Zootaxa 2049: 1–38 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Complicated histories: three new species of poison frogs of the genus *Ameerega* (Anura: Dendrobatidae) from north-central Peru

JASON L. BROWN[1] & EVAN TWOMEY[2]
Department of Biology, East Carolina University, Greenville NC, U.S.A.
E –mail: [1]jasonleebrown@hotmail.com, [2]evan.twomey@gmail.com

Abstract

We describe three new species of poison frogs discovered on recent expeditions throughout Central Peru. Two of these were discovered from the Serranía de Contamana, a small mountain range near the town of Contamana on the Río Ucayali in Central Peru. The first of these species is similar in appearance to *Ameerega petersi*, but the two species differ in call and size, have allopatric distributions, and are not close relatives according to a Bayesian phylogeny. This species is also similar morphologically to *A. pongoensis,* although the latter lacks flash marks above the groin and has a distinct advertisement call. Our phylogenetic data suggest that this species is closely related to *A. bassleri*, a species which is much larger and usually possesses a brightly-colored yellow or orange dorsum. The second new species has a red dorsum and is similar in appearance to *A. bassleri, A. cainarachi*, and the third new species described herein, but can be distinguished by its unusual advertisement call. The third new species in this paper was discovered in the upper Huallaga Valley near the towns of Tocache and Juanjui. This species resembles certain populations of *A. bassleri* and the second new species (mentioned above), but can be diagnosed by its advertisement call which is significantly slower than its closest relatives; approximately one-half the speed of *A. bassleri* and one-quarter the speed of the second new species. Finally, we discuss the biogeography of the *A. bassleri* clade in light of these new discoveries. The biogeography of this clade is consistent with the disturbance-vicariance hypothesis, and climatic fluctuations were likely a major driver in the divergence and speciation of this group. We also present a phylogeny with increased sampling in the *A. petersi* clade and discuss the taxonomy of the group.

Key words: *Ameerega ignipedis* **sp. nov.**, *Ameerega pepperi* **sp. nov.**, *Ameerega yoshina* **sp. nov.**, coalescence, Contamana, Dendrobatid frogs, disturbance-vicariance hypothesis, incomplete lineage sorting, introgressive hybridization, new species, Peru, poison frogs, taxonomy

Resumen

Se describe tres especies nuevas de ranas venenosas que fueron descubiertos en expediciones recientes por el Perú Central. Dos fueron descubiertos en el Serranía de Contamana, una cordillera pequeña cerca al pueblo de Contamana en el Río Ucayali en el parte central del Perú. La primera de estas especies es similar en su aspecto a *Ameerega petersi*, pero esas dos especies se distingue por sus cantos y tamaños, tienen distribuciones alopátricos, y no están cercanamente emparentadas en base de un filogénio Bayesiano. También es similar morfológicamente a *A. pongoensis*, pero la última especie le falta puntos sobre su ingle y tiene un canto distinto. Nuestros datos filogenéticos sugieren que esta especie está cercanamente emparentada a *A. bassleri*, una especie mucho más grande y que usualmente tiene un dorso amarillo o anaranjado brillante. La segunda especie nueva es similar en su aspecto a *A. bassleri*, *A. cainarachi*, y la tercera especie descrito aquí, pero se puede distinguirse por su canto raro. La tercera especie nueva en este papel fue descubierto en la parte alta del valle del Río Huallaga cerca los pueblos de Tocache y Juanjui. Esta especie es parecido a algunas poblaciones de *A. bassleri* y la segunda especie (mencionado arriba), pero se puede distinguirse por su canto cual es significamente más lento de sus parientes cercanas; aproximadamente la mitad de la velocidad de *A. bassleri* y un cuarto de la velocidad de la segunda especie nueva. Finalmente, discutimos la biogeografía del clado *A. bassleri* siguiendo estos

Accepted by M. Vences: 9 Feb. 2009; published: 23 Mar. 2009

descubrimientos nuevos. La biogeografía de este clado concuerde con la hipótesis disturbio-vicariancia y fluctuaciones climáticos probablemente eran un factor capital en la divergencia y especiación de ese grupo. También presentamos una filogenia con muestreo aumentado en el clado de *A. petersi* y discutimos la taxonomía del grupo.

Introduction

The past two decades have witnessed a dramatic increase in the number of described species, in part due to advances in molecular taxonomy. Prior to molecular phylogenetics (and other genetic-based techniques), researchers relied primarily on morphological characters to diagnose species. Unfortunately, many taxonomic groups have limited morphological variation and the available morphological characters are often more indicative of species groups rather than single species. Many systematic relationships based solely on morphological relationships have since been demonstrated to be incomplete or inaccurate (e.g. Stockman and Bond 2007); and convergences have been relatively abundant (Myers 1982, Schulte 1986, Morales 1992, Myers *et al.* 1998). Molecular phylogenetic estimates often provide robust species tree estimates and have proven a useful tool for taxonomic studies; however they should not be used an absolute barometer of species (Stockman & Bond 2007) as it is widely recognized that under certain circumstances, species trees and gene trees can be discordant (Pamilio and Nei 1988, Doyle 1997, McGuire *et al.* 2007), particularly in analyses based solely on mitochondrial data.

Two predominant processes affecting species tree and gene tree discordances are introgressive hybridization and incomplete lineage sorting (Patton and Smith 1994, Maddison 1997, Funk and Omland 2003), and an increasing number of studies are detecting these processes (Weisrock *et al.* 2005, McGuire *et al.* 2007). Under certain circumstances, both processes can result in similar phylogenetic patterns (Avise and Ball 1990, Morando *et al.* 2004). However, in many situations, the phylogeographic patterns are able to distinguish between these two phenomena. If the pattern is a result of incomplete lineage sorting, then alleles present in a common ancestor have been maintained in descendant species and the alleles are expected be distributed randomly among the descendant populations. Conversely, if the resulting pattern is a result of mitochondrial introgression, then the incongruence between mitochondrial phylogenies and the estimated species trees are concentrated geographically near species boundaries or observed hybrid zones (McGuire *et al.* 2007).

Gene tree polyphyly is common in initially-arisen species; as time progresses, lineage sorting typically converts most gene trees to a status of paraphyly and then reciprocal monophyly (Avise 2000). The rate this occurs depends on population size and reproduction rate, however there are also instances where stable lineages fail to sort completely despite considerable time. For example, the Brown Bear, *Ursus arctos*, is rendered paraphyletic by the Polar Bear, *U. maritimus* (Talbot and Shields 1996). *Bufo marinus*, the Cane Toad, is paraphyletic to *B. paracnemis* where the two clades of *B. marinus* display a deep phylogenetic separation based on the uplift of the Andes (Slade and Moritz 1998). The North American Deer Mouse, *Peromyscus maniculatus*, is rendered paraphyletic by the Old-field Mouse, *P. polionotus* (Avise *et al.* 1983). Under the strictest implementation of the phylogenetic species concept, each of the cases of paraphyly could be reconciled by elevating unique phylogenetic lineages to species. In these cases the utility of multiple species is questionable, particularly when gene flow continues to occur between members of the two (or more) clades, or the clades have not diverged ecologically or morphologically.

Detailed investigations of Peruvian poison frogs have consistently revealed cases where a single, widespread ‘species’ actually represents multiple cryptic species (Myers 1982, Schulte 1986, Morales 1992, Myers *et al.* 1998, Schulte 1999, Brown *et al.* 2006, Twomey and Brown 2008a). While certain taxonomic groups have undergone substantial revisions and restrictions (e.g. *Dendrobates quinquevittatus* sensu Silverstone), others have persisted as ‘taxonomic dustbins’ (e.g. *Ranitomeya ventrimaculata*, *Ameerega hahneli, A. picta*). Particularly confusing has been the *Ameerega petersi* group, a motley assortment of frogs sharing a dark dorsum with greenish dorsolateral stripes, which was first referred to as *Phyllobates petersi* and *P. smaragdinus* by Silverstone (1976). Subsequent studies (Myers *et al.* 1998, Schulte 1999) demonstrated that *Ameerega* (= *Phyllobates*) *petersi* sensu Silverstone in fact included two additional species (*A. simulans* and *A. pongoensis*) which were diagnosable from *A. petersi* mainly on the basis of size and advertisement call parameters. Recent exploration in central Peru has led us to the discovery of three undescribed species of *Ameerega*. We discovered two of these species during a 2006 reconnoiter of Contamana, an isolated town on Río Ucayali 125 km NNW of Pucallpa. The area between Contamana and the Brazilian border is unique in that it contains three distinct mountain ranges (collectively known as the Sierra del Divisor) which are isolated from the Andes and largely isolated from each other. Few herpetological surveys have occurred in these mountains until recently, although a rapid biological inventory in 2005 (Chicago Field Museum 2006) identified 109 species of reptiles and amphibians, about a dozen of which were new to science. However, the westernmost of these mountain chains, the Serranía de Contamana, was neglected in their survey. This 47 km-long chain of hills runs in a NW-SE direction, rises up to a maximum elevation of 774 m above sea level, and is characterized by intense geothermal activity, lush rainforest, and a rich dendrobatid community.

The first of these new species represents yet another cryptic species of *Ameerega* from upper Amazonia which superficially resembles members of the *petersi* group. It is similar in appearance to *A. petersi*, *A. pongoensis, A. simulans*, and *A. smaragdina*, can be diagnosed on the basis of call and color pattern, and is a close relative of *A. bassleri*, a species occurring in the Andean foothills of north-central Peru. This species represents the fourth to be teased apart from *A. petersi* sensu Silverstone (1976), and is now the fifth Peruvian *Ameerega* species to possess a dark dorsum and greenish dorsolateral stripes.

The second new species does not resemble any other frogs in the vicinity of Contamana, having a red dorsum and a relatively large SVL. It is similar in appearance to *A. cainarachi* and *A. bassleri*, two species which occur throughout the Cordillera Oriental in northern San Martín. This species has a distinct advertisement call which can be used to distinguish it from *A. bassleri*, its closest relative. We discovered an additional population of this species in 2007 in the Huallaga Canyon east of Chazuta, approximately 130 km NW of Contamana. This population occurs in close proximity (< 5 km) to populations of *A. bassleri*, yet these frogs have an advertisement call identical to that of the Contamana population.

The third new species is found in the upper Huallaga river valley (approximately between Juanjui and Tocache). It is very similar in appearance to *A. cainarachi*, *A. bassleri*, and the second new species (mentioned above), none of which occur in the upper Huallaga river valley. This species has a distinct advertisement call which can be used to diagnose it from its two sister taxa (the second new species and *A. bassleri*).

The purpose of this paper is to describe these three new species of *Ameerega* on the basis of molecular, morphological, and call data. Also, we describe one apparent instance of mitochondrial introgression and two apparent cases of incomplete linage sorting in the Peruvian poison frog genus *Ameerega*. The two instances of incomplete lineage sorting are likely the result of a speciation event involving a small population isolated from an ancestral stock with a large geographic distribution. The resulting daughter species renders the mother species paraphyletic, and despite considerable time, lineage sorting has not completed. In both our examples, the mother species possesses a considerable amount of morphological variation (within and between populations). In the one instance where the mother and daughter species are sympatric (or nearly sympatric; the case regarding *A. bassleri* and the third new species, respectively), the daughter species possesses dramatically different call characters that have likely reinforced the species boundaries.

We also include a phylogeny containing *A. petersi* and *A. smaragdina* individuals from several new localities. Our phylogenetic analysis supports the notion that *A. petersi* and *A. smaragdina* may be distinct species; however, this hypothesis is not supported by ecological or morphological data. Lastly, we discuss the biogeography of the *A. bassleri* clade, as it appears now that the Serranía de Contamana may have been colonized by members of this clade on two separate occasions.

Materials and methods

The type series of the new species are deposited in 70% ethanol in the Museo de Historia Natural San Marcos, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM). The following measurements were made with mechanical calipers and a micrometer to the nearest 0.1 mm, following Myers (1982) and Brown *et al.* (2006): snout-vent length (SVL), femur length from vent to lateral edge of knee (FL), tibia length from medial edge of heel to lateral edge of knee (TL), knee-knee distance with both legs extended straight (KK), foot length from proximal edge of metatarsal tubercle to tip of toe IV (FoL), hand length from proximal edge of metacarpal tubercle to tip of longest finger (HaL), head length from most exposed corner of occipitum to tip of snout (HL), head width between tympana (HW), body width under axillae (BW), upper eyelid width (UEW), interorbital distance (IOD), internarial distance (IND); horizontal tympanum diameter (TD), horizontal eye diameter (ED), distance from outer corner of eye to tympanum (DET), length of finger I from proximal edge of median palmar tubercle to tip of finger disc (L1F), length of finger II from proximal edge of median palmar tubercle to tip of finger disc (L2F), width of disc of finger III (W3D), and width of finger III just below disc (W3F). Sex was determined by checking for presence of vocal slits.

Advertisement calls for the new species and other species used in the analyses were recorded with a Sony DCR-JC42 camera and Sony ECM-HGZ1 gun microphone or a Marantz PMD660 recorder and Sennheiser microphone. Calls were analyzed in Raven 1.2 (Charif 2004) and compared to vocalizations of closely related or morphologically similar species. We measured the following characteristics: notes per second, notes per minute, note duration, space between notes, and dominant frequency. Notes per second were calculated by counting the numbers of notes within the center of a call (excluding start-up and ending notes which tend to be slower) and dividing that by the period of time; notes per minute were calculated by multiplying the notes per second calculation by 60. The note duration and space between notes were the average time of the duration of 8 notes and the average of time between those notes, respectively. Results were analyzed in SPSS 15.0 (2008) using one-way ANOVAs. If the assumption of homogeneity of variance was not met, data were *ln* transformed. Tukey's post hoc tests were used for pairwise comparisons since sample sizes were unbalanced.

Genetic data used for the phylogenetic analyses were adopted from previous studies (Grant *et al.* 2006, Roberts *et al.* 2006, Twomey and Brown 2008a) with the exception of 45 individuals including: five individuals of the first new species, six individuals from the second new species, nine individuals from the third new species, 11 *A. bassleri* from four localities, two *A. pongoensis* from a previously unknown locality, eight *A. petersi* from five localities, and four *A. smaragdina* from the type locality. Tissue collection, DNA extraction, amplification, and sequence analysis of new samples followed the methods of Roberts *et al.* (2006).

Phylogenetic analysis was done in MrBayes using methods outlined in Twomey and Brown (2008b). Briefly, the final phylogenetic data set included 135 individuals from 67 localities and contained 1682 bp of the following mitochondrial regions: 12s, 16s, cytochrome b (CytB). The dataset was partitioned into five partitions: codons (1st, 2nd, and 3rd positions for CytB) and separate, single partitions each for 12s and 16s. MrModeltest version 2.2 (Nylander 2004) was used to identify a substitution model, nucleotide frequencies, and optimal priors for the gamma parameter and the proportion of invariant sites for each partition. We used two species of *Allobates*, four species of *Colostethus*, five species of *Epipedobates* and two species of *Silverstoneia* as outgroups. MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was run for twelve million generations using the following mixed models indicated by MrModeltest 2.2: 12S (nst = 6, I + Γ), 16s (nst = 6, I + Γ), CytB-1st (nst = 2, I + Γ), CytB-2nd (nst = 2, I + Γ), CytB-3rd (nst=6, Γ). Negative log-likelihood (2ln) scores were used to identify the burn-in phase at 1.8 million generations, after which the remaining trees were summed. The standard deviation of split frequencies for the Bayesian analysis reached 0.01 to ensure consistency of the resulting tree topology. All *A. smaragdina* and individuals from the second new species were sequenced twice each direction (forward and reverse) to confirm resulting haplotypes.

The historical distributions of the *bassleri* and *petersi* clades were analyzed using dispersal-vicariance analysis (DIVA; Ronquist 1996). In the analysis of the *bassleri* clade, we divided the species distributions into six geographic groups: upper Huallaga river valley, Cordillera Azul, Cordillera Oriental, lower Huallaga Canyon and Pampas del Sacramento, Serranía de Contamana, and Ecuador (for outgroup, see below). The two river barriers depicted in Fig. 7 (The Ucayali River Valley and Huayabama River Valley) were not included because none of the species currently occur in these areas. Each taxon in the *bassleri* clade (individuals in Fig. 6 and *Ameerega bilinguis* as an outgroup) were placed into one of the six groups. In the analysis of the *petersi* clade, we divided the species distributions into four geographic groups: North Panguana (all localities of north of Puerto Inca that are within the Pachitea and Aguaytia drainages), South Panguana (all localities south of Puerto Inca), Cordillera Oriental (including Cordillera Azul), and Bolivia (for outgroup, see below). Each individual in the *petersi* clade was placed into one of the four groups (individuals in Fig. 6 and *A. yungicola* as outgroup). To simplify the analysis, clades composed of many individuals of the same species and same geographic group were reduced to a single individual.

To evaluate the environmental interchangibility and ecological divergence of *Ameerega bassleri* and *A.* **sp. nov.** 3, we used ecological niche modeling (Maxent version 3.2.1; Phillips *et al.* 2006; Stockman and Bond 2007). Maxent was run using the following parameters: random test percentage 25%, regularization multiplier = 1, maximum iterations = 500, convergences threshold = 0.00001, maximum number of background points = 10000. We used 107 presence points of *Ameerega bassleri* and 16 presence points of *A.* **sp. nov.** 3 for each species model. The models were built from the 19 standard bioclimatic environmental (Worldclim 1.4; Hijmans *et al.* 2005). Environmental layers were reduced in geographic extent to an area about 50% larger than the known distribution of each species. This is important for selection of realistic pseudo-absence points by Maxent (R. P. Anderson pers. comm.). The resulting models were then projected into a larger geographic area that encompassed the distributions of both species. The environmental variables were jackknifed and each final model was run using the variables that contributed 5% or more to the initial jackknifed model. The resulting model was converted to a binary absence-presence model using lowest habitat suitability value of observed present points as a cutoff (Phillips *et al.* 2006).

All holotype and paratype specimens are deposited in the Museo de Historia Natural San Marcos, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM). Addition specimens mentioned were from the following museums: the Field Museum of Natural History, Chicago, IL, USA (FMNH) and the American Museum of Natural History, New York, NY, USA (AMNH).

***Ameerega ignipedis* sp. nov.**

Figures 1, 11, 16

Phyllobates petersi: Silverstone 1976 p. 37 (FMNH 56248 collected in 1947 by J. M. Schunke at "Cerro Azul, 24 km E Contamaná").

Holotype. MUSM 24948, an adult female (Fig. 1) collected on 19 July 2006 by M. Pepper, E. Twomey, and J. Brown in Departamento Loreto, Peru, 17.5 km NE Contamana at the western foot of the Serranía de Contamana, 240 m elevation, 7° 11' 55.46" S, 74° 57' 35.28" W. Type locality near "El Unión", a campsite located at the confluence of a hot-water and cold-water stream.

Paratypes. Five adults (MUSM 24947, 24949–24952) collected on 19 July 2006, same locality as holotype.

Etymology. The species name *ignipedis* is a Latin adjective meaning 'fiery-footed', referring to the fact that the type locality is located alongside a geothermal stream. Our campsite in the Serranía de Contamana was located at the confluence of two streams, one of which was fed by hot springs and reached temperatures exceeding 90° C in some places. We found *A. ignipedis* along the cool-water stream in low abundances, but along the hot-water stream they were much more common.

Definition and diagnosis. Assigned to the genus *Ameerega* on the basis of the following: first finger longer than second, webbing absent between the toes, dorsal skin granular (Myers 1982, Grant *et al.* 2006).

This is a small species of *Ameerega* with an adult SVL of approximately 20–24 mm. Dorsum granular and brown medially, black laterally; pale yellowish-green dorsolateral stripes extending from loreal region to groin. Pale yellowish-green labial stripe present starting behind nares and terminating above forelimb as a yellow patch. Yellow spots present and distinct above groin, most individuals (~60%) also possess a yellow spot on the medial face of the tibia. Venter sky-blue with black marbling. Teeth present. Appressed first finger longer than second; finger discs weakly expanded; hands and feet lacking webbing between digits. Vocalization consists of a series of regularly-spaced notes occurring at a rate of ~2 notes per second; note length short (97 ms), dominant frequency of 4700 Hz.

FIGURE 1. (a) Holotype of *Ameerega ignipedis* **sp. nov.** (MUSM 24948); (b) *A. pepperi* **sp. nov.** (uncollected individual from Polvora, San Martín, Peru); (c) Holotype of *A. yoshina* **sp. nov.** (MUSM 24945); (d) *A. bassleri* (MUSM 26965) from type locality (Roque, San Martín, Peru).

Ameerega ignipedis is the sister taxon to a larger clade containing *Ameerega bassleri* and the two additional new species described in this paper. However, *A. ignipedis* bears little resemblance to any of these species, as they are larger and usually have a yellow, orange, or red dorsum. *Ameerega ignipedis* bears a pattern most similar to *A. pongoensis*, *A. petersi*, *A. simulans*, and *A. smaragdina*, all of which have a brown or black dorsum with green or yellow dorsolateral stripes. For *A. ignipedis* individuals that possess a spot on the medial face of the tibia, diagnosis can be made on this character alone, as none of the four similar species possess such spots. Individuals that lack this shank spot can be diagnosed on the basis of body size, advertisement call, and ventral coloration. Both *A. petersi* and *A. smaragdina* have a larger SVL than *A. ignipedis* (Table 1). Furthermore, the dorsolateral stripes of *A. smaragdina* (and many populations of *A. petersi*) are emerald-green as opposed to pale yellowish-green in *A. ignipedis*. Both *A. petersi* and *A. smaragdina* have ventral coloration that is green and blue (Figures 11 & 12) (vs. uniform sky-blue in *A.*

ignipedis), and *A. smaragdina* lacks black marbling on the venter (vs. black marbling present in *A. ignipedis*). Diagnosis against *A. simulans* can be made since *A. simulans* is smaller than *A. ignipedis* with respect to SVL (Table 1). *Ameerega simulans* also lacks spots above the axillae and groin (as opposed spots above axillae and groin present in *A. ignipedis*). *Ameerega ignipedis* can be distinguished from *A. pongoensis* in that the latter species lacks distinct yellow spots above the groin (vs. large, distinct spots in *A. ignipedis*), and by differences in advertisement call (Fig. 2). *Ameerega pongoensis* has a call consisting of a single- or double-peep which is repeated irregularly at a rate of one note (or note couplet) every ~2 seconds (vs. notes repeated regularly at a rate of two notes per second in *A. ignipedis*). *Ameerega ignipedis* can furthermore be diagnosed on the basis of 20 unambiguous nucleotide substitutions in the 16s and CytB regions of the mitochondrial genome.

Measurements (in mm) of holotype. The female holotype (Fig. 1) has SVL 23.8; FL 10.5; TL 11.1; KK 20.9; FoL 9.9; HaL 6.0; HL 6.0 HW 7.1; BW 7.0; UEW 4.6; IOD 3.4; IND 2.8; TD 2.6; ED 4.1; DET 1.4; L1F 4.7; L2F 2.9; W3D 0.9; W3F 0.6. Measurements of paratypes are given in Table 3.

Description of holotype. Size medium, SVL 23.8 mm. Dorsal skin of head, body, and hind limbs granular; skin smooth or nearly smooth on forelimbs and smooth on sides of head and body and ventral surfaces. In life, the dorsum is brown medially with weak black marbling; laterally the dorsum is black. A pale yellow dorsolateral stripe present on either side beginning at nares, passing over the eyelids, and continuing posteriorly to terminate above the groin as a bright yellow spot. Stripe starts as pale yellow and blends to bright yellow towards the groin. Dorsolateral lines are ~50% wider at groin than head. Pale yellow labial stripe present beginning near nares, extending posteriorly, terminating above axillae as a weakly defined yellow patch. Flanks black from groin to snout, ventral margin is pale yellow and fades to sky-blue ventrally. Upper forelimbs are yellow-bronze dorsally and conspicuously yellow ventrally; lower part of forelimbs brown dorsally and sky-blue with black reticulation ventrally. Hind limbs brown with irregular black markings dorsally; sky-blue with black reticulation ventrally. A conspicuous yellow spot is present on the medial surface of the tibia near the knee. Ventral surface of limbs, belly, and head sky-blue with a coarse reticulum of irregular black lines. Hands and feet brown dorsally. Iris black.

Widest part of head between jaw articulations, head narrower than body; greatest head-width 29.9% of SVL. Tongue medium sized, oval. Premaxillary and maxillary teeth present. Vocal slits absent. Snout narrow, sloping from lateral view; bluntly rounded from dorsal view; truncate from ventral view. Nares situated and directed posterolaterally to the tip of snout; nares visible from front and below but not from above. Canthus rostralis sloped, slightly rounded; loreal region nearly vertical and slightly concave. Upper eyelid 1.4 times wider than interorbital distance. Eye large and prominent with a maximum diameter of 17.2 % of the snout vent length, pupil rounded and horizontally elliptical. Tympanum circular, partially concealed posterodorsally, lacking tympanic annulus; its diameter less than 63.4 % of ED; supratympanic fold absent.

Hands relatively small, length being 25.2% of SVL. Relative length of appressed fingers: I ≈ II ≈ IV < III (numbers starting interiorly). Discs moderately expanded on all fingers but finger II, which has a weakly expanded disc. Disc on finger III is 1.5 times wider than distal end of adjacent phalanx. A large, circular outer metacarpal tubercle on median base of palm; a smaller inner metacarpal tubercle on base of finger I; one well developed and prominent subarticular tubercle on fingers I and II, two on fingers III and IV.

Hind limbs relatively short; heel reaches shoulder when appressed anteriorly. Tibia length 46.6% of SVL. Relative lengths of appressed toes I < II < V < III < IV; first toe short (but conspicuously present), barely reaching bottom of subarticular tubercle on base of second toe, with unexpanded disc; toes II and V barely expanded (much smaller than finger discs); toe III and IV expanded (disc 1.5 times broader than adjacent phalanx). Moderate-sized inner and small outer metatarsal tubercles, somewhat protuberant with rounded surfaces. One protuberant subarticular tubercle on toes I and II, two on toes III and V, and three on IV, however the proximal tubercle on toe IV is reduced. Hands and feet lacking supernumerary tubercles, lateral fringes, and webbing. No basal webbing on toe fringes. Tarsal tubercles absent.

In 70% alcohol the color is almost identical to the living animal described above. The only differences are that the brown on dorsum changed to grey, cream yellow dorsolateral stripes changed to silver-white. Flash marks changed from yellow to cream yellow. Blue coloration on the venter changed to slate-blue.

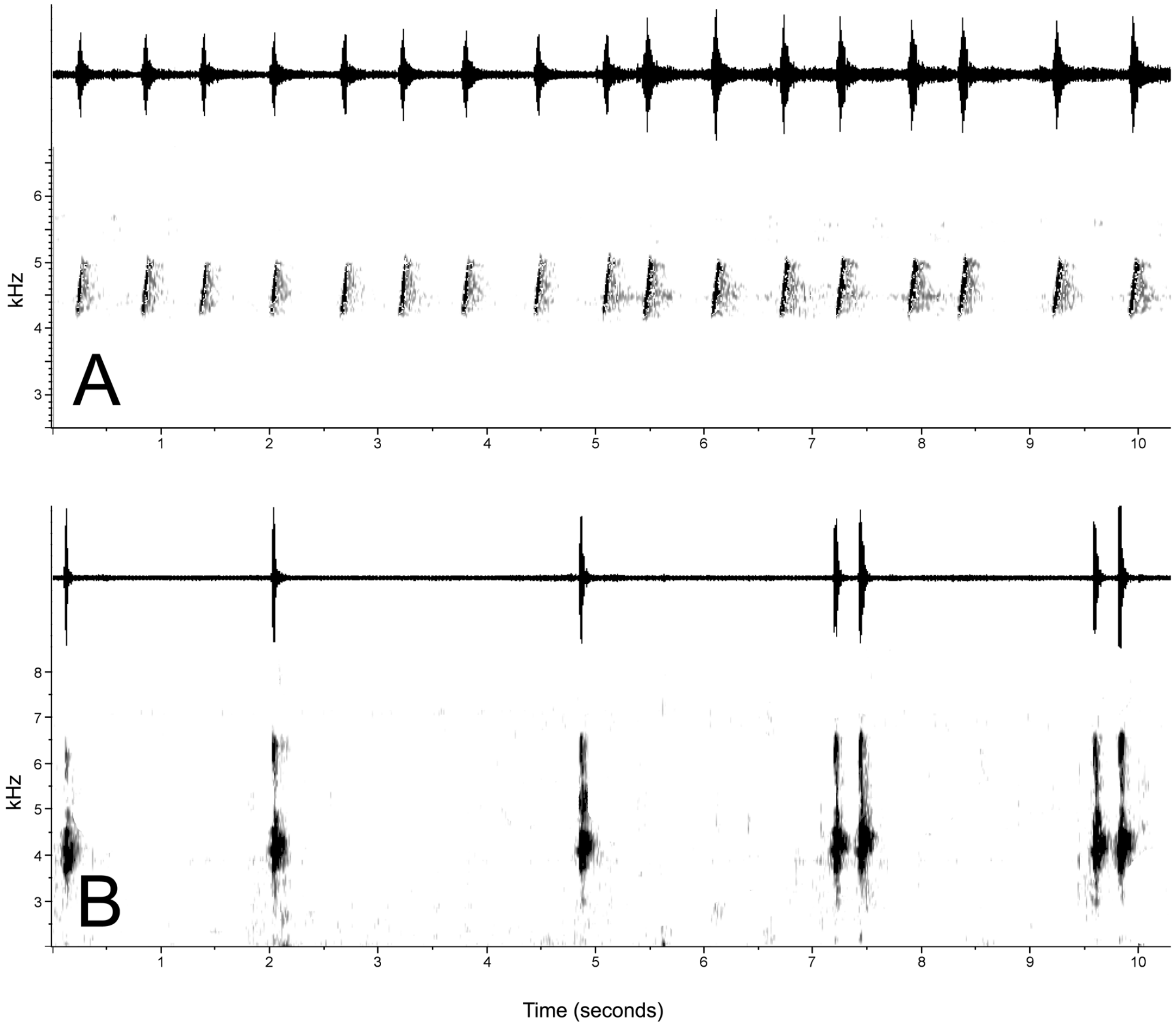

FIGURE 2. Advertisement calls of *Ameerega ignipedis* **sp. nov.** and *A. pongoensis*, spectrograms for calls with matching waveforms. (A) *A. ignipedis*, field recording of a male in type locality, 24 C, 5 May 2007. Dominant frequency 4583 Hz. (B) *A. pongoensis*, captive recording of a male from type locality (Huallaga Canyon). Dominant frequency 4233 Hz.

TABLE 1. Comparison of body size and advertisement call parameters for *Ameerega ignipedis* and species similar in appearance. Although this table and Fig. 10 would suggest that *A. petersi* and *A. smaragdina* have very different calls, we emphasize that the calls in these species are variable and do not appear to consistently differ in any parameter we have measured.

	Body size		Call parameters	
Species	Mean SVL	SVL Range	Notes/sec	Dominant frequency (Hz)
A. ignipedis	22.4 (*N* = 7)	20.3–24.2	1.7	4583
A. petersi	26.2 (*N* = 28)	23.2–30.3	9.0	3570
A. pongoensis	22.9 (*N* = 5)	20.5–26.0	0.4	4233
A. simulans	20.0 (*N* = 13)	18.9–22.4	2.0	4250
A. smaragdina	26.5 (*N* = 2)	26.5–26.5	5.2	3594

Variation in type series. The most notable variation in the type series is the yellow shank-spot present in four individuals and absent in two. The width of the oblique dorsolateral stripe and degree of reticulation varies between individuals (Figures 1 & 11); in some individuals this stripe blends completely with ventral reticulation. Within populations, the anterior terminus of dorsolateral lines varies between exterior edges of the eyelids to the superior part of the snout.

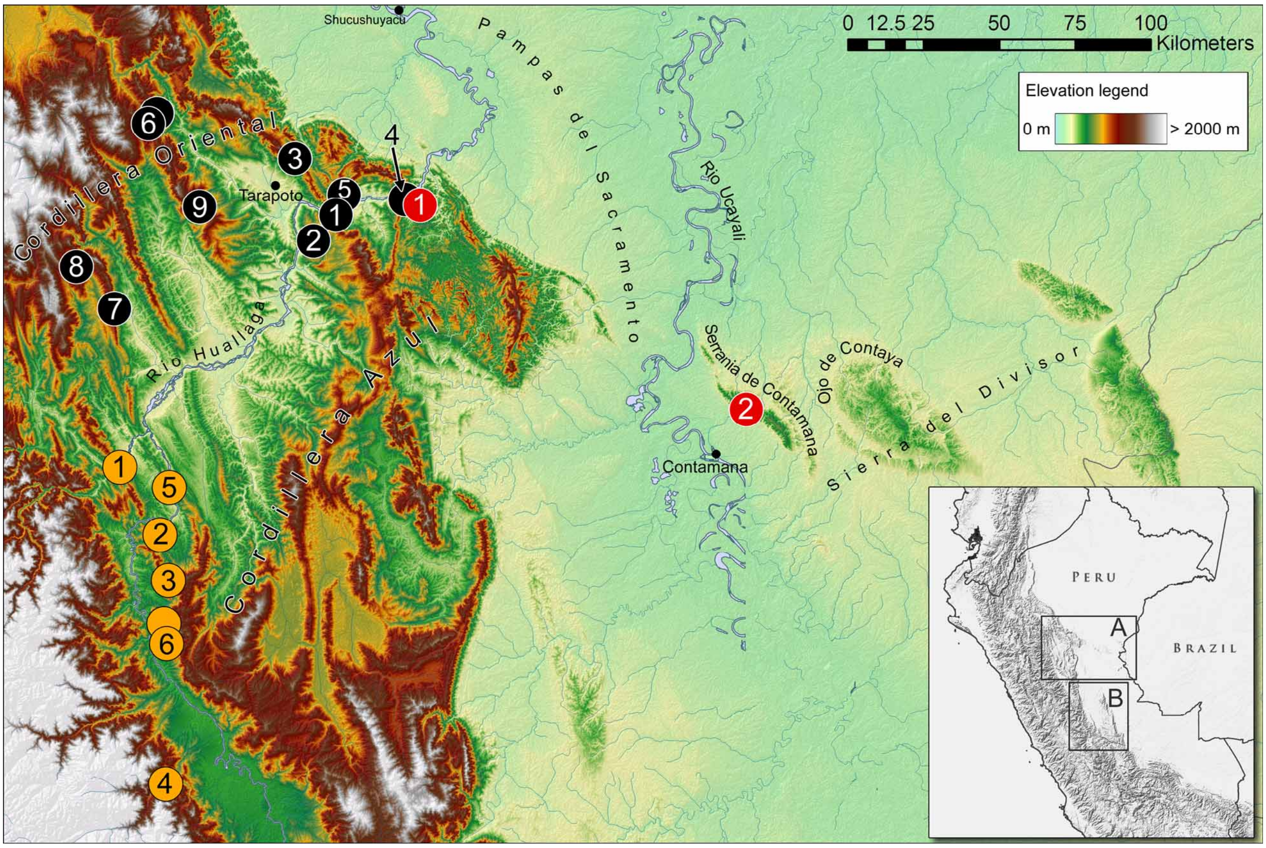

FIGURE 3. Map of study areas. Inset in bottom right shows the two primary regions dealt with in this paper. Box B corresponds to Fig. 8. Box A corresponds to the detailed map shown here. Black dots represent *A. bassleri* localities, red dots represent *A. yoshina* localities, and orange dots represent *A. pepperi* localities. All localities are in Departamento San Martín unless stated otherwise. *Ameerega bassleri*: (1) Chumia (6° 37' 5.10" S, 76° 10' 12.22" W); (2) Sauce (6° 41' 41.90" S, 76° 14' 11.87" W); (3) Cainarachi Valley (6° 27' 4.54" S, 76° 17' 30.84" W); (4) Huallaga Canyon (6° 34' 18.76" S, 75° 57' 51.12" W, north side of river); (5) Chazuta (6° 33' 30.68" S, 76° 8' 44.88" W); (6) Roque (6° 20' 48.54" S, 76° 43' 38.60" W, type locality of *A. bassleri*); (7) Saposoa (6° 53' 46.85" S, 76° 49' 41.41" W); (8) Alto Shima (6° 46' 15.85" S, 76° 56' 28.32" W); (9) Sisa (6° 35' 37.10" S, 76° 34' 29.41" W). *Ameerega yoshina*: (1) Callanayacu (6° 35' 17.61" S, 75° 55' 2.47" W; south side of river); (2) El Unión, Loreto (7° 11' 7.43" S, 74° 57' 13.12" W, type locality of *A. yoshina*). *Ameerega pepperi*: (1) Río Abiseo near Huicungo (7° 22' 2.88" S, 76° 48' 42.81" W); (2) Chumanza (7° 34' 7.8" S, 76° 41' 34.4" W); (3) Nuevo San Martín (7° 42' 13.64" S, 76° 40' 3.15" W; (4) San Francisco (8° 18' 30.3" S, 76° 40'40' 37.6" W, type locality of *A. pepperi*); (5) Campanilla (7° 25' 38.6" S, 76° 39' 53.3" W); (6) Polvora (7° 53' 28.75" S, 76° 40' 21.49" W). Note: Shucushuyacu, Loreto (shown near the top of the map) is the locality of a newly-discovered population of *A. pongoensis* (Fig. 11 c & d) and represents a substantial northward extension of the known range of this species.

Vocalizations. The advertisement call for *A. ignipedis* (Fig. 2) can be characterized as a ‘retarded trill’ following Lötters *et al.* (2003). The call consists of regularly-spaced ‘peeps’ which are repeated at a rate of about 1.7 notes per second. Note duration is 97 ms on average and notes are spaced 287–752 ms apart. Dominant frequency is slightly modulated, starting at 4398 Hz at the beginning of the note and ending at 4730 Hz. We witnessed a single male calling in the type locality after a heavy rain. Calling lasted for several minutes. The call sounds very similar to *A. altamazonica* (in fact their calls are nearly indistinguishable), although we have never encountered *A. altamazonica* in Contamana, or in any other site east of Río Ucayali.

In the type locality we also heard an unidentified species of *Allobates* calling whose call resembled that of *A. ignipedis*, but differed by having notes about 1000 Hz higher in pitch and half the duration of *A. ignipedis*. The call differs from that of *A. pongoensis* (Fig. 2), which consists of irregularly-spaced 'peeps' repeated at a rate of 1 note every 2–3 seconds. These notes are sometimes given in couplets. Note duration 9–11 ms, dominant frequency 4233 Hz.

Distribution and natural history. *Ameerega ignipedis* is known from two localities in the Serranía de Contamana but probably occurs more widely (Fig. 3). Because these localities lie at the foot of low mountains, we suspect that this species may occur widely throughout the foothills of the Serranía de Contamana, as well as other parts of the Sierra del Divisor. Few dendrobatid species are known to occur on both sides of Rio Ucayali, so it is probable that *A. ignipedis* occurs only to the east of this river.

This species occurs in lower montane rainforest. Although the elevation is relatively low (240 m), this habitat is more reminiscent of higher elevation forests in the east Andean versant than the surrounding lowlands. For example, at the foot of the Serranía de Contamana there is an abrupt change from typical lowland forest to a lush habitat which is characterized by abundant epiphytic plants, large tree ferns, and a comparatively moist leaf litter. This may be in part attributable to a slight Massenerhebung effect of these isolated hills, trapping moisture as clouds pass across the lowland plain. These hills also have a surprisingly diverse poison frog community. In addition to *A. ignipedis*, we found *A. hahneli*, *A. trivittata*, *A. yoshina* **sp. nov.**, *Ranitomeya lamasi*, and *R. ventrimaculata*.

Within this habitat, we found *A. ignipedis* near a small stream (Fig. 15h). Most individuals were observed and collected in the forest near the stream, although we also saw a few individuals around the vegetation growing in the sandy banks, one of which was a male transporting tadpoles (which were preserved but later damaged). Since we found no other bodies of water nearby, we presume that he was in the process of depositing tadpoles in this stream. The use of flowing water for tadpole deposition is rare in *Ameerega* species, although has been observed in a few species such as *A. cainarachi* (pers. obsv.), *A. macero* (Rodriguez and Myers 1993), and *A. pongoensis* (M. Pepper, pers. comm.). Additionally, we found tadpoles of *A. trivittata* and what appeared to be a relatively large species of *Leptodactylus*. Fish were observed in the deeper pools of this stream. Males were heard calling throughout the day, particularly after rain.

Conservation status. Following the IUCN Red List criteria (IUCN 2001), this species should be listed as Data Deficient (DD). Being known from only two localities that are less than 3 km apart, its extent of occurrence is unknown but probably occurs more widely. It is fairly common in the type locality and its habitat appears to be remote enough that deforestation risk is low.

Ameerega yoshina sp. nov.

Figures 1, 14, 16

Holotype. MUSM 24945, an adult female (Fig. 1) collected on 20 July 2006 by J. Brown in Departamento Loreto, Peru, 17.5 km NE Contamana at the western foot of the Serranía de Contamana, 310 m elevation, 7° 11' 7.43" S, 74° 57' 13.12" W. Found near El Unión, on the ground near a small creek flowing into the cold-water stream.

Paratypes. All from Peru. Loreto: Two adult females (MUSM 26953-26954) and one adult male (MUSM 26955), collected 3 May 2007 by J. Brown and E. Twomey, 283 m elevation, approximately 1 km S of holotype locality at 7° 11' 38.40" S, 74° 57' 7.20" W. San Martín: Three adult females (MUSM 26985–26987), collected 19 July 2007 by J. Brown, E. Twomey, and K. Fieselman, 261 m elevation, 6° 35' 17.61" S, 75° 55' 2.47" W.

Etymology. The species name is formed as an adjective, derived from the Panoan word '*Yoshín*' which means 'devil or evil spirit', in reference to the cryptic nature of this species and its haunting, penetrating call. The Panoan language is spoken by the Shipibo-Conibo people who are indigenous to the upper Río Ucayali and the Sierra del Divisor.

Definition and diagnosis. Assigned to the genus *Ameerega* on the basis of the following: first finger longer than second, webbing absent between the toes, dorsal skin granular (Myers 1982, Grant *et al.* 2006). This is a medium-large species of *Ameerega* with an adult SVL of approximately 26–35 mm. Dorsum has a black ground color, stippled brick-red to bright red or orange, stippling less dense near sacral region. Bright yellow dorsolateral stripes extend from the eyelid to the groin; a yellow labial stripe is also present starting near nares and extending posterior to the axillary region. Bright yellow-green spot present above groin in some populations, shank spots and axillary spots absent. Limbs greenish-bronze or black; venter ranges from greenish-gold to blue, black marbling weak or absent. Teeth present. Appressed first finger longer than second; finger discs moderately expanded, hands and feet lacking webbing between digits. Vocalization consists of a 'burst' of loud, whistle-like notes, each burst consisting of 4–31 notes, given at a rate of 4.0–4.5 notes per second; dominant frequency 3210– 3060 Hz (Fig. 4, Table 2).

Ameerega yoshina can be distinguished from most other species of *Ameerega* by its red dorsum. It is similar in appearance to the following species: *A. bassleri, A. bilinguis, A. cainarachi, A. macero, A. pepperi* **sp. nov.** (description below), and *A. parvula*, all of which have (or can have) a red dorsum. *Ameerega parvula* and *A. bilinguis* both lack distinct dorsolateral stripes (vs. dorsolateral stripes present and conspicuous in *A. yoshina*). *Ameerega cainarachi* is smaller, females up to 31.3 mm SVL (vs. 35 mm SVL in *A. yoshina*), and has an advertisement call consisting of a regular chain of notes which are not frequency–modulated, repeated at a rate of 9 notes per second (vs. 'bursts' of frequency-modulated notes, repeated 3.7–4.1 notes per second in *A. yoshina*). *Ameerega macero* is smaller than *A. yoshina*, females up to 29.5 mm SVL, and has a call consisting of a series of harsh 'peeps', repeated regularly at a rate of 10 notes per second. *Ameerega bassleri* and *A. pepperi* have an advertisement call consisting of a series of regularly-spaced whistle-like notes given continuously for several minutes at a rate of 1.6–2.1 notes per second in *A. bassleri* and 0.9–1.3 notes per second in *A. pepperi* (vs. bursts of 4–31 notes, given at a rate of 3.7–4.1 notes per second, each burst less than 8 seconds in total duration in *A. yoshina*).

TABLE 2. Relationships of call structures of *A. pepperi*, *A. bassleri* and *A. yoshina*. Mean ± standard deviation (min, max, pairwise significance).

Species	Calling rate (notes/min)	Note Duration (sec)	Silence between notes (sec)	Dominant Frequency (Hz)	*N*
A. pepperi	62.1 ± 9.4 (51.0–75.9, a)	0.17 ± 0.05 (0.13–0.22, a)	0.80 ± 0.20 (0.56–1.03, a)	2970 ± 130 (2800–3160, a)	6 indiv., 4 local.
A. bassleri	95.3 ± 10.0 (95.3–127.8, b)	0.15 ± 0.03 (0.09–0.19, a)	0.42 ± 0.08 (0.30–0.42, b)	2750 ± 190 (2400–3070, b)	18 indiv., 6 local.
A. yoshina	235.2 ± 5.6 (227.3–243.2, c)	0.13 ± 0.02 (0.09–0.15, a)	0.13 ± 0.02 (0.11–0.15, c)	3140 ± 50 (3060–3210, a)	6 indiv., 2 local.
ANOVA	$F_{2,27}=597.02$, $P < 0.001$	$F_{2,21}=2.54$, $P = 0.102$	$F_{2,21}=56.97$, $P < 0.001$	$F_{2,27} = 13.83$, $P < 0.001$	

Measurements (in mm) of holotype. The female holotype (Fig. 1) has SVL 34.8; FL 16.3; TL 17.6; KK 29.5; FoL 14.6; HaL 8.3; HL 8.1; HW 10.2; BW 12.1; UEW 7.3; IOD 4.9; TD 3.2; ED 5.3; DET 0.8; L1F 7.6; L2F 4.5; W3D 1.0; W3F 0.5. Measurements of paratypes are given in Table 4.

Description of holotype. Size large, SVL 34.8 mm (Table 4). Dorsal skin coarsely granular, pigmented black with brick red mottling densest near the snout. Thin light-yellow dorsolateral stripes extend from eyelids to groin; posterior to eyes the stripes blend into the red dorsum. Dorsolateral stripes slightly wider at groin. Flanks are solid black along the entire length of the body and head. Yellow labial stripe present extending from nares to axillae and upper forelimb. Forelimbs are weakly granular and are metallic-brown in color. Legs are granular, dark brown with faint olive green stippling. On each leg there is a large yellow spot present on the anterodorsal surface of the thigh. Hands, feet, and digits colored as limbs. The ventral skin is smooth and uniformly olive-gold in color on the belly and limbs; the head and chest are slightly darker than the belly. No distinct black markings present on the venter. Iris black.

Widest part of head between jaw articulations and tympanum, head narrower than body; greatest head-width 29.3% of SVL. Eyes protuberant. Tongue medium sized, oval. Premaxillary and maxillary teeth present. Vocal slits absent. Snout sloping laterally; bluntly rounded dorsally; truncate ventrally. Nares situated and directed posterolaterally to the tip of snout; nares visible from front and below but not from above. Canthus rostralis sloped, slightly rounded; loreal region nearly vertical and slightly concave. Upper eyelid 1.5 times wider than interorbital distance. Eye large and prominent with a maximum diameter of 9.3 % of the snout vent length; pupil rounded and horizontally elliptical. Tympanum circular; without tympanic annulus its diameter is less than 60.2 % of ED; supratympanic fold present.

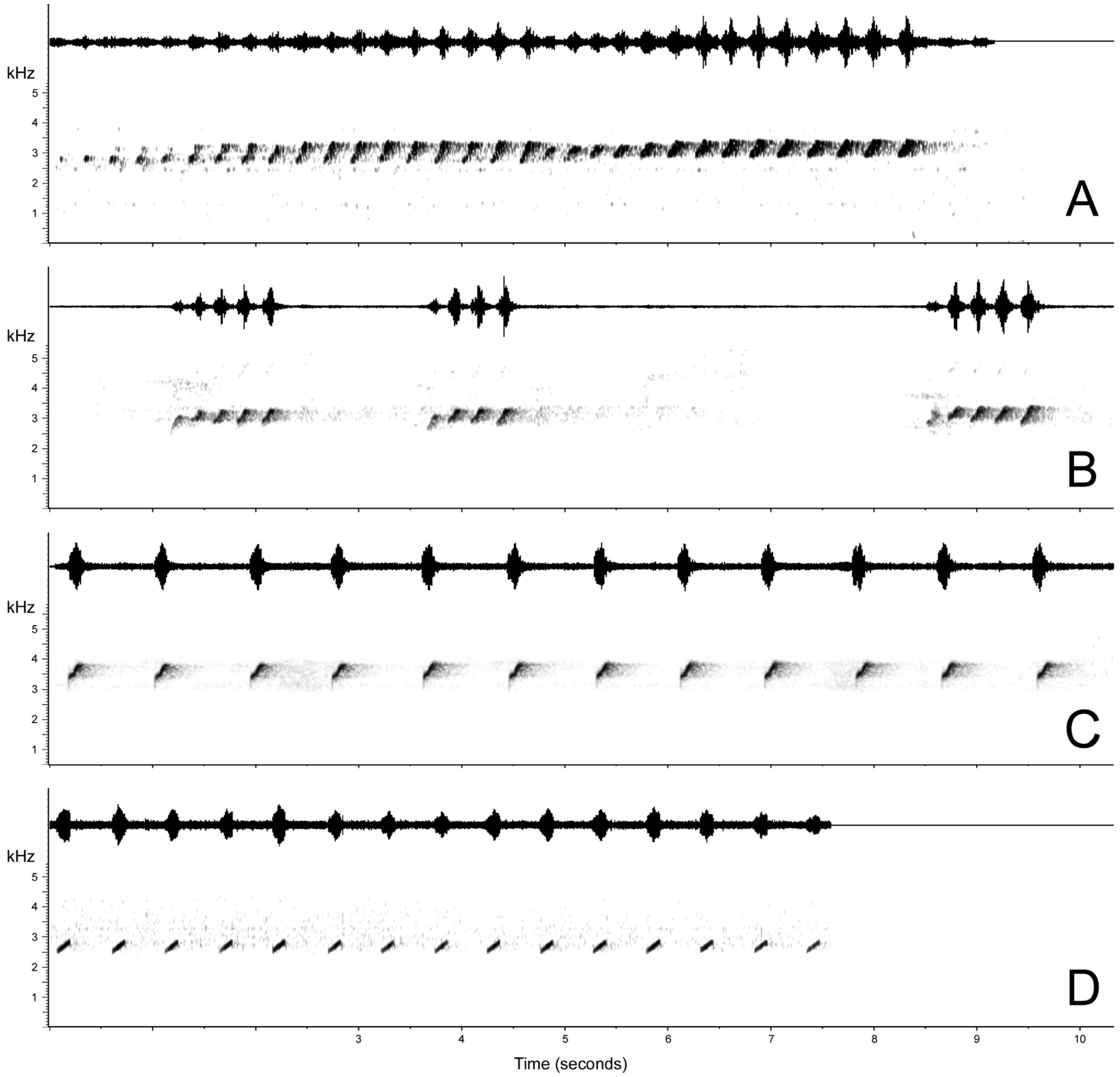

FIGURE 4. Advertisement calls of *Ameerega yoshina*, *A. pepperi*, and *A. bassleri*. (A) *A. yoshina* standard advertisement call, field recording from 1.6 km SE of Callanayacu, San Martín, 25.5 C, 19 July 2007. Dominant frequency 3244 Hz. (B) *A. yoshina* short call, same location, temperature, and date as previous. Dominant frequency 3141 Hz. (C) *A. pepperi* advertisement call, field recording from near Polvora, San Martín, 24.5 C, 8 November 2008. Dominant frequency 2749 Hz. (D) *A. bassleri* advertisement call, field recording from 5.7 km NE of Roque, San Martín, 26.5 C, 16 June 2007. Dominant frequency 2714 Hz. See Fig. 3 for map of localities.

Hands relatively small, length being 23.7% of SVL. Relative length of appressed fingers II ≈ IV < I < III (with I being interior-most finger). Discs moderately expanded on all fingers. Disc on finger III is twice as wide as distal end of adjacent phalanx. A large, circular outer metacarpal tubercle present on median base of palm; a smaller inner metacarpal tubercle on base of finger I absent; one well developed subarticular tubercle on fingers I and II, two on fingers III and IV.

Hind limbs short, with heel of appressed limb reaching the axillary region. Tibia length 50.7% of SVL. Relative lengths of appressed toes I < II < V< III < IV; first toe extending past subarticular tubercle on base of second toe; discs on toes I, II, III, IV barely expanded (much smaller than finger discs), and toe V expanded (disc 1.2 times wider than adjacent phalanx). Moderate-sized inner and outer metatarsal tubercles, protuberant with rounded surfaces. One protuberant subarticular tubercle on toes I and II, two on toes III, IV, and V. Hands and feet lacking supernumerary tubercles and lateral fringes. No basal webbing on toe fringes. Tarsal tubercles absent.

In 70% alcohol the color is similar to the living animal described above, except the red dorsum changed to black, yellow dorsolateral stripes changed to silver-white, and gold coloration on venter changed to black and grey-green.

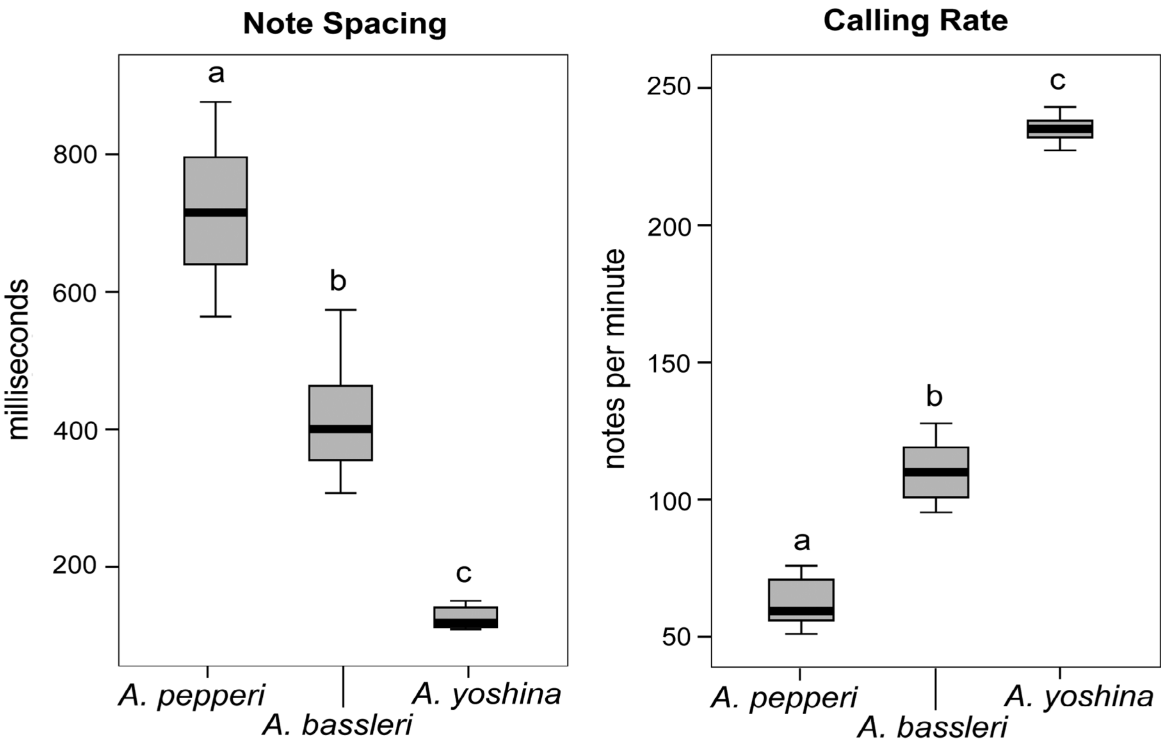

FIGURE 5. Call variation between *Ameerega bassleri*, *A. pepperi* and *A. yoshina*. Box plots show period of silence between notes (L) and the calling rate (notes per minute) of the three species (R).

Variation. Little variation has been observed in the two known populations of *A. yoshina*. Frogs from near Contamana vary somewhat with respect to dorsal coloration; some individuals are brick-red while others are more orange. These frogs also possess a venter which varies from greenish-gold to pale yellow-green, although black ventral markings are absent or not well-defined. Frogs from Callanayacu tend to be more orange as opposed to red on the dorsum (Fig. 14), and have blue venters that have diffuse and irregular black markings. The limbs vary in color from black to pale olive.

Vocalizations. The advertisement call for *A. yoshina* (Fig. 4) can be classified as a ‘retarded trill’ following Lötters *et al.* (2003). The call consists of a series of musical notes resembling short ‘whistles’ which are given in short bursts of 4–31 notes at a rate of 3.7–4.1 notes per second. Notes are 90–156 ms long, spaced 110–150 ms apart, and frequency-modulated by about 325 Hz (notes start at 2925 Hz, end at 3250 Hz, overall dominant frequency 3140 Hz).

The call of *A. yoshina* can be very easily distinguished from the advertisement call of *A. bassleri*, which is a series of whistle-like notes repeated at a much slower rate and for much longer. Our recordings of *A. bassleri* have notes repeated at a rate of 1.6–2.1 notes per second for 7.5 seconds or longer, although on several occasions we have heard *A. bassleri* calling continuously for several minutes at a time. *Ameerega bassleri* also has an aggressive call which consists of 3–5 notes given in quick succession. However, even this aggressive call differs from the advertisement call of *A. yoshina*, as notes are repeated at a slower rate (1.6 notes per second in *A. bassleri* vs. over 4 notes per second in *A. yoshina*) and are spaced further apart (notes spaced 420 ms in *A. bassleri* vs. 130 ms in *A. yoshina*). In the field, the call differences between the two species are immediately recognizable and fixed in a given geographic region. For example, during several days in Callanayacu and Contamana, we never heard a call which sounded like *A. bassleri*. Conversely, in areas containing *A. bassleri*, we have never heard a call which sounded like *A. yoshina*.

Distribution and natural history. *Ameerega yoshina* is known from two localities, one in the Serranía de Contamana and the other in the Huallaga Canyon[1] in the northern Cordillera Azul (Fig. 3). These two localities are 130 km apart and separated by the Ucayali floodplain, which may represent a significant barrier between these two populations. We have not found *A. yoshina* in lowland habitats and expect that this species is restricted to premontane forests of the Serranía de Contamana and northeastern Cordillera Azul. At their closest points, the Cordillera Azul and Serranía de Contamana are separated by 26 km of lowland forest (and Río Ucayali), and it is unlikely that there is currently gene flow across this barrier. In the lower Huallaga Canyon, we have only found *A. bassleri* on the north-bank and *A. yoshina* on the south-bank of Río Huallaga. Therefore, it is possible that *A. bassleri* is absent from the northeastern Cordillera Azul and that this area is inhabited exclusively by *A. yoshina*.

Ameerega yoshina occurs in undisturbed premontane forests, particularly in habitats which are adjacent to streams (Fig. 15h & j). This species appears to have extremely specific habitat requirements, as we were only able to find them in isolated pockets near Contamana and Callanayacu. For example, in Callanayacu we hiked for several kilometers along Quebrada Pacuyacu (a small stream), and only heard *A. yoshina* calling along a ~200-m stretch of habitat adjacent to the stream. In Contamana we were able to find this species alongside three different streams, but only in small areas along these streams. This species is exceedingly cryptic and is nearly impossible to detect if males are not calling. In Callanayacu we spent roughly 68 person-hours searching within the 200-m stretch of habitat and were only able to capture three individuals, despite the fact that numerous males were calling in the vicinity the entire time we were searching. In Contamana we searched for 60 person-hours in 2006 and 48 person-hours in 2007, yet we were only able to encounter a total of six frogs, one of which was a metamorph. Most adults were found under logs or under piles of leaf litter, although two were found moving about the leaf litter after a heavy rain.

Reproduction appears to take place near small streams and forest pools. In the same spot where we found *A. ignipedis* transporting tadpoles, we also found one *A. yoshina* metamorph at the edge of the water. There were no other water sources nearby and we assume this metamorph emerged from the stream. We also found a shallow forest pool at the base of a small waterfall, around which many *A. yoshina* males were calling. In this pool were numerous tadpoles which were *A. yoshina* (confimed by DNA sequencing). Males appear to call from the leaf litter throughout the day. In Callanayacu males called incessantly the entire day, but were well-hidden.

---

[1] The term “Huallaga Canyon” refers to a narrow section of the river which forms between the Cordillera Oriental and Cordillera Azul, starting at Chumia and ending when the river reaches the lowlands. Callanayacu and Chazuta are both towns located within this section of river.

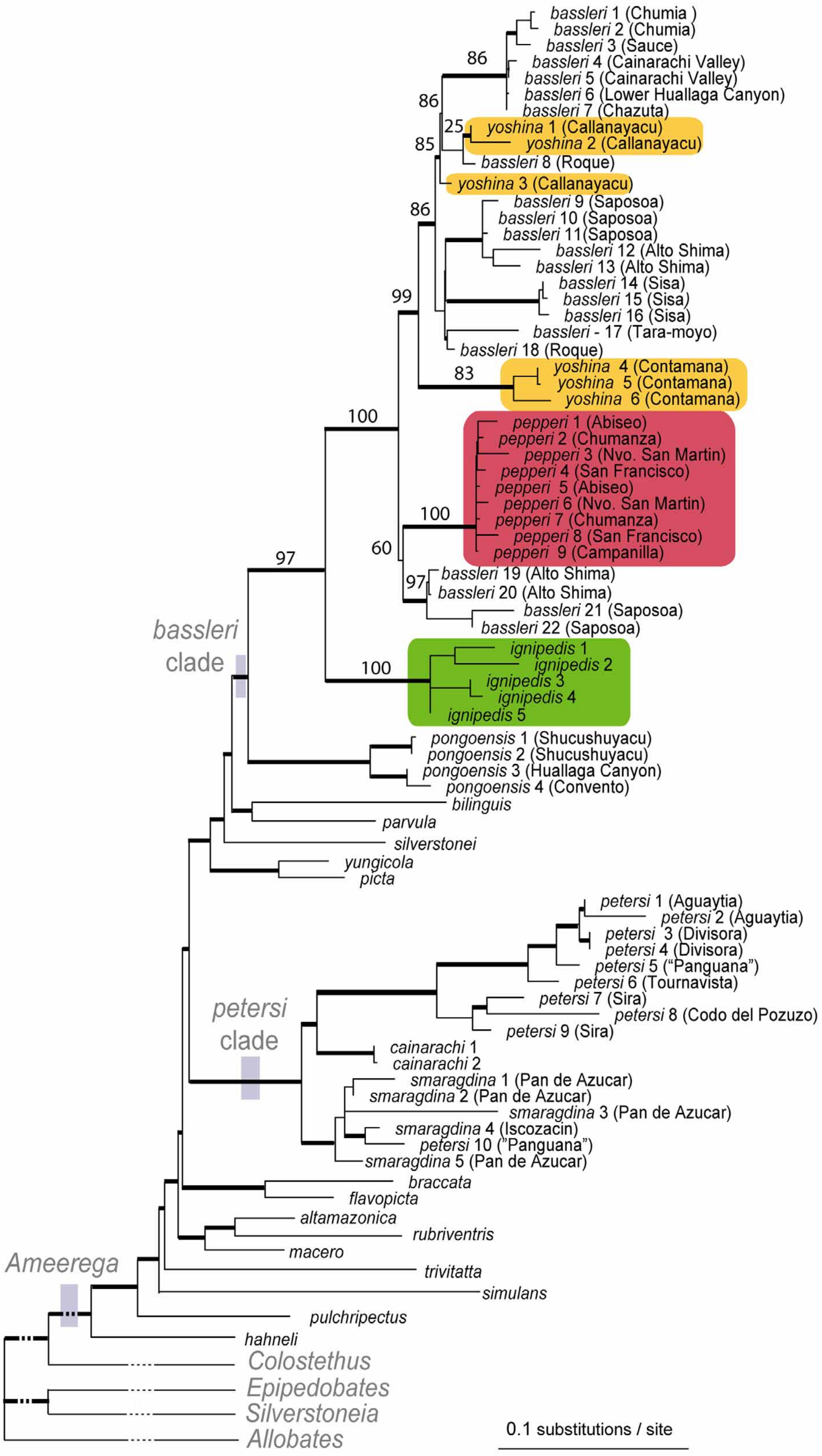

FIGURE 6. Our Bayesian phylogenetic hypothesis. Thicker branches represent posterior probabilities greater than 80 (nodes of particular interest to this study are labeled with observed posterior probabilities). *Ameerega ignipedis* **sp. nov.**, *A. yoshina* **sp. nov.**, and *A. pepperi* **sp. nov.** are highlighted with green, orange and red boxes, respectively. Since this study focused on the *bassleri* and *petersi* clades, to simplify visualization of the tree we reduced individuals of other species of *Ameerega* and other genera to a single representative (reducing the number of terminals to 76 from 135, and in every case, each clade comprised a monophyletic lineage).

Conservation status. Following the IUCN Red List criteria (IUCN 2001), we tentatively suggest that *A. yoshina* should be listed as Near Threatened (NT) based on the following: (1) we estimate the extent of occurrence to be less than 4,500 km^2, (2) populations appear to be fragmented, as we have only encountered two disjunct populations which are restricted to small areas, and (3) there has been a small amount of potential habitat loss in the Cordillera Azul near Río Huallaga, although this species probably occurs throughout the northern part of the Cordillera Azul National Park. Further investigation may warrant a revised classification, as the above estimate on extent of occurrence assumes that this species occurs at elevations from 280–600 m and also occurs in the Ojo de Contaya (Fig. 3). While this species may range widely throughout the Cordillera Azul and Sierra del Divisor, it may just as likely be restricted to the Serranía de Contamana and extreme northern Cordillera Azul.

Ameerega pepperi sp. nov.

Figures 1, 14–16

Phyllobates bassleri: Silverstone 1976 p. 45 (AMNH 42327, 42867, 43402 collected in 1926 and 1928 by H. Bassler at “Pachiza”).

Holotype. MUSM 26940 (Fig. 16), an adult male collected on 14 July 2006 by M. Pepper, Provincia Tocache, Departamento San Martín, Peru, 2 km NE of San Francisco, 980 m elevation, 8° 18' 30.3" S, 76° 40' 37.6" W. Found on the ground near a small waterfall.

Paratypes. All from San Martín, Peru. One adult female (MUSM 26942), collected 21 July 2006 by M. Pepper and E. Twomey, and two adult females (MUSM 26975, 26976), collected 25 June 2007 by J. Brown, E. Twomey, and K. Fieselman, 390 m elevation, 7.0 km SW of Huicungo at 7° 21' 58.6" S, 76° 48' 44.1" W: Two adult females (MUSM 26979, 26980), collected on 25 June by J. Brown, E. Twomey, and K. Fieselman, 384 elevation, 6.6 km N of Campanilla at 7° 25' 38.6" S, 76° 39' 53.3" W: One adult female (MUSM 26968), collected on 15 July 2006 by M. Pepper, 570 m elevation, SW of Chumanza (alternate spelling: Shumanza) at 7° 34' 7.8" S, 76° 41' 34.4" W: One adult male (MUSM 26941), collected at same locality of holotype, 980 m elevation, 2 km NE of San Francisco at 8° 18' 30.3" S, 76° 40' 37.6" W.

Etymology. The specific epithet is a patronym for the discoverer of this species, Mark Pepper, an enthusiastic Canadian explorer and conservationist whose discoveries have greatly contributed to the authors’ understanding of poison frog biogeography and taxonomy.

Definition and diagnosis. Assigned to the genus *Ameerega* on the basis of the following: first finger longer than second, webbing absent between the toes, dorsal skin granular (Myers 1982, Grant *et al.* 2006). This is a medium-large species of *Ameerega* with an adult SVL of approximately 28–34 mm. Dorsum has a black ground color, finely stippled brick-red to bright red in southern populations (e.g. San Francisco) to coarsely stippled orange or yellow northern populations (e.g. Campanilla), stippling concentrated near head and is less dense near sacral region. Bright yellow dorsolateral stripes extend from the eyelid to the groin; a yellow labial stripe is also present starting near nares and extending posterior to the axillary region. Faded yellow-green to white spot present above groin in some populations, shank spots and axillary spots absent. Limbs greenish-bronze or black; venter blue with strong black marbling. Teeth present. Appressed first finger longer than second; finger discs moderately expanded, hands and feet lacking webbing between digits. Vocalization consists of a loud retarded trill of whistle-like notes given at a rate of 0.9–1.3 notes per second; dominant frequency 2800–3160 Hz (Fig. 4, Table 2).

Ameerega pepperi can be distinguished from most other species of *Ameerega* by its red, orange, or yellow dorsum. It is similar in appearance to the following species: *A. bassleri, A. bilinguis, A. cainarachi, A. macero, A. parvula,* and *A. yoshina,* all of which have (or can have) a solid red, orange, or yellow dorsum. *Ameerega parvula* and *A. bilinguis* both lack distinct dorsolateral stripes (vs. dorsolateral stripes present and conspicuous in *A. pepperi*). *Ameerega cainarachi* is smaller, females up to 31.3 mm SVL (vs. 34 mm SVL in

A. pepperi), and has an advertisement call consisting of a regular chain of notes which are not frequency modulated, repeated at a rate of 9 notes per second (vs. 'bursts' of frequency-modulated notes, repeated 0.9–1.3 notes per second in *A. pepperi*). *Ameerega macero* is smaller than *A. pepperi*, females up to 29.5 mm SVL, and has a call consisting of a series of harsh 'peeps', repeated regularly at a rate of 10 notes per second. *Ameerega bassleri* has an advertisement call consisting of a series of regularly-spaced whistle-like notes given continuously for several minutes at a rate of 1.6–2.1 notes per second (vs. notes given at a rate of 0.9–1.3 notes per second in *A. pepperi*). *Ameerega yoshina* has an advertisement call given in bursts of 4–31 notes, given at a rate of 3.7–4.1 notes per second, each burst less than 8 seconds in total duration (vs. notes given at a rate of 0.9–1.3 notes per second in *A. pepperi*).

Measurements (in mm) of holotype. The male holotype (Fig. 16) has SVL 28.6; FL 13.4; TL 16.1; KK 28.2; FoL 13.3; HaL 7.5; HL 7.7; HW 7.8; BW 8.3; UEW 5.5; IOD 3.9; IND 4.0; TD 3.0; ED 4.2; DET 1.6; L1F 6.9; L2F 6.1; W3D 0.8; W3F 0.6. Measurements of paratypes are given in Table 5.

Description of holotype. Size medium, SVL 28.6 mm (Table 5). Dorsal skin is coarsely granular and is pigmented black with brick red mottling densest near the snout. Thin yellow dorsolateral stripes extend from groin, extending dorsally around snout; posterior to eyes the stripes blend into the red dorsum. Dorsolateral stripe is sightly wider at groin. Flanks are black extending from groin to nares. Forelimbs lack granulation and are colored tan and flecked in olive. Yellow coloration extends up upper arm surface, anteriorly around lip. Legs are coarsely granulated; contain yellow femoral spots, dorsal surface colored olive green. The venter is yellow-gold with olive marbling; marbling extends primarily on throat and margins of legs. Iris black.

Widest part of head between jaw articulations and tympanum, head narrower than body. Greatest head width 27.0 % of SVL. Eyes very protuberant. Tongue medium sized, oval. Premaxillary and maxillary teeth present. Vocal slits present. Dorsal skin of head and body is finely granular; skin smooth or nearly smooth on limbs and smooth on sides of head and body and ventral surfaces. Snout sloping laterally; bluntly rounded dorsally; truncate ventrally. Nares situated and directed posterolaterally to the tip of snout; nares visible from front and below but not from above. Canthus rostralis sloped, slightly rounded; loreal region nearly vertical and slightly concave. Upper eyelid 1.4 times wider than interorbital distance. Eye large and prominent with a maximum diameter of 14.6 % of SVL, pupil rounded and horizontally elliptical. Tympanum circular, without tympanic annulus its diameter less than 73.0 % of ED, supratympanic fold absent.

Hands relatively small, length being 26.2 % of SVL. Relative length of appressed fingers I ≈ II ≈ IV < III (numbers starting interiorly). Discs moderately expanded on all fingers. In adults, disc on finger III is 1.25 times wider than distal end of adjacent phalanx A large, circular outer metacarpal tubercle present on median base of palm; smaller inner metacarpal tubercle on base of finger I absent; one well developed subarticular tubercle on fingers I and II, two on fingers III and IV.

Hind limbs short, small, with heel of appressed limb reaching the interior axilla. Tibia length 56.3% of SVL. Relative lengths of appressed toes I < II < V < III < IV; first toe terminates at subarticular tubercle on base of second toe; toes I, II barely expanded (much smaller than finger discs), and toe III, IV, and V expanded (disc 1.5 times broader than adjacent phalanx). Moderate-sized inner and outer metatarsal tubercles, protuberant with rounded surfaces. One protuberant subarticular tubercle on toes I and II, two on toes III, IV, and V. Hands and feet lacking supernumerary tubercles and lateral fringes. Basal webbing absent. Tarsal tubercles absent.

In 70% alcohol the color is similar to the living animal described above, except that the red on the dorsum changed to black and the yellow dorsolateral lines changed to silver-white.

Variation. The dorsal coloration varies over a cline from the north to south. In southern populations, the dorsum is finely stippled brick-red to bright red and gradually changes to coarsely stippled orange or yellow in northern populations (Figures 14 & 15).

Vocalizations. The advertisement call for *A. pepperi* (Fig. 4) can be classified as a 'retarded trill' following Lötters *et al.* (2003). The call consists of a series of musical notes resembling short 'whistles' at a rate of 0.9–1.3 notes per second. Notes are 130–220 ms long, spaced 560–1030 ms apart, overall dominant frequency 2970 Hz. The call of *A. pepperi* can be distinguished from the advertisement call of *A. bassleri* by the rate the notes are repeated, with *A. bassleri* repeating notes at 1.6–2.1 notes per second (vs. 0.9–1.3 in *A. pepperi*).

Distribution and natural history. *Ameerega pepperi* is known from throughout the upper Huallaga Valley, south of Río Huayabama (near Huicungo) to the southern border of San Martín at elevations from 380 m to approximately 1000 m elevation. Most of the known localities for this species run along the main road to Tingo Maria, which is primarily on the eastern side of Río Huallaga (i.e., the western slope of the Cordillera Azul). This species also occurs on the western side of the Huallaga (i.e., the eastern slope of the Andes) in San Francisco and Huicungo, and likely occurs throughout the area between these two sites based on the results of our niche model (Fig. 17). This model also predicts suitable habitat throughout much of the Río Biabo valley in the Cordillera Azul, although no surveys have taken place in this area. There appears to be very little predicted overlap in the distributions of *A. pepperi* and *A. bassleri* in the niche model (Fig. 17), with the only overlapping areas occurring near the town of Juanjui. In 2007 we spent a day in the mountains just west of Juanjui and did not detect either species. The area near Juanjui appears to be somewhat drier than surrounding areas, and this may function as a weak barrier that separates the distributions of these two species.

Ameerega pepperi occurs in undisturbed premontane forests and secondary premontane forest, particularly in habitats which are adjacent to streams (Fig. 15i). Unlike *A. bassleri,* which we have often observed far from streams (> 300 m), we have never observed *A. pepperi* greater than ~30 m from a stream. This species can occur in moderately disturbed areas provided the streamside habitats are relatively shaded. During the day, adults tend to hide amongst streamside boulders. Calling activity seems to peak during late evening, just prior to dusk. Males call from elevated positions on boulders, although courtship and oviposition appears to take place on the ground in the leaf litter. Clutches contain 22-44 eggs and are guarded by males (M. Pepper pers. comm.). These eggs typically hatch in approximately 18 days. Tadpoles have been found in shallow eddies or streamside pools which are sometimes left by receding water levels. Clay-bottomed streams appear to be most commonly used, and tadpoles will often submerge themselves under the sediment, particularly during the hotter hours in the afternoon. These pools also contain tadpoles of the sympatric *A. altamazonica* and *A. trivittata*. Tadpoles of *Ameerega pepperi* complete development in as little as 5-6 weeks.

Conservation status. Following the IUCN Red List criteria (IUCN 2001), we tentatively suggest that *A. pepperi* should be listed as Least Concern (LC) based on the following: (1) we estimate the extent of occurrence to be at least 5,500 km^2, (2) populations appear to be continuous and (3) this species occurs in largely undisturbed forests, though there has been a small amount of potential habitat loss in the Cordillera Azul and in the upper Huallaga Valley.

Discussion

Mitochondrial Introgression in *A. bassleri*

The observed phylogenetic relationship of *A. pepperi* and four *A. bassleri* individuals from Saposoa/Alto Shima (individuals 19–22 in Fig. 6) does not reflect the geographical extent of *A. pepperi*. Further, other *A. bassleri* (individuals 9–13) from the same localities are sister to nearby populations (Sisa). Since it is unlikely that the two distinct Saposoa/Alto Shima clades represent two species that are identical in all regards (sharing characteristics of *A. bassleri*), it is likely that the observed phylogenetic relationship is not reflective of the "true" species tree. The most likely explanation for this pattern is the historic hybridization and introgression of the mitochondrial genome of *Ameerega pepperi* into the adjacent population of the common ancestor to both *A. bassleri* and *A. yoshina*.

Incomplete Lineage Sorting in *A. yoshina*

Given the dramatic acoustic differences, and their unlikely convergences, species with similar calls likely share common ancestry; however our phylogeny does not reflect this relationship. The three *A. yoshina* individuals from Callanayacu are not sister to the other individuals from Contamana, nor are they sister to the individuals of *A. bassleri* from close proximity, such as individuals from the Huallaga Canyon (separated by 8 km) and Chazuta (separated by 25 km), a pattern predicted in mitochondrial introgression (McGuire *et al.* 2007). The three *A. yoshina* individuals are more closely related to an individual from near Roque (which is separated by 100 km and three mountain ranges). This supports the pattern of incomplete lineage sorting, as alleles present in a common ancestor have been retained in the Callanayacu populations of *A. yoshina* and are randomly distributed in the *A. bassleri* clade.

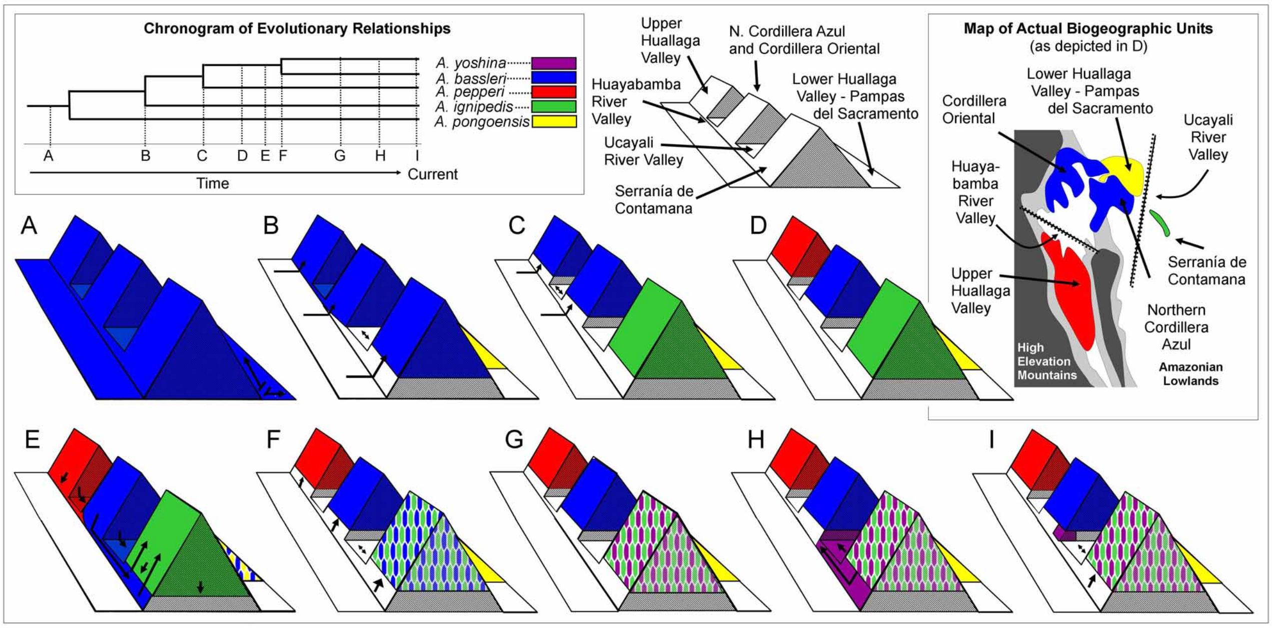

FIGURE 7. The biogeography of the *bassleri* clade is likely the result of multiple disturbance-vicariance events that resulted in periodic connections between the Cordillera Oriental and the Serranía de Contamana, followed by longer periods of isolation. This scenario assumes three periods of connectivity between the Cordillera Oriental and Contamana and would provide a mechanism for allopatric speciation in this group. Each species' distribution (and their ancestors) is depicted by the corresponding color. The bicolored pattern depicts the sympatry of two species.

Speciation in the *bassleri* clade: the importance of call characteristics

The divergence of call characteristics appears to be important factor in the speciation of members of the *bassleri* clade. This group is distributed through a relatively small area (throughout San Martín and nearby Loreto), but contains an incredible amount of diversity, being composed of the three new species plus *A. bassleri* and *A. pongoensis*. The three larger, more colorful species (*Ameerega bassleri*, *A. pepperi*, and *A. yoshina*) are very similar in appearance, suggesting divergences in behavior and acoustic characteristics have played a major role in their speciation. We suspect a major factor reinforcing species boundaries in this group is the divergence in call characteristics, particularly in calling rate. The individual notes that compose the calls of *A. bassleri*, *A. pepperi*, and *A. yoshina* are very similar in pulse duration, structure, and frequency (Table 2, Fig. 4); though between each species, there has been tremendous divergence in the rate that notes are given (Table 2, Fig. 4). In the case of *A. bassleri* and *A. yoshina*, the divergence in call characteristics likely quickly reinforced species boundaries, so fast that many other genes, including the mitochondrial genes sampled for the phylogeny, have yet to coalesce. Preliminary call playback experiments on *A. bassleri* males (from Chazuta) show that males will aggressively respond to recorded calls of the same species from different localities (Saposoa and Cainarachi Valley). However, males do not respond to the calls of *A. pepperi* (JLB unpub. data). This suggests that divergence in calls is recognized, at least, by *A. bassleri* males.

Although we are hesitant to describe a species without reciprocally monophyletic gene trees, we are faced with a situation where we have acoustic data which strongly suggests the existence of three species. Advertisement calls are thought to be important intraspecific signals (Boul *et al.* 2007) and have frequently

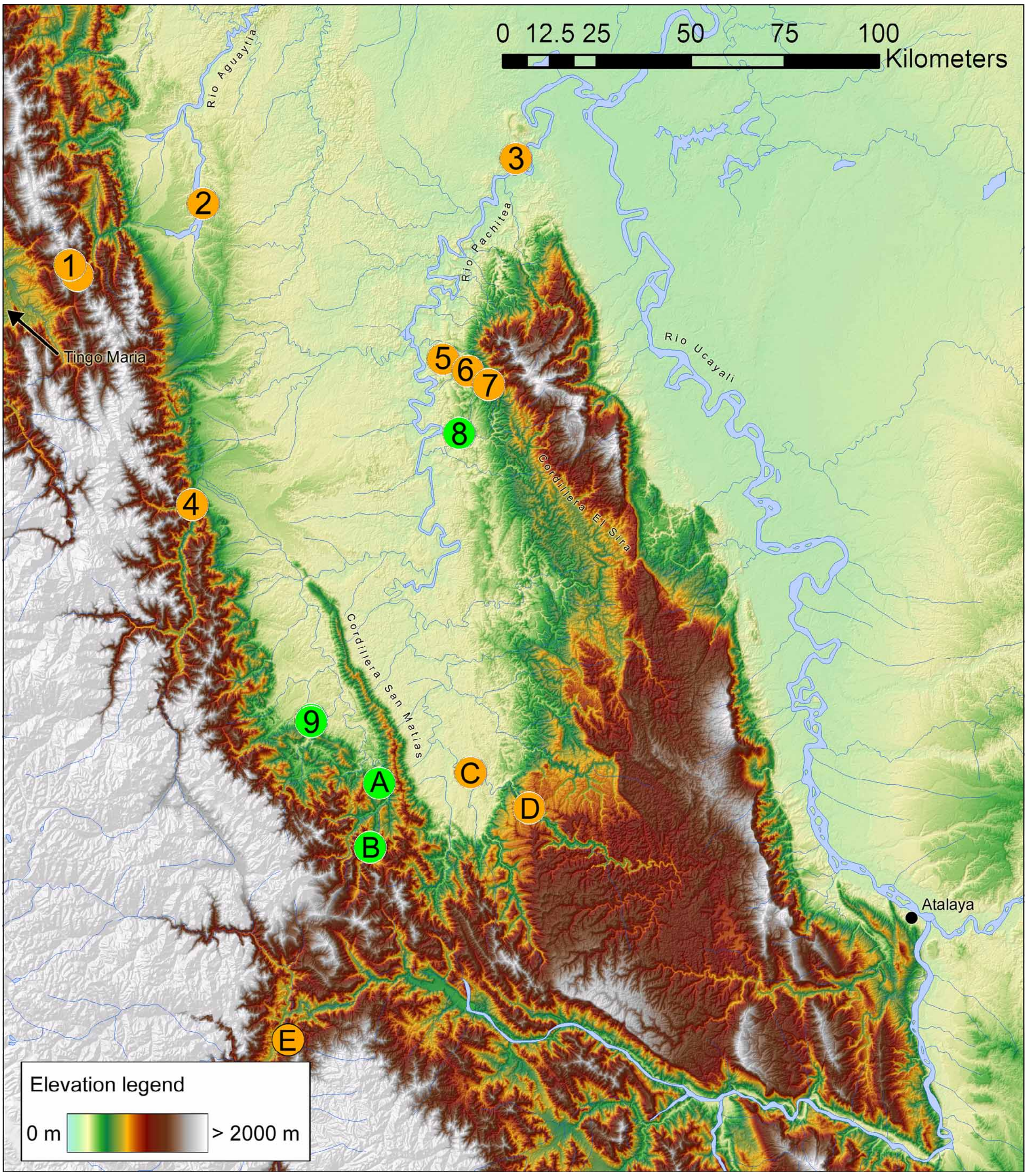

FIGURE 8. Map of central Peru. Orange circles represent localities of *Ameerega petersi*, green circles represent *A. smaragdina* localities. Localities are based on personal observations and Silverstone (1976). Localities labeled with numbers are those which were included in the phylogenetic analysis; localities with letters are those for which individuals have not been sequenced. (1) Miguel Grau, Ucayali (9° 11' 11.47" S, 75° 47' 4.13" W); (2) Aguaytia, Ucayali (9° 0' 54.00" S, 75° 29' 2.40" W); (3) Tournavista, Huánuco (8° 54' 23.50" S, 74° 44' 7.20" W); (4) Codo del Pozuzo, Huánuco (9° 44' 5.22" S, 75° 30' 37.59" W); (5) Puerto Inca, Huánuco (9° 23' 10.39" S, 74° 54' 37.21" W); (6,7) Cordillera El Sira, Huánuco (9° 26' 50.86" S, 74° 48' 1.91" W); (8) Panguana, Huánuco (9° 33' 51.40" S, 74° 52' 16.29" W); (9) Pan de Azucar, Pasco (10° 14' 56.34" S, 75° 13' 32.09" W, type locality of *A. smaragdina*); (A) Río Iscozacin, Pasco (10° 24' 5.66" S, 75° 3' 47.29" W); (B) Cacazú, Pasco (10° 33' 16.29" S, 75° 5' 4.79" W); (C) Santa Isabel, Pasco (approximate location; type locality of *A. petersi*); (D) Nevati, Pasco (approximate location); (E) Río Vitoc, Junín (approximate location).

been used as fundamental characters in anuran systematics (Platz and Forester 1988, Platz 1989, Sullivan *et al.* 1996, Gamble *et al.* 2008, Twomey and Brown 2008a). The distinct calls of these species are ecologically relevant characters, which we believe justify full-species status, despite contradictory phylogenetic evidence.

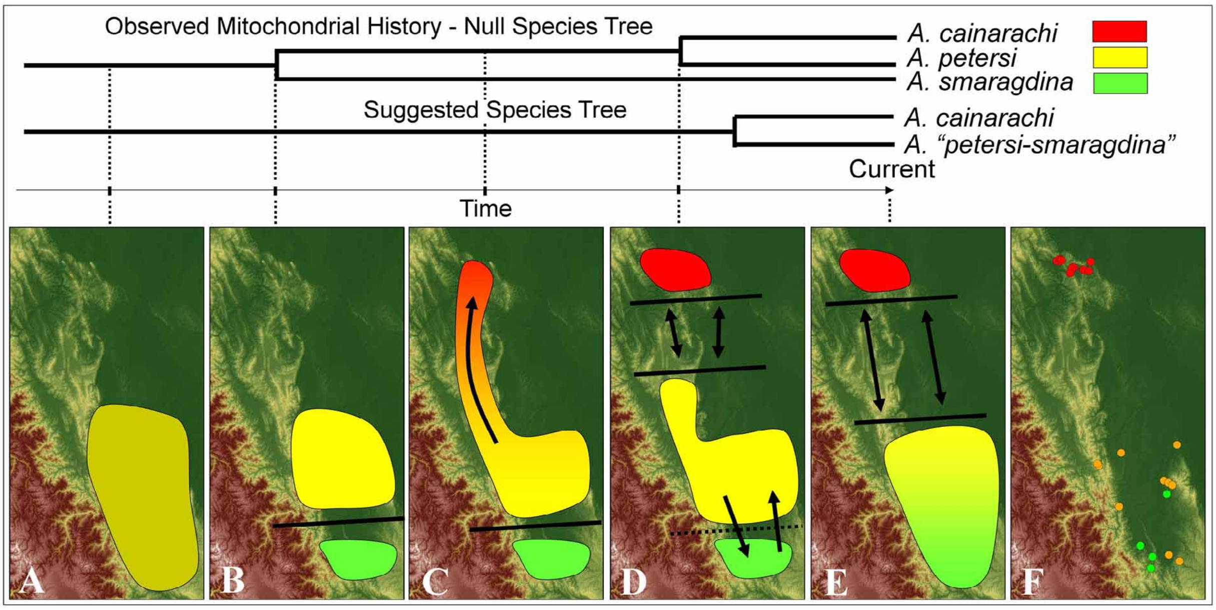

FIGURE 9. Speciation and biogeography of the *petersi* clade. The series of maps depicts the putative ancestral distributions of the *petersi* clade through time, with (A) being the oldest and (E) being present-day. This hypothetical scenario could explain the discordance between our observed mitochondrial gene tree and the suggested species tree (in which *A. petersi* and *A. smaragdina* are the same species). Each species' distribution (and their ancestors' distribution) is depicted by the corresponding color. (F) Depiction of the known localities for each of these species.

The biogeography of *bassleri* clade

The results from our DIVA analysis of the *bassleri* clade are consistent with the disturbance-vicariance hypothesis (Colinvaux 1993, Noonan and Chippindale 2006, Noonan and Gaucher 2006, Noonan and Wray 2006), which predicts that climatic oscillations throughout the quaternary period (1.8 MYA to present) resulted in periods of connectivity and isolation between highland refuges. In our example, the biogeography of the *bassleri* clade can be explained through multiple disturbance-vicariance events that resulted in recurring connections between the Cordillera Oriental (for simplicity, this definition includes the Cordillera Azul) and the Serranía de Contamana, followed by longer periods of isolation. One possible scenario, which is congruent with the ancestral range estimates from our DIVA analysis (unpublished data), is that the common ancestor to the *bassleri* clade (as defined in Fig. 6) was a widespread montane species whose distribution extended into the adjacent lowlands and into Serranía de Contamana (Fig. 7a) (Roberts *et al.* 2006). Due to a vicariant event, a lowland population became isolated and diverged into *A. pongoensis* (Fig. 7b). During this time, the Contamana populations of the *bassleri-ignipedis-yoshina-pepperi* common ancestor could have become isolated (Fig. 7b) and eventually diverged into *A. ignipedis* (Fig. 7c). This divergence may have been facilitated by selection for aposematic coloration or sexual selection for brightly-colored frogs (Roberts *et al.* 2007), thus explaining the more brightly-colored frogs of the *bassleri-yoshina-pepperi* group. Soon after, the Upper Huallaga population of the *bassleri-yoshina-pepperi* group became isolated from the Cordillera Oriental populations (Fig. 7c) and eventually diverged into *A. pepperi* (Fig. 7d). Note that all *A. pepperi* individuals are closely related, despite occupying a relatively large area. This suggests that gene flow continues among sampled populations or that this species recently experienced a range expansion throughout the Upper Huallaga Valley (see Fig. 6 for phylogenetic relationships). Later, a subsequent connection between the Cordillera Oriental and the Upper Huallaga Valley allowed *A. pepperi* to re-colonize the Saposoa region (where it possibly hybridized with *A. bassleri*). During the same time period, Cordillera Oriental and

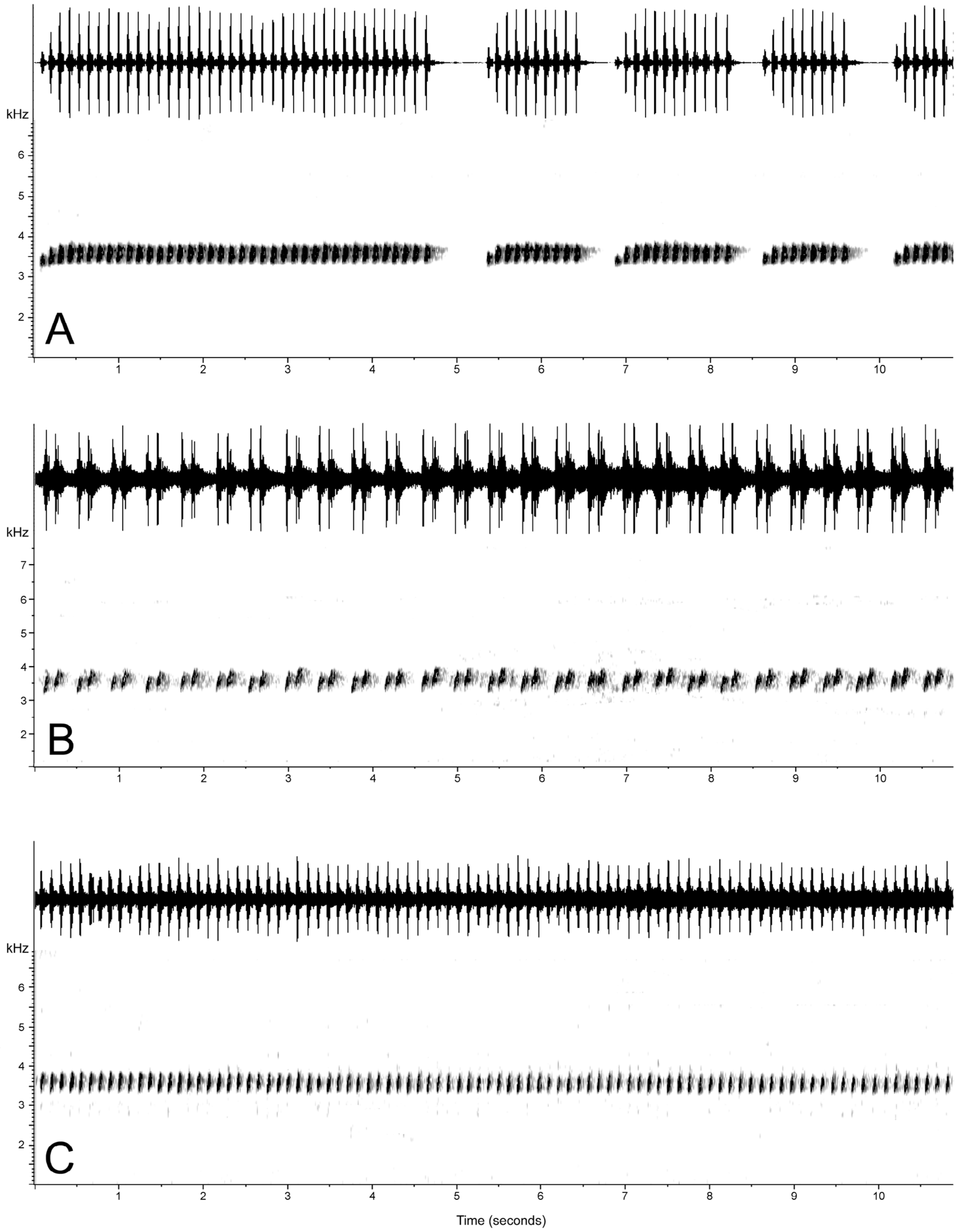

FIGURE 10. Advertisement calls of species in the *Ameerega petersi* group. (A) *A. petersi*, field recording in the Cordillera Azul 20 km NE of Tingo Maria, near the village of Miguel Grau (#1 on Fig. 8), 24.5 C, 27 April 2006. Dominant frequency 3570 Hz. (B) *A. smaragdina*, field recording from Pan de Azucar, near the town of Iscozacin (#9 on Fig. 8), 24.5 C, 9 August 2007. Dominant frequency 3594 Hz. (C) *A. cainarachi*, field recording from Chazuta, San Martin, Peru, 25 C, 7 July 2007, Dominant frequency 3590 Hz.

Contamana likely became connected and the common ancestor of *A. bassleri* and *A. yoshina* re-colonized Contamana (Fig. 7e), became isolated (Fig. 7f), and diverged into *A. yoshina* (Fig. 7g). Finally, a relatively recent connection between Contamana and the Cordillera Oriental may have allowed *A. yoshina* to disperse back into the Cordillera Oriental (Fig. 7h), although this population may be currently isolated from the Contamana population (Fig. 7i). This scenario requires at least three connections and subsequent dispersal events between the Cordillera Oriental and Contamana. Support for this hypothesis is provided by our phylogenetic results and the results of the DIVA analysis.

Remarks on the taxonomy of the *petersi* clade

A single frog collected from Contamana in 1947 was previously thought to represent an outlying population of *A. petersi* (Silverstone 1976), a species which, sensu Silverstone (1976), ranged from northern San Martín, Peru, south to Puno, Peru, near Bolivia. Subsequent revisions (Myers and Rodriguez 1998, Schulte 1999) described these outlying northern and southern populations as *A. pongoensis* and *A. simulans*, respectively. As currently defined, *A. petersi* is distributed throughout the Pachitea drainage in central Peru (Fig. 8), where it occurs extensively throughout lowland forests and well into the montane forests of the east Andean versant. Our record from the Cordillera Azul represents the highest-altitude locality for *A. petersi* by at least 780 m. Here, we found several individuals at 1247 m (e.g. Fig. 11 i–k), and a single male was heard calling at 1580 m in a site where *A. silverstonei* was present. Silverstone (1976) also referred specimens from the upper Río Ene, Perené, and Chanchamayo drainages in Junín to *A. petersi*, although further phylogenetic studies in this area are needed to confirm whether these frogs are, in fact, *A. petersi*, whether they represent outlying populations of *A. smaragdina*, or whether they represent an undescribed species.

Characters originally used to diagnose *A. smaragdina* from *A. petersi* now appear to be present in populations of both species. For example, Silverstone (1976) used the lack of ventral marbling in *A. smaragdina* as the character to distinguish this species from *A. petersi*. In 2006, we found a population of *A. petersi* (diagnosis based on genetic data) from Aguaytia, Huánuco (Fig. 11 l & m), that completely lacks ventral marbling and is morphologically indistinguishable from *A. smaragdina*. Analysis of advertisement calls has not proven to be useful in diagnosing these two species. *Ameerega petersi* is notorious for having highly variable advertisement calls, which may consist of notes given as couplets, triplets, quadruplets (Myers *et al*. 1998), or a continuous series (Fig. 10). During our field investigations we have not noticed any particular trends with respect to call types, and in fact, a single male of either species will often make all different types, typically "warming up" with couplets near the beginning of a calling bout, and eventually progressing to a continuous chain of notes which may last for 5 seconds or more. We recorded a male *A. smaragdina* from Pan de Azucar which had a call consisting of note couplets (Fig. 10), although other males were heard calling with long chains of notes.

A potential case of incomplete lineage sorting: *A. petersi* and *A. smaragdina*

There is a distinct possibility, despite the observed high level of mitochondrial divergence, that *A. smaragdina* and *A. petersi* represent a single paraphyletic species that underwent several disturbance-vicariance events, and the observed phylogenetic pattern is a result of mitochondrial introgression between two divergent populations of *A.*"*petersi-smaragdina*". First, the two species are essentially sympatric, or at least parapatric with no barrier separating them. Second, the two species appear to be indistinguishable ecologically, morphologically, and acoustically, so we cannot envision a scenario where they would not freely interbreed in nature. However, because phylogenetics support the current taxonomy, and because we have no topotypic material for *A. petersi* (from nearby Santa Isabel), further investigation is needed before any taxonomic revision is made in this group.

One possible scenario to explain our observed phylogeny could be that the common ancestor of *A. cainarachi*, *A. smaragdina*, and *A. petersi* was widely distributed throughout the Pachitea drainage (Fig. 9a). A primary disturbance-vicariance event separated the southern and northern populations, giving rise to *A. smaragdina* and the common ancestor *A. cainarachi* and *A. petersi* (Fig. 9b). The northern population then likely radiated into San Martín, but soon after, a second disturbance-vicariance event separated these populations (Fig. 9c). Due to different selective regimes, the ancestor to *A. cainarachi* diverged from the ancestor of *A. petersi*, giving rise to the distinct morphologies that we see today. Around the same time, populations of *A. petersi* (northern populations) and *A. smaragdina* (southern populations) came back into contact with each other and due to the high morphological and high acoustic variability, "species" boundaries were weak and the northern and southern populations introgressed (Fig. 9d and 9e). This example is just one of many hypothetical biogeographic scenarios, although this scenario is consistent with the results from our DIVA analysis on this group (unpublished data). If *A. smaragdina* and *A. petersi* are two divergent populations of the same species, we expect there to be an area of introgression at the margins of both "species" where *A. petersi* and *A. smaragdina* haplotypes are abundant and further away from this area, the haplotypes will be dominated by the historic species' haplotype. There is some support for this hypothesis as one individual of *A. smaragdina* (classified as *A. petersi* by its collectors) was collected near Panguana Research Station, an area largely composed of *A. petersi* haplotypes (Fig. 8 locality 8), however, more distant haplotypes are entirely *A. petersi* (to the north) or *A. smaragdina* (to the south).

Acknowledgements

We thank Brian Sexton who generously donated money which helped finance our Contamana expeditions. We thank Bryan Richardson and Wouter Olthof for their assistance in the field and Karen Siu Ting for her help in the museum and help dissecting the type series of *A. yoshina*. We especially wish to thank Mark Pepper for providing life history information on these frogs, a photograph of *Ameerega pepperi*, and for assistance in the field. We also thank Justin Yeager and Marcos Ramírez Zárate for providing photos. We are grateful to Tim Green who alerted us to the existence of a red-backed *Ameerega* from Contamana in 2005. We also thank Manuel Sanchez Rodríguez for providing a Spanish translation of the abstract. Lastly, we are grateful to Kyle Summers for his support of our taxonomic studies. Permits to conduct field work and to export materials were issued by INRENA (N° 002765-AG-INRENA, N° 061-2003-INRENA-IFFS-DCB, N° 050-2006-INRENA-IFFS-DCB, and N° 067-2007-INRENA-IFFS-DCB).

Literature cited

Avise, J.C. (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA, U.S.A., 447 pp.

Avise, J.C. & Ball, R.M. (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology*, 7, 45–67.

Avise, J.C., Shapira, J.F., Daniel, S.W., Aquadro, C.F. & Lansman, R.A. (1983) The use of restriction endonucleases to measure mitochondrial-DNA sequence relatedness in natural-populations. 1. Mitochondrial-DNA differentiation during the speciation process in *Peromyscus*. *Molecular Biology and Evolution*, 1, 38–56.

Boul, K.E., Funk, W.C., Darst, C.R., Cannatella, D.C. & Ryan, M.J. (2007) Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society of London Series B*, 274, 399–406.

Brown, J.L., Schulte, R. & Summers, K. (2006) A new species of *Dendrobates* (Anura: Dendrobatidae) from the Amazonian lowlands in Perú. *Zootaxa*, 1152, 45–58.

Charif, R.A., Clark, C.W. & Fristrup, K.M. (2004) *Raven 1.2 User's Manual*. Cornell Laboratory of Ornithology, Ithaca, New York, U.S.A, 205 pp.

Chicago Field Museum (2006) *Rapid biological inventory: Peru: Sierra del Divisor*. The Field Museum, Chicago, Illinois, U.S.A., 96 pp.

Colinvaux, P. (1993) Pleistocene biogeography and diversity in tropical forests of South America. *In* Goldblatt, P. (ed), *Biological relationships between Africa and South America*. Yale University Press, New Haven, Connecticut, U.S.A., pp. 473–499.

Doyle, J.J. (1997) Trees within trees: Genes and species, molecules and morphology. *Systematic Biology*, 46, 537–553.

IUCN (2001) *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK, 14 pp.

Funk, D.J. & Omland, K.E. (2003) Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics*, 34, 397–423.

Gamble, T., Berendzen, P.B., Shaffer, H.B., Starkey, D.E. & Simons, A.M. (2008) Species limits and phylogeography of

North American cricket frogs (*Acris*, Hylidae). *Molecular Phylogenetics and Evolution*, 48, 112–125.

Graham, C.H., Ron, S.R., Santos, J.C., Schneider, C.J. & Moritz, C. (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, 58, 178–193.

Grant, T., Frost, D.R., Caldwell, J.P., Gagliardo, R., Haddad, C.F.B., Kok, P.J.R., Means, B.D., Noonan, B.P., Schargel, W.C. & Wheeler, W.C. (2006) Phylogenetic systematics of dart-poison frogs and their relatives (Anura: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History*, 299, 1–262.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis., A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny, Version 3.1.2. *Bioinformatics* (Oxford England), 17, 754–755.

Lötters, S., Reichle, S. & Jungfer, K.-H. (2003) Advertisement calls of neotropical poison frogs (Amphibia: Dendrobatidae) of the genera *Colostethus*, *Dendrobates* and *Epipedobates*, with notes on dendrobatid call classification. *Journal of Natural History*, 37, 1899–1911.

Lötters, S. & Vences, M. (2000) Bemerkungen zur Nomenklatur und taxonomie peruanischer Pfeilgiftfrösche (Anura: Dendrobatidae: *Dendrobates*, *Epipedobates*) [Remarks on the nomenclature and taxonomy of Peruvian poison frogs (Anura: Dendrobatidae: *Dendrobates*, *Epipedobates*)]. *Salamandra*, 36, 247–260.

Maddison, W.P. (1997) Gene trees in species trees. *Systematic Biology*, 46, 523–536.

McGuire, J.A., Linkem, C.W., Koo, M.S., Hutchison, D.W., Lappin, A.K., Orange, D.I., Lemos-Espinal, J., Riddle, B.R. & Jaeger, J.R. (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of Crotaphytid lizards. *Evolution*, 61, 2879–2897.

Morales, V.R. (1992) Dos especies nuevas de *Dendrobates* (Anura: Dendrobatidae) para Perú. *Caribbean Journal of Science*, 28, 191–199.

Morando, M., Avila, L.J., Baker, J. & Sites, J.W. (2004) Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): Evidence for introgression and incomplete lineage sorting. *Evolution*, 58, 842–861.

Myers, C.W. (1982) Spotted poison frogs: Descriptions of three new *Dendrobates* from western Amazonia, and resurrection of a lost species from "Chiriqui". *American Museum Novitates*, 272, 1–23.

Myers, C.W., Rodriguez, L.O. & Icochea, J. (1998) *Epipedobates simulans*, a new cryptic species of poison frog from southeastern Peru, with notes on *E. macero* and *E. petersi* (Dendrobatidae). *American Museum Novitates*, 3238, 1–20.

Noonan, B.P. & Chippindale, P.T. (2006) Dispersal and vicariance: The complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution*, 40, 347–358.

Noonan, B.P. & Gaucher, P. (2006) Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. *Molecular Ecology*, 15, 4425–4435.

Noonan, B.P. & Wray, K.P. (2006) Neotropical diversification: the effects of a complex history on diversity within the poison frog genus *Dendrobates*. *Journal of Biogeography*, 33, 1007–1020.

Nylander, J.A.A. (2004) MrModeltest 2.2. Program distributed by author. Evolutionary Biology Centre, Uppsala University.

Pamilo, P. & Nei, M. (1988) Relationships between gene trees and species trees. *Molecular Biology and Evolution*, 5, 568–583.

Patton, J.L. & Smith, M.F. (1994) Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (Genus *Thomomys*). *Systematic Biology*, 43, 11–26.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190, 231–259.

Platz, J.E. (1989) Speciation within the chorus frog *Pseudacris triseriata*: morphometric and mating call analyses of the boreal and western subspecies. *Copeia*, 1989, 704–712.

Platz, J.E. & Forester, D.C. (1988) Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). *Copeia*, 1988, 1062–1066.

Rodríguez, L. & Myers, C.W. (1993) A new poison frog from Manu National Park, southeastern Peru (Dendrobatidae, *Epipedobates*). *American Museum Novitates*, 3068, 1–15.

Roberts, J.L., Brown, J.L., Arizabal, W., von May, R. Schulte, R. & Summers, K. (2006) Genetic divergence and speciation in lowland and montane Peruvian poison frogs. *Molecular Phylogenetics and Evolution*, 41, 149–164.

Roberts, J.L., Brown, J.L., Schulte, R., Arizabal, W. & Summers, K. (2007) Rapid diversification of colouration among populations of a poison frog isolated on sky-peninsulas in the central cordilleras of Peru. *Journal of Biogeography*, 34, 417–426.

Ronquist, F. (1996) DIVA version 1.1. Computer program and manual available by anonymous FTP from Uppsala University (ftp.uu.se or ftp.systbot.uu.se). Downloaded on September 23, 2008.

Santos, J., Coloma, L. & Cannatella, D. (2003) Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 12792–12797.

Silverstone, P.A. (1976) A revision of the poison-arrow frogs of the genus *Phyllobates* Bibron in Sagra (Family Dendrobatidae). *Bulletin of the Natural History Museum of Los Angeles County*, 27, 1–53.

Schulte, R. (1986) Ein neue *Dendrobates*- art aus Ostperu (Amphibia: Salientia: Dendrobatidae). *Sauria*, 8, 11–20.

Schulte, R. (1999) *Pfeilgiftfrösche "Artenteil - Peru"*. Wailblingen, Germany, INBICO, 292 pp.

Slade, R.W. & Moritz, C. (1998) Phylogeography of *Bufo marinus* from its natural and introduced ranges. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 265, 769–777.

SPSS 15.0 (2008) SPSS for Windows, Rel. 15. 2008. Chicago: SPSS Inc.

Stockman, A.K. & Bond, J.E. (2007) Delimiting cohesion species: extreme population structuring and the role of ecological interchangeability. *Molecular Ecology*, 16, 3374–3392.

Sullivan, B.K., Malmos, K.B. & Given, M.F. (1996) Systematics of the *Bufo woodhousii* complex (Anura: Bufonidae): advertisement call variation. *Copeia*, 1996, 274–280.

Summers, K. & Clough, M. (2001) The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proceedings of the National Academy of Sciences of the United States of America*, 98, 6227–6232.

Talbot, S.L. & Shields, G.F. (1996) Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution*, 5, 477–494.

Twomey, E. & Brown, J.L. (2008a) A partial revision of the *Ameerega hahneli* complex (Anura: Dendrobatidae) and a new cryptic species from the east-Andean versant of central Peru. *Zootaxa*, 1757, 49–65.

Twomey, E. & Brown, J.L. (2008b) Spotted poison frogs: rediscovery of a lost species and a new genus (Anura: Dendrobatidae) from northwestern Peru. *Herpetologica*, 64, 121–137.

Vences, M., Kosuch, J., Boistel, R., Haddad, C.F.B., La Marca, E., Lotters, S. & Veith, M. (2003) Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. *Organisms Diversity & Evolution*, 3, 215–226.

Weisrock, D.W., Kozak, K.H. & Larson, A. (2005) Phylogeographic analysis of mitochondrial gene flow and introgression in the salamander, *Plethodon shermani*. *Molecular Ecology*, 14, 1457–1472.

Appendix figures

FIGURE 11. *Ameerega ignipedis* and similar species. (a,b) *A. pongoensis* from lower Cainarachi Valley, San Martin, Peru; (c,d) *A. pongoensis* from Shucushuyacu, Loreto, Peru; (e,f) *A. ignipedis* from type locality (holotype and MUSM 24952, respectively); (g) *Ameerega simulans* from Mazuko, Madre de Díos, Peru (photo by K. Summers); (h) *A. petersi* from Codo del Pozuzo, Huánuco, Peru; (i, k, j) *A. petersi* from Miguel Grau, Ucayali, Peru (photo k by J. Yeager); (l,m) *A. petersi* from Aguaytia, Ucayali, Peru; (n) *A. petersi* from Puerto Inca, Huánuco, Peru.

FIGURE 12. *Ameerega petersi*, *A. smaragdina*, and *A. bassleri*. (a) *A. petersi* from Puerto Inca, Huánuco, Peru; (b) *A. petersi* from Tournavista, Huánuco, Peru; (c,d) *A. smaragdina* from Pan de Azucar, Pasco, Peru (photo 'd' by K. Summers); (e-h) *A. bassleri* from 8.6 km E of San José de Sisa, San Martín, Peru; (i-o) *A. bassleri* from 7 km NW of Saposoa, San Martín, Peru.

FIGURE 13. *Ameerega bassleri*. (a,b) Roque, San Martín, Peru (type locality); (c-e) Cainarachi Valley, San Martín, Peru; (f-j) Chazuta, San Martín, Peru; (k) Sauce, San Martín, Peru; (l-m) Cordillera Azul, 14 km SE of Shamboyacu, San Martín, Peru (photo by M. Ramírez Zárate); (n-p) eggs and tadpoles of *A. bassleri*.

FIGURE 14. *Ameerega yoshina*, *A. cainarachi*, and *A. pepperi*. (a-c) *A. yoshina* from Callanayacu, San Martín, Peru (MUSM 26985-26987); (d-f) *A. yoshina* from type locality (MUSM 24945, 26953, 26954), (g) *A. yoshina* (left) and sympatric *A. cainarachi* (right) from Callanayacu, San Martín, Peru; (h) *A. cainarachi* from Cainarachi Valley, San Martín, Peru; (i) *A. cainarachi* from Chazuta, San Martín, Peru; (j,k) *A. pepperi* from type locality, (l) *A. pepperi* from Nuevo San Martín, San Martín, Peru.

FIGURE 15. *Ameerega pepperi* and habitat photos for the new species in this paper. (a,e) *A. pepperi* from Chumanza, San Martín, Peru (photo 'a' by M. Pepper); (b-d) *A. pepperi* from Río Abiseo near Huicungo, San Martín, Peru; (f,g) *A. pepperi* from 6.3 km N of Campanilla, San Martín, Peru; (h) type locality of *A. ignipedis*, a geothermal stream flowing out of the Serranía de Contamana. The entire type series (as well as one *A. yoshina* metamorph) was found in the forest adjacent to the stream or among the plants growing in the stream banks. (i) type locality of *A. pepperi* near San Francisco, San Martín, Peru. (j) Quebrada Pacuyacu near Callanayacu. Three *A. yoshina* paratypes (MUSM 26985-26987) were collected from amongst the rocks and vegetation alongside the stream.

FIGURE 16. Type series of new species in this paper. (a) *Ameerega ignipedis* (L-R, MUSM 24947–24952); (b) *A. yoshina* (L-R, MUSM 24945, 26953–26955, 26985–26987); (c) *A. pepperi* (L-R, MUSM 26940-26942, 26968, 26975, 26976, 26979, 26980).

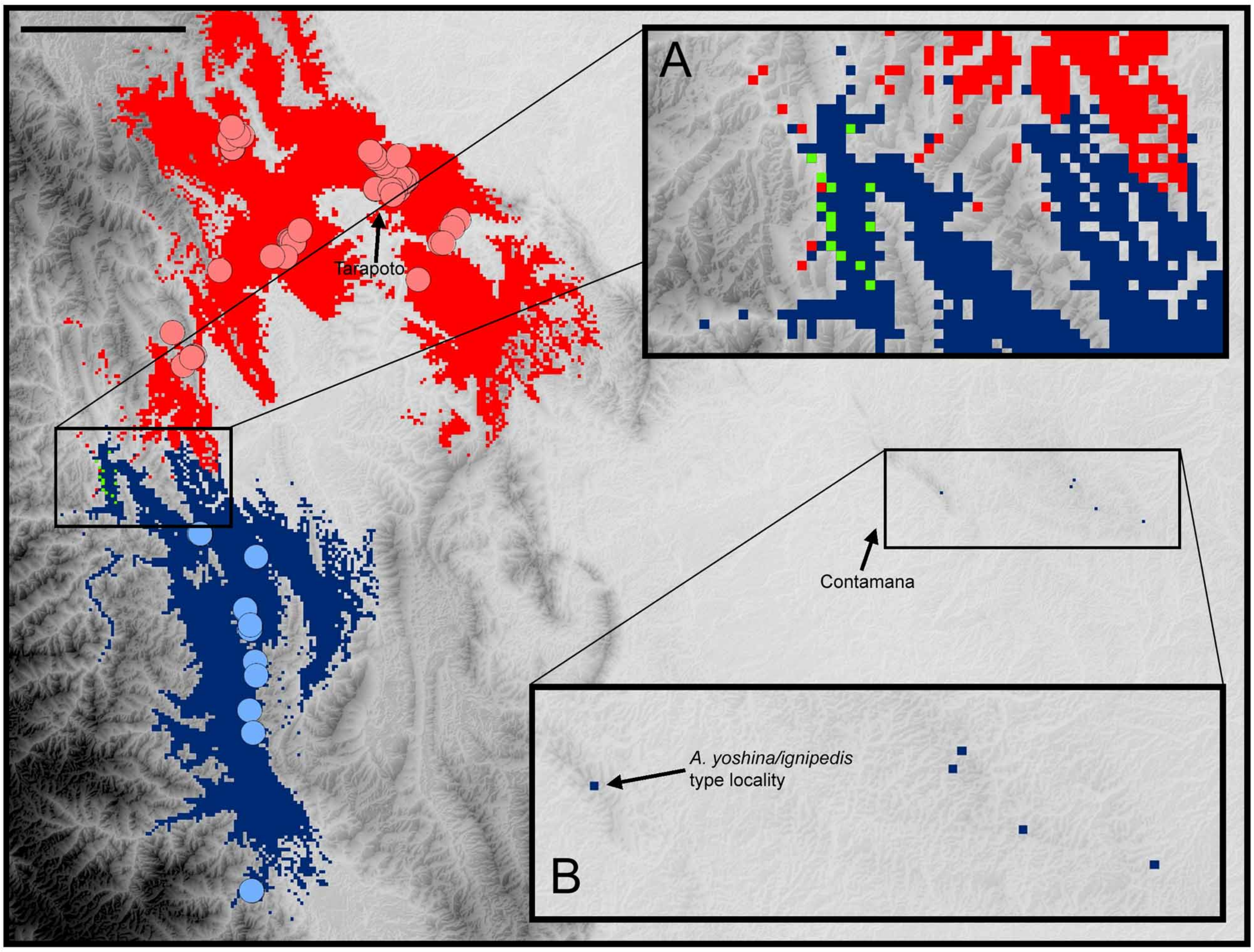

FIGURE 17. Ecological niche models of *Ameerega bassleri* (red) and *A. pepperi* (blue). (A) Only 13 ”presence” pixels overlapped between both species' models (depicted in green). This is 0.17% (pixel count = 7790) of the “presence” area in the model of *A. bassleri* and 0.28% (pixel count = 4622) of the “presence” area in the model of *A. pepperi*. These results support the hypothesis that these two species have diverged ecologically and currently occupy separate niches. (B) The model for *A. pepperi* also predicted this species' occurrence at the type localities of *A. yoshina* and *A. ignipedis* (near Contamana). This suggests that both *A. yoshina* and *A. pepperi* share similar niches, however due to limited locality data on *A. yoshina*, we were unable to calculate this species' distribution and therefore cannot make comparisons. Black bar = 50 km; 1 pixel in niche model = 1 km^2.

Appendix tables

TABLE 3. Measurements (in mm) of *Ameerega ignipedis* type series. Averages (with standard deviation) were calculated from the type series.

Character	MUSM 24947	MUSM 24948	MUSM 24949	MUSM 24950	MUSM 24951	MUSM 24952	Average (*N* = 6)
SVL	20.3	23.8	20.8	21.7	24.2	23.5	22.4 ± 1.6
FL	8.2	10.5	9.3	10.6	8.9	10.5	9.7 ± 1.0
TL	9.6	11.1	9.6	11.0	11.1	11.0	10.6 ± 0.8
KK	17.2	20.9	18.4	20.1	18.6	20.7	19.3 ± 1.5
FoL	8.2	9.9	7.8	9.5	9.0	9.8	9.0 ± 0.9
HaL	4.8	6.0	5.2	5.8	5.9	6.2	5.7 ± 0.5
HL	4.8	6.0	5.6	5.4	5.5	5.3	5.4 ± 0.4
HW	6.1	7.1	6.1	6.6	6.1	6.8	6.5 ± 0.4
BW	5.9	7.0	6.0	5.5	6.1	6.2	6.1 ± 0.5
IND	2.1	2.8	2.3	2.6	2.2	2.3	2.4 ± 0.3
UEW	4.3	4.6	5.3	4.8	4.3	5.3	4.8 ± 0.5
IOD	3.0	3.4	4.0	4.2	4.0	4.6	3.9 ± 0.6
TD	2.1	2.2	2.0	1.9	1.8	2.2	2.0 ± 0.2
ED	3.6	4.1	4.3	3.8	4.0	4.2	4.0 ± 0.3
DET	0.9	1.4	1.2	1.0	1.3	1.1	1.1 ± 0.2
L1F	4.5	4.7	4.6	4.6	3.8	5.4	4.6 ± 0.5
L2F	2.1	2.9	2.5	3.0	2.2	2.6	2.6 ± 0.4
W3D	0.9	0.9	0.8	0.9	0.9	0.9	0.9 ± 0.1
W3F	0.5	0.6	0.6	0.6	0.5	0.5	0.5 ± 0.1
SEX	M	F	F	F	M	F	

TABLE 4. Measurements (in mm) of *Ameerega yoshina* type series. Averages (with standard deviation) were calculated from the type series, excluding MUSM 26955, which was a sub-adult male. An asterisk indicates individuals were dissected to confirm sex.

Character	MUSM 24945	MUSM 26953	MUSM 26954	MUSM 26955	MUSM 26985	MUSM 26986	MUSM 26987	Average (*N* = 6)
SVL	34.8	29.2	33.1	20.8	31.5	33.9	26.2	31.4 ± 3.2
FL	16.3	13.4	15.2	11.5	14.0	14.6	14.4	14.7 ± 1.0
TL	17.6	15.9	17.0	11.6	16.5	17.5	17.8	17.1 ± 0.7
KK	29.5	28.7	30.6	11.3	29.7	29.6	31.0	28.3 ± 3.9
FoL	14.6	13.7	14.4	10.5	15.8	13.9	15.0	14.6 ± 0.8
HaL	8.3	8.5	8.2	6.1	9.0	8.8	8.8	8.6 ± 0.3
HL	8.1	9.2	9.7	7.3	8.5	9.1	9.8	9.1 ± 0.7
HW	10.2	8.8	9.8	6.7	9.3	10.3	10.7	9.9 ± 0.7
BW	12.1	9.5	11.1	5.7	8.7	11.8	12.8	11.0 ± 1.6
UEW	7.3	6.6	6.7	5.1	6.3	6.1	7.1	6.7 ± 0.5
IOD	4.9	3.9	4.7	3.2	4.0	4.8	5.6	4.6 ± 0.6
TD	3.2	1.9	1.9	1.8	1.9	1.9	2.6	2.2 ± 0.5

ED	5.3	5.8	5.8	4.5	5.8	4.5	5.1	5.4 ± 0.5
DET	0.8	1.4	1.6	1.1	1.6	1.4	1.6	1.4 ± 0.3
L1F	7.6	6.7	7.4	5.6	8.5	8.2	7.2	7.6 ± 0.6
L2F	4.5	6.7	7.2	5.0	8.2	7.2	5.6	6.6 ± 1.3
W3D	1.0	1.0	1.0	0.6	1.0	1.0	1.0	1.0 ± 0.1
W3F	0.5	0.5	0.8	0.5	0.5	0.5	0.6	0.6 ± 0.1
SEX	F	F*	F*	M*	F	F*	F	

TABLE 5. Measurements (in mm) of *Ameerega pepperi* type series. Averages (with standard deviation) were calculated from the type series.

Character	MUSM 26976	MUSM 26975	MUSM 26980	MUSM 26979	MUSM 26940	MUSM 26941	MUSM 26942	MUSM 26968	Average (*N* = 7)
SVL	32.5	32.8	31.5	30.8	28.6	28.6	34.4	34.1	31.7 ± 2.2
FL	14.8	15.7	16.1	16.0	13.9	13.4	15.6	15.8	15.2 ± 1.0
TL	15.7	16.6	16.5	16.3	15.0	16.1	16.8	16.8	16.2 ± 0.6
KK	30.1	30.5	30.4	29.7	27.7	28.2	31.4	30.8	29.9 ± 1.3
FoL	13.9	15.5	14.9	14.4	13.7	13.3	15.1	16.0	14.6 ± 0.9
HaL	8.9	8.6	8.6	8.2	7.8	7.5	8.0	9.3	8.4 ± 0.6
HL	9.8	9.8	9.3	8.5	8.2	7.7	9.1	8.6	8.9 ± 0.8
HW	9.2	9.7	9.5	9.1	8.6	7.8	9.1	9.7	9.1 ± 0.6
BW	8.6	9.8	8.7	8.9	8.9	8.3	11.4	9.9	9.3 ± 1.0
UEW	5.6	6.1	5.6	5.1	5.5	5.5	5.1	6.1	5.6 ± 0.4
IOD	3.9	3.7	4.0	3.9	4.2	3.9	4.2	3.5	3.9 ± 0.2
IND	4.7	5.0	4.0	4.2	4.7	4.0	4.8	4.8	4.5 ± 0.4
TD	2.7	1.8	2.6	2.1	1.8	3.0	1.6	2.1	2.2 ± 0.5
ED	4.2	4.0	4.5	4.2	3.7	4.2	4.5	4.8	4.3 ± 0.3
DET	1.6	1.8	1.6	1.6	1.5	1.6	1.6	1.6	1.6 ± 0.1
L1F	7.2	7.6	7.1	7.2	7.4	6.9	8.4	7.4	7.4 ± 0.4
L2F	7.2	7.1	6.9	6.4	6.9	6.1	7.2	7.4	6.9 ± 0.4
W3D	0.8	1.0	0.8	1.0	1.0	0.8	1.1	1.1	0.9 ± 0.1
W3F	0.5	0.5	0.3	0.5	0.6	0.6	0.6	0.6	0.5 ± 0.1
SEX	F	F	F	F	M	M	F	F	

TABLE 6. List of localities and GenBank accession numbers for individuals included in the phylogeny.

Species	Locality	12s	16s	CytB	Reference
altamazonica 1	Chazuta, San Martín, PE	DQ522980	DQ523051	DQ523121	Roberts *et al.* 2006
altamazonica 2	Tarapoto, San Martin, PE	DQ522955	DQ523026	DQ523096	Roberts *et al.* 2006
altamazonica 3	San Jose de Sisa, San Martin, PE	DQ523008	DQ523079	DQ523149	Roberts *et al.* 2006
altamazonica 4	Tarapoto, San Martin, PE	DQ523007	DQ523078	DQ523148	Roberts *et al.* 2006
altamazonica 5	Chazuta, San Martin, PE	DQ522966	DQ523037	DQ523107	Roberts *et al.* 2006
altamazonica 6	Tocache, San Martin, PE	EU517662	EU517665	EU517671	Twomey & Brown 2008a
altamazonica 7	Tocache, San Martin, PE	EU517661	EU517663	N/A	Twomey & Brown 2008a
altamazonica 8	Pampa Hermosa, Loreto, PE	EU517660	EU517664	EU517672	Twomey & Brown 2008a
altamazonica 9	Pampa Hermosa, Loreto, PE	EU517659	EU517666	EU517677	Twomey & Brown 2008a
altamazonica 10	Saposoa, San Martin, PE	DQ523015	DQ523086	DQ523156	Roberts *et al.* 2006

altamazonica 11	Tarapoto, San Martin, PE	DQ522951	DQ523022	DQ523092	Roberts *et al.* 2006
altamazonica 12	Tarapoto, San Martin, PE	DQ522978	DQ523049	DQ523119	Roberts *et al.* 2006
bassleri 1	Chumia, San Martín, PE	FJ752366	FJ752289	FJ752333	this study
bassleri 2	Chumia, San Martín, PE	FJ752367	FJ752290	FJ752334	this study
bassleri 3	Sauce, San Martín, PE	DQ522989	DQ523060	DQ523130	Roberts *et al.* 2006
bassleri 4	Cainarachi valley, San Martín, PE	DQ522974	DQ523045	DQ523115	Roberts *et al.* 2006
bassleri 5	Cainarachi valley, San Martín, PE	DQ523009	DQ523080	DQ523150	Roberts *et al.* 2006
bassleri 6	Huallaga Canyon, San Martín, PE	DQ522986	DQ523057	DQ523127	Roberts *et al.* 2006
bassleri 7	Chazuta, San Martín, PE	DQ523016	DQ523087	DQ523157	Roberts *et al.* 2006
bassleri 8	Roque, San Martín, PE	FJ752352	FJ752273	FJ752318	this study
bassleri 9	Saposoa, San Martín, PE	N/A	FJ752266	FJ752310	this study
bassleri 10	Saposoa, San Martín, PE	N/A	FJ752267	FJ752311	this study
bassleri 11	Saposoa, San Martín, PE	N/A	FJ752268	FJ752312	this study
bassleri 12	Alto Shima, San Martín, PE	N/A	FJ752269	FJ752313	this study
bassleri 13	Alto Shima, San Martín, PE	N/A	FJ752270	FJ752314	this study
bassleri 14	San Jose de Sisa, San Martín, PE	DQ522999	DQ523070	DQ523140	Roberts *et al.* 2006
bassleri 15	San Jose de Sisa, San Martín, PE	DQ523002	DQ523073	DQ523143	Roberts *et al.* 2006
bassleri 16	San Jose de Sisa, San Martín, PE	DQ523014	DQ523085	DQ523155	Roberts *et al.* 2006
bassleri 17	Shanao, San Martín, PE	DQ522972	DQ523043	DQ523113	Roberts *et al.* 2006
bassleri 18	Roque, San Martín, PE	FJ752351	FJ752272	FJ752317	this study
bassleri 19	Alto Shima, San Martín, PE	DQ523012	DQ523083	DQ523153	Roberts *et al.* 2006
bassleri 20	Alto Shima, San Martín, PE	N/A	FJ752271	FJ752316	this study
bassleri 21	Saposoa, San Martín, PE	DQ523017	DQ523088	DQ523158	Roberts *et al.* 2006
bassleri 22	Saposoa, San Martín, PE	N/A	NA	FJ752315	this study
bilinguis 1	Primavera, Napo, EC	DQ523003	DQ523074	DQ523144	Roberts *et al.* 2006
bilinguis 2	Cuyabeno, Sucumbios, EC	DQ502095	DQ502095	DQ502527	Grant *et al.* 2006
bilinguis 3	Cuyabeno, Sucumbios, EC	DQ502073	DQ502073	DQ502504	Grant *et al.* 2006
braccata	Manso, Matto Grosso, BR	DQ502125	DQ502125	N/A	Grant *et al.* 2006
cainarachi 1	Cainarachi valley, San Martín, PE	DQ522953	DQ523024	DQ523094	Roberts *et al.* 2006
cainarachi 2	Cainarachi valley, San Martín, PE	DQ522982	DQ523053	DQ523123	Roberts *et al.* 2006
flavopicta	Rio Tocantins, Paraná, BR	DQ502124	DQ502124	N/A	Grant *et al.* 2006
hahneli 1	Convento, San Martín, PE	DQ522961	DQ523032	DQ523102	Roberts *et al.* 2006
hahneli 2	Puerto Inca, Huanuco, PE	N/A	EU517669	EU517676	Twomey & Brown 2008a
hahneli 3	Panguana, Huanuco, PE	N/A	AF282248	N/A	Lötters & Vences 2000
hahneli 4	Puente Itaya, Loreto, PE	DQ522990	DQ523061	DQ523131	Roberts *et al.* 2006
hahneli 5	Leticia, Amazonas, CO	DQ502270	DQ502270	DQ502701	Grant *et al.* 2006
hahneli 6	Yasuni, Orellana, EC	AY364573	AY364573	N/A	Santos *et al.* 2003
hahneli 7	Rio Manati, Loreto, PE	DQ523004	DQ523075	DQ523145	Roberts *et al.* 2006
hahneli 8	Alto Purus, Ucayali, PE	DQ522970	DQ523041	DQ523111	Roberts *et al.* 2006
hahneli 9	Boca Manu, Cusco, PE	DQ522956	DQ523027	DQ523097	Roberts *et al.* 2006
hahneli 10	Rio los Amigos, Madre de Dios, PE	DQ522985	DQ523056	DQ523126	Roberts *et al.* 2006
hahneli 11	Cobija, Pando, BO	N/A	AF282246	N/A	Lötters & Vences 2000
hahneli 12	Madre de Dios, PE	DQ501997	DQ501997	DQ502422	Grant *et al.* 2006
hahneli 13	Amazonas, BR	DQ522992	DQ523063	DQ523133	Roberts *et al.* 2006
hahneli 14	Amazonas, BR	DQ522996	DQ523067	DQ523137	Roberts *et al.* 2006
hahneli 15	near Manaus, Amazonas, BR	DQ502226	DQ502226	DQ502659	Grant *et al.* 2006
hahneli 16	near Manaus, Amazonas, BR	DQ502226	DQ502226	DQ502659	Grant *et al.* 2006

hahneli 17	Reserve Trinite, Saint-Élie, FG	N/A	AY263247	N/A	Vences *et al.* 2003
hahneli 18	Porto Walter, Acre, BR	DQ502084	DQ502084	DQ502515	Grant *et al.* 2006
hahneli 19	Porto Walter, Acre, BR	DQ502085	DQ502085	DQ502516	Grant *et al.* 2006
ignipedis 1	Contamana, Loreto, PE	FJ752368	FJ752291	N/A	this study
ignipedis 2	Contamana, Loreto, PE	FJ752369	FJ752292	N/A	this study
ignipedis 3	Contamana, Loreto, PE	FJ752370	FJ752293	N/A	this study
ignipedis 4	Contamana, Loreto, PE	N/A	FJ752294	FJ752335	this study
ignipedis 5	Contamana, Loreto, PE	FJ752371	FJ752295	FJ752336	this study
macero 1	Ivochote, Cusco, PE	DQ522968	DQ523039	DQ523109	Roberts *et al.* 2006
macero 2	near La Merced, Junin, PE	N/A	EU525852	EU525853	Twomey & Brown 2008a
macero 3	Manu, Madre de Dios, PE	DQ502155	DQ502155	DQ502591	Grant *et al.* 2006
macero 4	Alto Purus, Ucayali, PE	DQ523018	DQ523089	N/A	Roberts *et al.* 2006
parvula	Macas, Morona-Santiago, EC	N/A	AY364574	N/A	Santos *et al.* 2003
pepperi 1	Abiseo, San Martin, PE	FJ752357	FJ752280	FJ752324	this study
pepperi 2	Chumanza, San Martin, PE	FJ752358	FJ752281	FJ752325	this study
pepperi 3	Nuevo San Martin, San Martin, PE	FJ752359	FJ752282	FJ752326	this study
pepperi 4	San Francisco, San Martin, PE	FJ752360	FJ752283	FJ752327	this study
pepperi 5	Abiseo, San Martin, PE	FJ752361	FJ752284	FJ752328	this study
pepperi 6	Nuevo San Martin, San Martin, PE	FJ752362	FJ752285	FJ752329	this study
pepperi 7	Chumanza, San Martin, PE	FJ752363	FJ752286	FJ752330	this study
pepperi 8	San Francisco, San Martin, PE	FJ752364	FJ752287	FJ752331	this study
pepperi 9	Campanilla, San Martin, PE	FJ752365	FJ752288	FJ752332	this study
petersi 1	Aguaytia, Ucayali, PE	N/A	FJ752300	FJ752341	this study
petersi 2	Aguaytia, Ucayali, PE	FJ752372	FJ752301	FJ752342	this study
petersi 3	Divisoria, Ucayali, PE	FJ752373	FJ752302	FJ752343	this study
petersi 4	Divisoria, Ucayali, PE	FJ752374	FJ752303	FJ752344	this study
petersi 5	Panguana, Huánuco, PE	DQ502116	DQ502116	N/A	Grant *et al.* 2006
petersi 6	Tournavista, Huánuco, PE	FJ752375	FJ752304	FJ752345	this study
petersi 7	Cordillera El Sira, Huánuco, PE	FJ752378	FJ752305	FJ752346	this study
petersi 8	Codo del Pozuzo, Huánuco, PE	FJ752379	FJ752306	FJ752347	this study
petersi 9	Cordillera El Sira, Huánuco, PE	FJ752380	FJ752307	FJ752348	this study
petersi 10	Panguana, Huánuco, PE	DQ502114	DQ502114	N/A	Grant *et al.* 2006
picta	Kartabo Pt., Mazaruni-Potaro, GY	DQ502252	DQ502252	N/A	Grant *et al.* 2006
pongoensis 1	Shucushuyacu, Loreto, PE	FJ752381	FJ752308	FJ752349	this study
pongoensis 2	Shucushuyacu, Loreto, PE	FJ752382	FJ752309	FJ752350	this study
pongoensis 3	Huallaga Canyon, San Martín, PE	DQ523005	DQ523076	DQ523146	Roberts *et al.* 2006
pongoensis 4	Convento, San Martín, PE	DQ522973	DQ523044	DQ523114	Roberts *et al.* 2006
pulchripectus	Serra do Navio, Amapá, BR	DQ502033	DQ502033	N/A	Grant *et al.* 2006
rubriventris 1	near Aguaytia, Ucayali, PE	N/A	EU517668	EU517674	Twomey & Brown 2008a
rubriventris 2	near Aguaytia, Ucayali, PE	N/A	EU517667	EU517673	Twomey & Brown 2008a
rubriventris 3	near Aguaytia, Ucayali, PE	N/A	AF282247	N/A	Lötters & Vences 2000
silverstonei 1	Tingo Maria, Huánuco, PE	DQ523013	DQ523084	DQ523154	Roberts *et al.* 2006
silverstonei 2	Captive bred, no data	N/A	N/A	DQ502582	Grant *et al.* 2006
simulans 1	Quincemil, Cusco, PE	DQ523020	DQ523090	DQ523160	Roberts *et al.* 2006
simulans 2	Mazuko, Madre de Dios, PE	DQ523019	N/A	DQ523159	Roberts *et al.* 2006
smaragdina 1	Pan de Azucar, Pasco, PE	N/A	FJ752296	FJ752337	this study
smaragdina 2	Pan de Azucar, Pasco, PE	N/A	FJ752297	FJ752338	this study

smaragdina 3	Pan de Azucar, Pasco, PE	FJ752376	FJ752298	FJ752339	this study
smaragdina 4	Iscozacin, Pasco, PE	DQ522971	DQ523042	DQ533112	Roberts *et al.* 2006
smaragdina 5	Pan de Azucar, Pasco, PE	FJ752377	FJ752299	FJ752340	this study
trivittata 1	Tarapoto, San Martín, PE	DQ522950	DQ523021	DQ523091	Roberts *et al.* 2006
trivittata 2	Alto Purus, Ucayali, PE	DQ522957	DQ523028	DQ523098	Roberts *et al.* 2006
trivittata 3	French Guiana	DQ523006	DQ523077	DQ523147	Roberts *et al.* 2006
yoshina 1	Callanayacu, San Martín, PE	FJ752353	FJ752274	FJ752319	this study
yoshina 2	Callanayacu, San Martín, PE	FJ752354	FJ752275	FJ752320	this study
yoshina 3	Callanayacu, San Martín, PE	N/A	FJ752276	FJ752321	this study
yoshina 4	Contamana, Loreto, PE	FJ752355	FJ752277	FJ752322	this study
yoshina 5	Contamana, Loreto, PE	FJ752356	FJ752278	FJ752323	this study
yoshina 6	Contamana, Loreto, PE	N/A	FJ752279	N/A	this study
yungicola	Carnavi, La Paz, BO	N/A	AY263239	N/A	Vences *et al.* 2003
Allobates femoralis 1	Boca Manu , Cusco, PE	DQ523069	DQ523139	DQ522998	Roberts *et al.* 2006
A. femoralis 2	Rio Sucusari, Loreto, PE	DQ522952	DQ523023	DQ523093	Roberts *et al.* 2006
A. marchesianus	Bonilla, San Martín, PE	DQ522977	DQ523048	DQ523118	Roberts *et al.* 2006
Colostethus inguinalis	San Roque, Caldas, CO	DQ502265	DQ502265	DQ502696	Grant *et al.* 2006
C. cf. pratti	Cana, Darien, PA	DQ502173	DQ502173	N/A	Grant *et al.* 2006
C. fugax	Morona-Santiago, EC	AY364547	AY364547	N/A	Santos *et al.* 2003
C. panamensis	Cana, Darien, PA	DQ502172	DQ502172	DQ502608	Grant *et al.* 2006
Epipedobates anthonyi	Ecuador	DQ502151	DQ502151	DQ502584	Grant *et al.* 2006
E. boulengeri 1	Ecuador	N/A	N/A	DQ502447	Grant *et al.* 2006
E. boulengeri 2	Ecuador	AF128555	AF128554	AF128556	Summers & Clough 2001
E. espinosai	Santo Domingo, Pichincha, EC	DQ502158	N/A	DQ502594	Grant *et al.* 2006
E. sp. QCAZ16589	Mindo, Pichincha, EC	AY364575	N/A	N/A	Santos *et al.* 2003
E. tricolor	Moraspungo, Bolivar, EC	AY395961	N/A	N/A	Graham *et al.* 2004
Silverstoneia flotator	El Cope, Cocle, PA	DQ502164	DQ502164	DQ502599	Grant *et al.* 2006
S. nubicola	El Cope, Cocle, PA	DQ502165	DQ502165	DQ502600	Grant *et al.* 2006