DQ502252	Grant <i>et al.</i> 2006
<i>Ameerega picta</i>	Bolivia: Mataracu	AF124126	Vences <i>et al.</i> 2000
<i>Ameerega pongoensis</i>	Peru: San Martin, Convento	DQ523044	Roberts <i>et al.</i> 2006
<i>Ameerega pongoensis</i>	Peru: Loreto: Shucushuyacu	FJ752308	Brown & Twomey 2009
<i>Ameerega pongoensis</i>	Peru: San Martin, Huallaga Canyon	DQ523076	Roberts <i>et al.</i> 2006
<i>Ameerega PortoWalteri</i>	Brazil: Acre, Porto Walter	DQ502229	Grant <i>et al.</i> 2006
<i>Ameerega PortoWalteri</i>	Brazil: Acre, Porto Walter	DQ502230	Grant <i>et al.</i> 2006
<i>Ameerega pulchripecta</i>	Brazil: Amapa, Serra do Navio	DQ502033	Grant <i>et al.</i> 2006
<i>Ameerega rubriventris</i>	Peru: Ucayali, near Aguaytia	AF282247	Lötters & Vences 2000
<i>Ameerega rubriventris</i>	Peru: Ucayali, near Aguaytia	EU517667	Twomey & Brown 2008
<i>Ameerega shihuemoy</i>	Peru: Madre de Dios, Manu Learning Centre	KX898435	This study
<i>Ameerega shihuemoy</i>	Peru: Madre de Dios, Manu Learning Centre	KX898436	This study
<i>Ameerega shihuemoy</i>	Peru: Madre de Dios, Manu Learning Centre	KX898437	This study
<i>Ameerega silverstonei</i>	Peru: Huanuco, Tingo Maria	DQ523084	Roberts <i>et al.</i> 2006
<i>Ameerega simulans</i>	Peru: Cusco, Quincemil	DQ523090	Roberts <i>et al.</i> 2006
<i>Ameerega smaragdina</i>	Peru: Pasco, Pan de Azucar	EU517670	Twomey & Brown 2008
<i>Ameerega smaragdina</i>	Peru: Pasco, Iscozacin	DQ523042	Roberts <i>et al.</i> 2006
<i>Ameerega smaragdina</i>	Peru: Pasco, Pan de Azucar	FJ752296	Brown & Twomey 2009
<i>Ameerega smaragdina</i>	Peru: Pasco, Pan de Azucar	FJ752297	Brown & Twomey 2009
<i>Ameerega smaragdina</i>	Peru: Pasco, Pan de Azucar	FJ752299	Brown & Twomey 2009
<i>Ameerega sp</i>	Peru: Cusco, Ivochote	DQ523038	Roberts <i>et al.</i> 2006
<i>Ameerega trivittata</i>	Peru: Ucayali, Alto Purus	DQ523028	Roberts <i>et al.</i> 2006
<i>Ameerega trivittata</i>	Peru: Madre de Dios, Tambopata	DQ502023	Grant <i>et al.</i> 2006
<i>Ameerega yoshina</i>	Peru: San Martin, Callanayacu	FJ752274	Brown & Twomey 2009
<i>Ameerega yoshina</i>	Peru: San Martin, Callanayacu	FJ752275	Brown & Twomey 2009
<i>Ameerega yoshina</i>	Peru: Loreto, Contamana	FJ752278	Brown & Twomey 2009
<i>Ameerega yoshina</i>	Peru: Loreto, Contamana	FJ752279	Brown & Twomey 2009
<i>Ameerega yungicola</i>	Bolivia: La Paz, Carnavi	AY263239	Vences <i>et al.</i> 2003
<i>Colostethus fugax</i>	Ecuador: Morona-Santiago	AY364547	Santos <i>et al.</i> 2003

APPENDIX III

Variance inflation factors (VIFs) for the environmental variables, where VIF values greater than 10 indicates that there is some problem of collinearity.

Environmental variables	VIF	Remarks
Number of potential refuges	1.72	Non-collinearity
Percentage of large rocks	1.44	Non-collinearity
Leaf litter cover	2.84	Non-collinearity
Leaf litter depth	2.06	Non-collinearity
Canopy cover	1.51	Non-collinearity
Presence of a still body of water	1.51	Non-collinearity
Stream flow	1.11	Non-collinearity
Wood-debris cover	1.30	Non-collinearity

APPENDIX IV

Results of the variables influencing frog presence/absence within SLR and CCR habitat; where positive β -estimates indicate the positive effect of a variable on frog presence and negative β -estimates indicate the negative effect of a variable on frog presence (e.g. a negative β -estimate of stream flow means that low stream flow is related to frog presence, while fast stream flow is related to frog absence).

Parameter	β -Estimate	SE	95% CI
Leaf litter cover	0.198	0.077	0.091 to 0.410
Number of potential refuges	0.279	0.109	0.104 to 0.555
Percentage of large rocks	0.403	0.131	0.201 to 0.745
Still body of water presence	3.677	1.649	1.032 to 7.986
Stream flow	-16.057	6.532	-33.559 to -6.520
Canopy cover	0.069	0.034	-0.007 to 0.148
Wood debris percentage	-	-	-
Leaf litter depth	-	-	-

APPENDIX V

Model averaging results of the variables influencing frog presence/absence within SLR habitat; where positive β -estimates indicate the positive effect of a variable on frog presence and negative β -estimates indicate the negative effect of a variable on frog presence (e.g. a negative β -estimate of stream flow means that low stream flow is related to frog presence, while fast stream flow is related to frog absence).

Parameter	Σ	β -Estimate	SE	95% CI
Leaf litter cover	1.00	0.186	0.068	0.051 to 0.322
Number of potential refuges	1.00	0.221	0.107	0.008 to 0.433
Percentage of large rocks	1.00	0.319	0.125	0.072 to 0.567
Still body of water presence	1.00	2.979	1.629	-0.246 to 6.205
Stream flow	1.00	-13.399	5.980	-25.241 to -1.558
Canopy cover	0.69	0.042	0.040	-0.006 to 0.129
Wood debris percentage	0.00	-	-	-
Leaf litter depth	0.00	-	-	-

Model averaged parameters from the best supported models defined as all models within $\Delta AICc < 2$ of the top model. The strength of support (Σ) for a given parameter: 1 "full support", >0.9 "Strong support", >0.6 "some support", and <0.6 "weak support"