

SPOTTED POISON FROGS: REDISCOVERY OF A LOST SPECIES AND A NEW GENUS (ANURA: DENDROBATIDAE) FROM NORTHWESTERN PERU

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ABSTRACT: The Santiago poison frog, *Adelphobates captivus*, a species not seen in life since 1929, was recently rediscovered on an expedition to its type locality in northwestern Peru. The colors of this species, previously unknown, consist of a black dorsum with bright red-orange spots and yellow spots ventrally. We provide amendments to the original description as well as the first accounts of tadpole morphology, vocalization, and natural history. A Bayesian phylogenetic analysis suggests *Adelphobates captivus* and a species originally described as *Dendrobates mysteriosus* are sister species that form a monophyletic clade sister to *Ranitomeya*. We propose to clarify the taxonomic status of *D. mysteriosus* incertae sedis by erecting a new genus, *Excidobates*, to include *mysteriosus* and its sister taxon *captivus*. Members of this genus are distinguished from *Ranitomeya* by 11 site substitutions in their *rrnS* and *rrnL* sequences, well-developed first fingers, and pale spots on the ventral surfaces of the thighs.

RESUMEN: La rana venenosa del Santiago, *Adelphobates captivus*, una especie no vista en vida desde 1929, fue redescubierta recientemente en una expedición a su localidad típica en el noroccidente de Perú. Los colores de esta especie, previamente desconocidos, consisten en un dorso negro con puntos rojos brillantes y puntos amarillos en el vientre. Presentamos rectificaciones a la descripción original, así como los primeros datos sobre morfología de los renacuajos, vocalización e historia natural. Un análisis filogenético Bayesiano sugiere *A. captivus* y una especie originalmente descrito como *Dendrobates mysteriosus* son especies hermanas que forman un clado monofilético, hermano a *Ranitomeya*. Proponemos aclarar el estado taxonómico de *D. mysteriosus* incertae sedis, erigiendo un nuevo género, *Excidobates*, para incluir *mysteriosus* y su taxón hermano *captivus*. Los miembros de este género se distinguen de *Ranitomeya* por 11 substituciones de lugar en sus secuencias de *rrnS* y *rrnL*, por un primer dedo bien desarrollado y puntos pálidos en las superficies ventrales de los muslos.

Key words: Dendrobatidae; Dendrobatoidea; *Excidobates captivus* comb. nov.; *Excidobates* gen. nov.; *E. mysteriosus* comb. nov.; Peru; Poison frogs; Systematics; Taxonomy

DESPITE widespread amphibian declines and extinctions over the past 20 yr (Stuart et al., 2004), the number of known species of poison frogs (Aromobatidae, Dendrobatidae) has more than doubled during this time, bringing the current total up to 247 (Grant et al., 2006). Extensive field work (e.g., Myers and Daly, 1976) and numerous molecular studies (e.g., Noonan and Wray, 2006; Grant et al., 2006, and additional studies reviewed therein) have greatly improved understanding of dendrobatid biodiversity, particularly within the subfamily Dendrobatinae, half of whose 48 recognized species have been described since 1975.

Nonetheless, one species, originally described as *Dendrobates captivus* Myers (1982) (recently placed in the genus *Adelphobates* by Grant et al., 2006), has remarkably

eluded observation for 77 yr; it was last seen in 1929 by geologist and original collector Harvey Bassler. Bassler's collections were reviewed by Silverstone (1975), who designated three Peruvian specimens from the junction of the Río Santiago and Río Marañón as a transitional form of *Dendrobates quinquevittatus* sensu Silverstone. Myers (1982) concluded that these three specimens represented a distinct species possessing the following unusual characteristics: a dorsal pattern consisting of dots and dashes, no reticulation on the hind limbs, small hands with short fingers, and diminutive size (<16 mm snout-vent length [SVL]). No information was available as to the life colors of the species, natural history, call, or tadpole morphology.

The type locality described by Bassler is an isolated and poorly studied area. The confluence of the Río Santiago and Río Marañón lies within a wide, torrential valley sharply defined

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by the Cordillera del Condor to the west and the Cerros de Campanquis to the east, the latter rising abruptly to a height of 1200 m above sea level and separating the Santiago valley from the Amazon basin. From the east, this valley is accessible by river via the Pongo de Manseriche, a gorge that constricts the 750-m-wide Marañón to a 120-m-wide rapid. Furthermore, the Santiago valley is inhabited by Huambisa and Aguaruna natives, who, until recently, engaged in regular intertribal combat. Indeed the epithet *captivus* stems from Bassler's observation of an Aguaruna war-party returning from battle with two Huambisas held captive in the bottom of their canoes. The valley's inaccessible and dangerous nature has prohibited exploration; ours is the first known herpetological survey of this region.

Here we provide the first complete description of *Adelphobates captivus*, a species previously not known in life. We for the first time provide accounts of reproductive ecology, vocalization, tadpole morphology, distribution, a revised taxonomy, and discuss its conservation status, including tests for the epizootic amphibian parasite *Batrachochytrium dendrobatidis*.

MATERIALS AND METHODS

Specimens were observed and collected during an expedition to the Río Santiago, Departamento Amazonas, Peru, 25–29 June 2006. The primary field site was located near the Aguaruna village Tsamajain, on the south side of the Río Marañón directly across from the mouth of the Santiago. Other surveys were made on the north side of the Marañón along both east and west banks of the Santiago, and in the Cerros de Campanquis. The forest in this region is undisturbed and very wet, as evidenced by abundant moss and epiphytes, and frequent rains experienced during the expedition.

We observed a total of 16 adult and 1 juvenile *Adelphobates captivus* during the survey. Four adults were preserved in 70% ethanol for permanent storage. We later identified these specimens as *A. captivus* based on the distinct patterning on the dorsal and ventral surfaces, and by a series of measurements corresponding to the original description (Myers, 1982). Measurements were made with mechanical calipers and a

micrometer to the nearest 0.01 mm, following Myers (1982) and Brown et al., (2006): snout-vent length (SVL), femur length from vent to lateral surface of knee (FL), tibia length from heel to lateral surface 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 tympanum (HW), body width under axillae (BW), upper eyelid width (UEW), interorbital distance (IOD), 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 (LIF), 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). All voucher specimens were deposited in the Museo de Historia Natural San Marcos, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM).

Two calling males were recorded with a Sony DCR-JC42 camera and Sony ECM-HGZ1 gun microphone. Calls were analyzed in Raven 1.2 (Charif et al., 2004) and compared to vocalizations of a Peruvian species originally described as *Dendrobates mysteriosus* Myers, 1982 (currently *incertae sedis*, Grant et al., 2006), which we consider the sister taxon to *A. captivus* (see below).

Four tadpoles were collected from the backs of male parent frogs, preserved in 70% ethanol for description, and staged according to Gosner (1960). Mouthpart formulas follow McDiarmid and Altig (1999). Photographs of the preserved tadpoles were taken with Nikon D50 and a Nikon Coolpix 2100 attached to a microscope.

The distributions of *Adelphobates captivus* and its putative sister taxon *Dendrobates mysteriosus* were estimated using the niche-modeling program Maxent 3.0 Beta (Phillips et al., 2004). The niche model for *E. captivus* was built using six presence points; the *E. mysteriosus* model used 13 presence points. Occurrence data were obtained through the

2006 survey in the Santiago valley and a 2007 survey in the Cordillera del Condor. BIOCLIM climatic data were downloaded from the WORLDCLIM website (<http://www.worldclim.org/>) for current climatic conditions (ca. 1950–2000). Maxent was run with the following parameters: random test percentage = 50%, regularization multiplier = 1.0, maximum iterations = 500, convergence threshold = 0.00001, number of background points = 10,000. Environmental variables were jackknifed to determine relative heuristic contribution to the model.

DNA Extraction, Amplification, and Sequencing

Tissue samples, collected by clipping tail tips from tadpoles, were preserved in a buffer solution of 20% DMSO saturated with sodium chloride and EDTA. Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Tissue Kit. The cytochrome-b (*cob*), cytochrome oxidase 1 (*cox1*), recombinant-activating gene 1 (*RAG-1*), *rrnS*, and *rrnL* gene regions (nomenclature following Mueller, 2006) were amplified using the following primer sets for a total of 2124 base pairs: *cob*: CB1-L, CB2-H (Kocher et al., 1989); KSCYB1(A)-L, KSCYB1-H (Clough and Summers, 2000); *rrnS*: 12SA-L (Palumbi et al., 1991), 12SK-H (Goebel et al., 1999); *rrnL*: LGL 286, LGL 381 (Palumbi et al., 1991). We amplified 493 base pairs of the *RAG-1* gene using specially designed primers DendroR-AG1F (TACCGGAGAAGGCTGTCCGCTTCTC) and DendroRAG1R (GGGTTTCAA-TAAATGGCTTTG-CAG). All loci used are from the mitochondrial genome with the exception of *RAG-1* which is from the nuclear genome. PCR amplifications and DNA sequencing protocols followed Roberts et al., (2006b).

Samples were sequenced in both directions and aligned using Autoassembler version 1.4.0 (Applied Biosystems Inc., 1995). Consensus sequences were transferred to Gene Jockey (Taylor, 1990) for alignment with a sequence of the same region from a different individual. Sequences from *rrnS* and *rrnL* were aligned using the Probabilistic Alignment Kit (PRANK; Löytynoja and Goldman, 2005; <http://www.ebi.ac.uk/goldman/prank>). Because

PRANK keeps track of gaps introduced into a multiple sequence alignment rather than automatically penalizing them, it is expected to more accurately reflect the history of indel events than other methods (Higgins et al., 2005; Löytynoja and Goldman, 2005). Protein coding genes (*cob*, *RAG-1* and *cox1*) were aligned using Clustal X. All sequences were translated to confirm proper reading frame and absence of stop codons.

Phylogenetic Analysis

With the exception of *RAG-1* sequences, and all sequence data for *Adelphobates captivus*, sequences used in this analysis were derived from previous studies (Clough and Summers, 2000; Darst and Cannatella, 2004; Graham et al., 2004; Grant et al., 2006; Noonan and Wray, 2006; Roberts et al., 2006a; Santos et al., 2003; Summers et al., 1999; Symula et al., 2001; Symula et al., 2003). Representative species of the genera *Allobates*, *Ameerega*, and *Phyllobates* were used as outgroups for a phylogenetic analysis using Bayesian inference (Huelsenbeck and Ronquist, 2001). The dataset was partitioned into codon-position specific sets of nucleotides (1st, 2nd, and 3rd positions for *cob*, *cox1*, and *RAG-1*, with a separate, single partition each for *rrnS* and *rrnL*). 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. Sequence data may better be explained by partitioning a dataset than by applying an average model across genes and codon positions, as indicated by higher model likelihood scores in partitioned analyses (Nylander et al., 2004). MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was run for eight million generations using the following mixed models indicated by MrModeltest 2.2: *rrnS* (nst = 6, I + Γ), *rrnL* (nst = 6, I + Γ), *cob-1st* (nst = 1, propinv), *cob-2nd* (nst = 6, I + Γ), *cob-3rd* (nst = 6, I + Γ), *cox1-1st* (nst = 2, Γ), *cox1-2nd* (nst = 2, Γ), *cox1-3rd* (nst = 6, I + Γ), *RAG-1-1st* (nst = 1, Γ), *RAG-1-2nd* (nst = 1, equal), *RAG-1-3rd* (nst = 6, I + Γ). Negative log-likelihood ($-\ln$) scores were used to identify the burn-in phase at 200,000 gener-

TABLE 1.—Hypotheses tested in a Bayes Factor Analysis. A $2\ln(B10)$ greater than 10 was used as a cutoff for rejecting the null hypothesis (constrained trees), a negative $2\ln(B10)$ was used as support for the null, and $2\ln(B10)$ of 0–10 was interpreted as support for both hypotheses. In all cases H_A = molecular tree depicted in Fig. 6. **Adelphobates* containing *A. quinquevittatus*, *A. sp* Matto Grosso, *A. galactonotus*, *A. castaneoticus*, and *M. steyermarki*.

Hypothesis (H_0)	LnL: unconstrained	LnL: constrained	LnL: difference	Bayes factor [$2\ln(B10)$]
H ₁ : <i>Adelphobates</i> * monophyletic	–16,597.61	–16,598.18	0.57	1.13
H ₂ : <i>Oophaga</i> monophyletic	–16,597.61	–16,597.80	0.19	0.37
H ₃ : <i>Dendrobates</i> monophyletic	–16,597.61	–16,598.49	0.88	1.76
H ₄ : <i>Ranitomeya</i> monophyletic	–16,597.61	–16,598.82	1.20	2.41
H ₅ : <i>Excidobates captivus</i> within <i>Adelphobates</i> *	–16,597.61	–16,633.64	36.03	72.05
H ₆ : <i>Excidobates captivus</i> with <i>E. mysteriosus</i>	–16,597.61	–16,597.97	0.35	0.71
H ₇ : <i>Excidobates</i> in <i>Oophaga</i> , <i>Dendrobates</i> , <i>Adelphobates</i> *	–16,597.61	–16,598.06	0.45	0.90
H ₈ : <i>Excidobates</i> within <i>Ranitomeya</i>	–16,597.61	–16,597.89	0.28	0.56

ations, after which all trees ($n = 159,600$) were summed. To ensure burn-in phase was properly defined, Bayesian parameter files were also analyzed in TRACER 1.3 (Rambaut and Drummond, 2003). The Bayesian analysis standard deviation of split frequencies reached <0.01 to ensure consistency of the resulting tree topology.

Genetic distances were calculated using the Kimura two-parameter model using MEGA 3.2 (Kumar et al., 2004) between dendrobatine genera (excluding *Phylllobates*). Character state reconstruction methods used to diagnose lineages was performed in PAUP* using ACCTRAN optimization (Bond, 2004; Swoford, 2002). All unambiguous characters were mapped for the node of interest in MacClade (Maddison and Maddison, 2001).

To test the monophyly of genera proposed by Grant et al., (2006) and the new genus described herein (*Excidobates*), we used Bayes factor analyses (BFA). This analysis has been used as an explicit test of monophyly in a Bayesian framework (e.g., Brandley et al., 2005). Bayes factor analyses were done in MrBayes by generating constraint trees of each test topology. The constraint trees each consisted of a tree containing two hypothetical clades, one of which is the test group (e.g., *Oophaga*), the other consisting of all other taxa included in the analysis. Post burn-in harmonic mean $-\ln$ likelihood was calculated for each hypothesis and the differences between the unconstrained topology (H_A) and constrained topology (H_0) were multiplied by two. Values above 10 were interpreted as strong evidence against H_0 . Values between 0 and 10 were interpreted as support

for both hypotheses, and values less than 0 were interpreted as support against H_A (Brandley et al., 2005; Kass and Raftery, 1995; Suchard et al., 2005). We tested 8 hypotheses (Table 1), testing the monophyly of: H₁, *Adelphobates* (containing *Minyobates steyermarki*); H₂, *Oophaga*; H₃, *Dendrobates*; H₄, *Ranitomeya*, H₅; *Excidobates captivus* with *Adelphobates* (as defined above); H₆, *Excidobates captivus* with *E. mysteriosus*; H₇, *Excidobates* within *Oophaga*, *Dendrobates*, *Adelphobates* (as defined above); H₈, *Excidobates* within *Ranitomeya*.

Chytrid Analysis

Eight adults and four tadpoles were screened for signs of *Batrachochytrium dendrobatidis* infection (Bd). Tadpole mouthparts were examined for signs of infection following Obendorf et al., (2005). Eight adults were swabbed in the field with sterile cotton swabs (stored at room temperature for three months until DNA extraction. DNA was extracted as described above). Presence of Bd was tested using PCR protocol and two pairs of primers described by Boyle et al., (2004) and Annis et al., (2004). Negative results were rerun two additional times, doubling the amount of template DNA each time to minimize the chance of false negatives. Results were compared to a known positive control for Bd.

SYSTEMATICS

Excidobates, **gen. nov.**

Type species.—*Dendrobates mysteriosus* Myers, 1982.

Etymology.—From the Latin *Excido* (fallen out of or excised from memory) + *bates* (a walker), in reference to both members of the genus having shared a similarly enigmatic past, being originally collected by Bassler in the late 1920's and described by Myers in 1982. *Excidobates mysteriosus* was later rediscovered (Schulte, 1990) 60 yr after its original collection, and our rediscovery of *E. captivus* took place 77 yr after its initial collection. Gender masculine. The second half of the name is common among poison frogs; furthermore, the two known species from this genus are hesitant to jump and tend to "walk" instead.

Definition and diagnosis.—Size small to medium, 15–17 mm in *E. captivus*, 27–29 mm SVL in *E. mysteriosus*. Dorsal coloration dark brown or black, dorsal spots present. Complete dorsolateral stripes absent. Pale spots present under the chin, on the ventral surface of the thighs, at dorsal insertions of limbs, and, in most individuals, above the eyelid. Dorsal skin texture granular or smooth. Head narrower than body, teeth absent, vocal slits present in males. Tongue ovoid, white in preservative, 3–4 mm long in adult *E. mysteriosus*, 2–2.5 mm long in *E. captivus*. General habitus of *E. captivus* resembling *Ranitomeya*, *E. mysteriosus* resembling *Dendrobates* and large *Oophaga*. Appressed first finger shorter than second but well developed, relative lengths of fingers $3 > 4 > 2 > 1$. Finger discs moderately expanded, webbing absent from hands and feet. Appressed relative toe lengths $4 > 3 > 5 > 2 > 1$, first toe well defined. Tadpoles with labial tooth row formula 2(2)/3(1) (formula following McDiarmid and Altig, 1999), keratinized jaw sheaths well developed, medial indentation in posterior jaw sheath present or absent, vent dextral, spiracle sinistral. Clutch size 8–13 eggs in *E. mysteriosus* (Lötters et al., 2007), unknown in *E. captivus*. Adult males have a buzz-call, in *E. captivus* the call sounds like a short "shriek", *E. mysteriosus* has a longer rattle-like call. Terrestrial or scansorial; diurnal.

Eleven molecular synapomorphies within *rrnS* and *rrnL* gene regions (Fig. 1) diagnose this genus from other dendrobatine genera. No unambiguous molecular synapomorphies were present in the other gene regions. The

lack of pale reticulation on the limbs in members of *Excidobates* and presence of a well-developed first finger distinguish this genus from Amazonian *Ranitomeya*. Both known *Excidobates* possess large spots on the ventral surfaces of the thighs, a character noted as a potential synapomorphy uniting these taxa (Myers, 1982). No known members of *Ranitomeya* possess well-defined ventral thigh spots.

Distribution.—Known only from the upper Río Marañón drainage in northwestern Peru (Departamento Amazonas) between 200 m and 1500 m above sea level.

Species included.—*Dendrobates mysteriosus* Myers, 1982, *Dendrobates captivus* Myers, 1982.

Remarks.—The morphological contrast between *Excidobates* and the *Ranitomeya* may eventually be strengthened pending further phylogenetic investigation of the *Ranitomeya minuta* group. Members of the *R. minuta* group (*Ranitomeya claudiae*, *R. fulgurita*, *R. minuta*, and putatively *R. abdita*, *R. alto-bueyensis*, *R. bombetes*, *R. daleswansonii*, *R. dorisswansonae*, *R. opisthomelas*, *R. tolimense*, *R. viridis*, and *R. virolinensis*) are currently contained within *Ranitomeya* but lack pale limb reticulation. This group occupies a trans-Andean distribution extending from northern Ecuador to Panama, whereas other members of *Ranitomeya* with pale limb reticulation have an Amazonian distribution.

Excidobates captivus Myers,
1982, **comb. nov.**

1975 *Dendrobates quinquevittatus*, non Steindachner, 1864; partim: Silverstone, p. 35., considered to be transitional between striped and spotted variants of *D. quinquevittatus* sensu Silverstone.

1982 *Dendrobates captivus* Myers. Holotype: American Museum of Natural History (AMNH) 42963, collected August 1929 by Harvey Bassler from the "mouth of the Río Santiago, 580 feet (177 m) elevation, Department of Amazonas, Peru."

2006 *Adelphobates captivus* Grant et al., assigned to genus on basis of dorsal pattern.

New specimens.—MUSM 24931–24934 collected 26–28 June 2006, south bank of the Río Marañón at the mouth of the Río

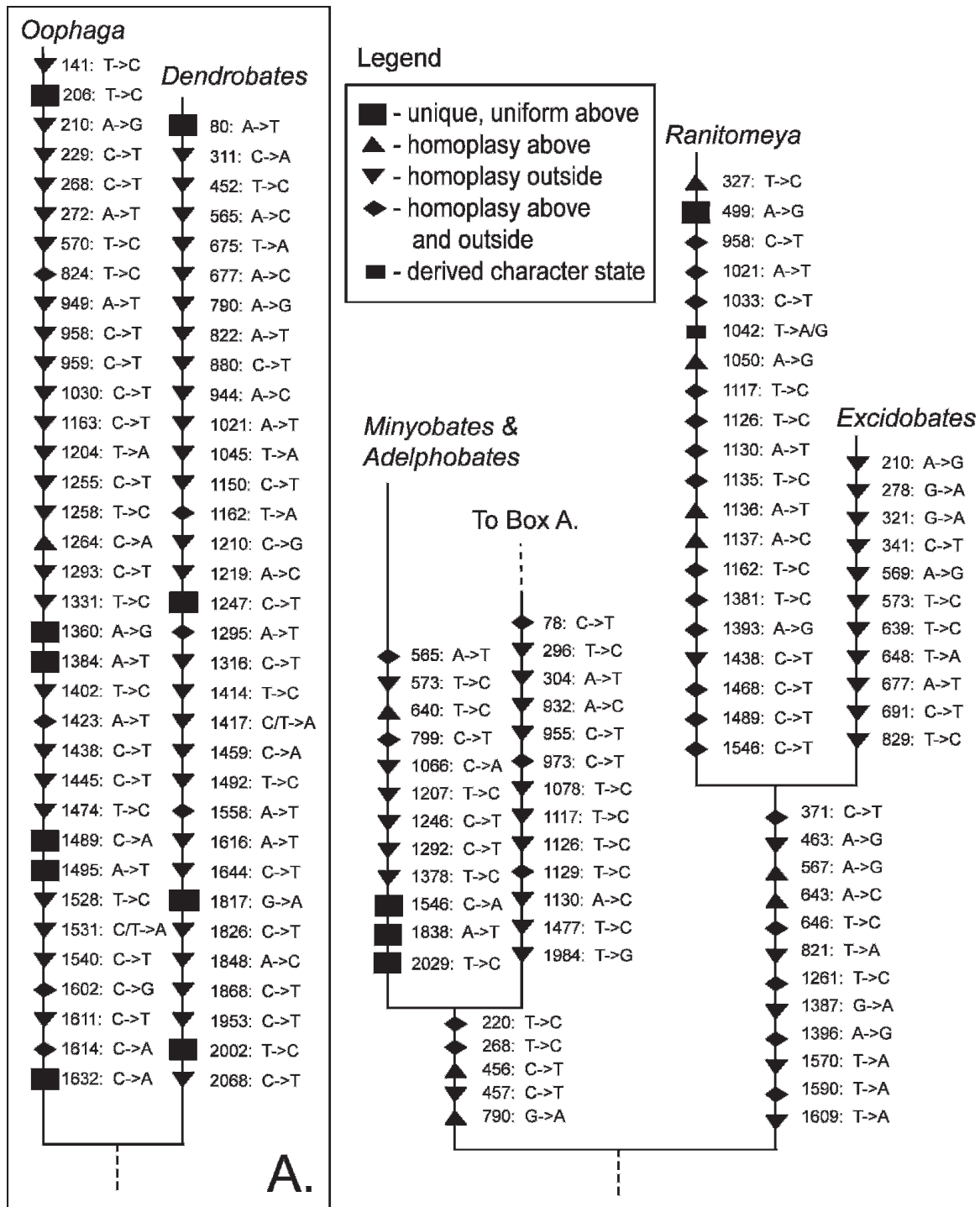


FIG. 1.—Character transformation for *Excidobates* gen. nov. diagnostic nucleotide site substitutions using ACCTRAN optimization. Alignment available by request.

Santiago, 213 m above sea level, Departamento Amazonas, Peru (4° 26' 48 S, 77° 38' 37 W).

Definition and diagnosis.—Small species with mean SVL 15.04 mm ($n = 12$), range

12.5–16.8 mm. Dorsum black with markings varying in color from orange to deep red.

These markings are a series of asymmetrical spots and elongated dashes aligned dorsolat-



FIG. 2.—*Excidobates captivus* from type locality, mouth of the Río Santiago, Departamento Amazonas, Peru. Bottom showing variation in *E. captivus* among eight uncollected adults photographed in the field with corresponding ventral photos.

erally (Fig. 2). Bright yellow spots are present above upper arms and thighs, on the sides anterior to the hind limbs, and on the head inferior to the eyes. Limbs dark brown, reticulation absent. One specimen with faint yellow flecks on lower legs and shanks. Ventral ground color is black with scattered pale-yellow spots of variable shapes. Chin spot and elongated thigh spots present, most specimens have a yellow bar across the chest and several

large spots on the abdomen. Vocal slits present in males, teeth absent. Larvae grey with a round body, jaw sheaths well-developed.

Distinguishing features given by Myers (1982) can be expanded here. Presence of well-defined red-orange spots and dashes on a black dorsum distinguishes *Excidobates captivus* from all other dendrobatines except *Adelphobates castaneoticus*. The latter pos-

TABLE 2.—Measurements of new *Excidobates captivus* specimens, character definitions are given in the methods.

Character	MUSM 24931	MUSM 24932	MUSM 24933	MUSM 24934	Average \pm 1 SD
SVL	15.02	16.48	12.62	12.52	14.16 \pm 1.93
FL	6.49	6.22	5.50	5.02	5.81 \pm 0.67
TL	6.91	6.88	6.15	6.32	6.57 \pm 0.39
KK	13.20	13.79	11.68	11.50	12.54 \pm 1.13
FoL	6.12	6.01	5.00	5.12	5.56 \pm 0.58
HaL	3.12	3.18	2.95	2.98	3.06 \pm 0.11
HL	5.52	5.58	4.90	4.92	5.23 \pm 0.37
HW	5.00	5.08	4.22	4.41	4.68 \pm 0.43
BW	4.48	4.49	3.98	4.10	4.26 \pm 0.26
UEW	1.59	1.89	1.80	1.71	1.75 \pm 0.13
IOD	1.87	1.95	1.83	1.74	1.85 \pm 0.09
TD	0.67	1.10	0.70	0.55	0.76 \pm 0.24
ED	1.71	1.59	1.53	1.50	1.58 \pm 0.09
DET	0.37	0.37	0.31	0.37	0.35 \pm 0.03
L1F	2.13	2.39	1.89	2.05	2.12 \pm 0.21
L2F	2.52	2.85	2.02	2.33	2.43 \pm 0.35
W3D	0.69	0.70	0.45	0.52	0.59 \pm 0.12
W3F	0.38	0.39	0.32	0.37	0.37 \pm 0.03
SEX	♂	♀	♀	♀	

sesses similar patterning but has white (versus orange to deep red) dorsal spots, as well as distinct orange spots on the dorsal surface of the shanks. The only other poison frog of similar size with which *E. captivus* lives sympatrically is *Ranitomeya variabilis*, a blue-green frog with black spots on the dorsum, venter, and limbs.

Description.—Of the four undissected individuals, one was diagnosed as male by the presence of vocal slits, the other three lacked such vocal slits and were designated females. Measurements of new specimens are given in Table 2. The original description was based on preserved specimens; we include here new measurements of additional specimens and characteristics of live animals. Size very small, 12.5–16.8 mm SVL. Hands small, 2.95–3.18 mm long, smaller than similarly-sized *Ranitomeya* (e.g., *R. uakarii*, mean SVL 15.4, mean hand length 3.80 mm).

Skin smooth on the sides and venter, finely granular on dorsum and dorsal surfaces of legs. Dorsum black with distinct red-orange dorsolateral dots and dashes, starting as a medial spot on anterior loreal region with a spot anterior to each eye and above each eyelid. A spot or dash is present immediately posterior to each eye, though fused in some individuals with the eyelid spot. Row of dots and dashes extending posteriorly at least to level of the ilium. Yellow spots are present

inferior to eye, and dorsally above upper arms and thighs at their point of insertion. Venter black with pale yellow spots of varying shape and number. All specimens have one large yellow chin spot, yellow spots between the axillae usually forming a bar, and yellow spots on the ventral surface of the thigh. Yellow abdominal spotting is also present but highly variable. Limbs dark brown proximally, lighter distally.

Vocalization.—The advertisement call of *E. captivus* is a series of short shrieks composed of 13–15 rapid notes (Fig. 3). The call is bitonal, with a mean dominant frequency of 5540 Hz, and another peak at 4107 Hz, though this lower peak can vary by up to 440 Hz between consecutive calls. Each shriek is short (0.23 s) and repeated about once per second for several minutes. The call does not carry far, being audible at ≤ 10 m, though the secretive calling habits of the males may muffle the call. Males were heard calling throughout the day from concealed positions near the ground or hidden in phytotelmata such as *Heliconia* bracts or Marantaceae. This call can be classified as a buzz call following Lötters et al., (2003), consisting of short pulses with little frequency modulation. The call of *Excidobates mysteriosus* (Fig. 3) can also be classified as a buzz call, consisting of rattle-like buzzes 1 s in duration repeated 8–10 times per minute

TABLE 3.—Species names, collection localities, and GenBank accession numbers (where available) for taxa included in the analysis.

Species	Location	<i>rns</i>	<i>rnl</i>	<i>cox1</i>	<i>cob</i>	<i>RAG-1</i>
<i>Adelphobates castaneoticus</i> 1	E. Brazil	AF482774	AF482789	AF482818	AF482804	EU325903
<i>A. castaneoticus</i> 2	E. Brazil	AF482775	AF482790	AF482819	AF482805	N/A
<i>A. galactonotus</i>	E. Brazil	DQ371300	DQ371311	N/A	DQ371330	EU325906
<i>A. quinquevittatus</i>	E. Brazil	AF482773	AY263253	N/A	AF482803	EU325912
<i>A. sp. Matto Grosso</i>	Matto Grosso, BR	DQ371309	DQ371320	N/A	DQ371339	N/A
<i>Allobates talamancae</i>	Costa Rica	AF128587	AF128586	AF097496	AF128588	N/A
<i>Ameerega trivittata</i>	Peru	AF128570	AF128569	AF128571	N/A	N/A
<i>Excidobates captivus</i> 1	Rio Santiago, PE	EU325898	EU325900	N/A	EU325902	EU325914
<i>E. captivus</i> 2	Rio Santiago, PE	EU325899	EU325901	N/A	N/A	N/A
<i>E. mysteriosus</i>	N. Peru	DQ371303	DQ371314	DQ371323	DQ371333	EU325908
<i>Dendrobates auratus</i>	Panama	AF128602	AF098745	AF097501	AF128603	EU325909
<i>D. leucomelas</i>	Venezuela	AF128593	AF124119	AF097499	AF128594	EU325923
<i>D. tinctorius</i>	French Guiana	AF128605	AF128604	N/A	AF128606	N/A
<i>Minyobates steyermarki</i>	Venezuela	DQ371310	DQ371321	DQ371329	DQ371340	EU325904
<i>Oophaga arborea</i>	Panama	AF128611	AF128610	AF097504	AF128612	N/A
<i>O. granulifera</i>	Costa Rica	AF128608	AF098749	AF097505	AF128609	N/A
<i>O. histrionica</i> 1	Ecuador	AF128617	AF128616	AF097498	U70154	EU325910
<i>O. histrionica</i> 2	Ecuador	AF124098	AF124117	N/A	AF173766	EU325915
<i>O. pumilio</i>	Bocas del Toro, PA	AF128614	AF128613	AF097500	U70147	EU325918
<i>O. speciosa</i>	Panama	AF128596	AF098747	AF097503	AF128597	EU325916
<i>O. sylvatica</i>	Ecuador	AY364569	AY364569	N/A	AF324041	N/A
<i>Phyllobates bicolor</i>	Choco, CO	AF128578	AF128577	N/A	AF128579	EU325920
<i>Ranitomeya amazonica</i>	Iquitos, Loreto, PE	AF482770	AF482785	AF482815	AF482800	N/A
<i>R. biolat</i>	S. Peru	AF482779	AF482794	AF482823	AF482809	EU325913
<i>R. claudiae</i>	Panama	DQ371304	DQ371315	DQ371324	DQ371334	EU325921
<i>R. duellmani</i>	Napo, EC	AY364566	AY263246	N/A	N/A	N/A
<i>R. fantastica</i> 1	Sauce, San Martin, PE	AF412444	AF412472	AF412416	AF412500	EU325919
<i>R. fantastica</i> 2	Cainarachi, San Martin, PE	AF412447	AF412475	AF412419	AF412503	N/A
<i>R. flavovittata</i>	Tahuayo, Loreto, PE	DQ371306	DQ371317	DQ371326	DQ371336	EU325917
<i>R. imitator</i> 1	Huallaga, San Martin, PE	AF412448	AF412476	AF412420	AF412504	EU325911
<i>R. imitator</i> 2	Pongo, San Martin, PE	AF412459	AF412487	AF412431	AF412515	N/A
<i>R. lamasi</i>	Tingo Maria, Huanuco, PE	AF482778	AF482793	AF482822	AF482808	N/A
<i>R. minuta</i>	Panama	AF128590	AF128589	AF128591	MMU70163	N/A
<i>R. reticulata</i> 1	Puente Itaya, Loreto, PE	AF482772	AF482787	AF482817	AF482802	N/A
<i>R. reticulata</i> 2	B. Achille, Loreto, PE	AF482771	AF482786	AF482816	AF482801	N/A
<i>R. uakarii</i>	Rio Tahuayo, Loreto, PE	DQ371305	DQ371316	DQ371325	DQ371335	N/A
<i>R. vanzolinii</i>	Peru	AF128599	AF128598	N/A	AF128600	N/A
<i>R. variabilis</i>	Cainarachi, San Martin, PE	AF412463	AF412491	AF412435	AF412519	EU325922
<i>R. ventrimaculata</i> B1	Solimoes, Amazonas, BR	DQ371307	DQ371318	DQ371327	DQ371337	N/A
<i>R. ventrimaculata</i> B2	Porto Walter, Acre, BR	DQ371301	DQ371312	DQ371322	DQ371331	N/A
<i>R. ventrimaculata</i> E1	Ecuador	AF482780	AF482795	AF482824	AF482810	EU325905
<i>R. ventrimaculata</i> E2	Ecuador	AF128620	AF128619	AF097502	AF120013	N/A
<i>R. ventrimaculata</i> FG	French Guiana	DQ371302	DQ371313	N/A	DQ371332	N/A
<i>R. ventrimaculata</i> P1	Bonilla, San Martin, PE	AF412466	AF412494	AF412438	AF412522	N/A
<i>R. ventrimaculata</i> P2	Near Rio Napo, Loreto, PE	AF482781	AF482796	AF482825	AF482811	N/A
<i>R. ventrimaculata</i> sp. aff.	Solimoes, Amazonas, BR	DQ371308	DQ371319	DQ371328	DQ371338	N/A

(recording at 24 C), with a dominant frequency of 3550 Hz.

Tadpole.—A stage 25 tadpole collected from the back of a male was chosen for the description (Fig. 4). Total length 12.56 mm, body length 4.78, maximum width 3.18, depth 2.04. Snout broadly rounded when viewed from above. Body almost perfectly round in dorsal view. Nares forming small tube, spaced

0.66 apart, 0.62 from eyes, directed anterolaterally. Eyes dorsal, black, 1.47 from snout. Spiracle sinistral, not forming free tube, vent dextral. Dorsal and ventral tail fins begin at tail base, deepest depth 1.63 measured 2/3 towards distal end. Caudal musculature deepest at tail base, musculature depth 0.81.

The mouth (Fig. 5) is directed anteroventrally. Oral disc not emarginated, 1.88 wide,

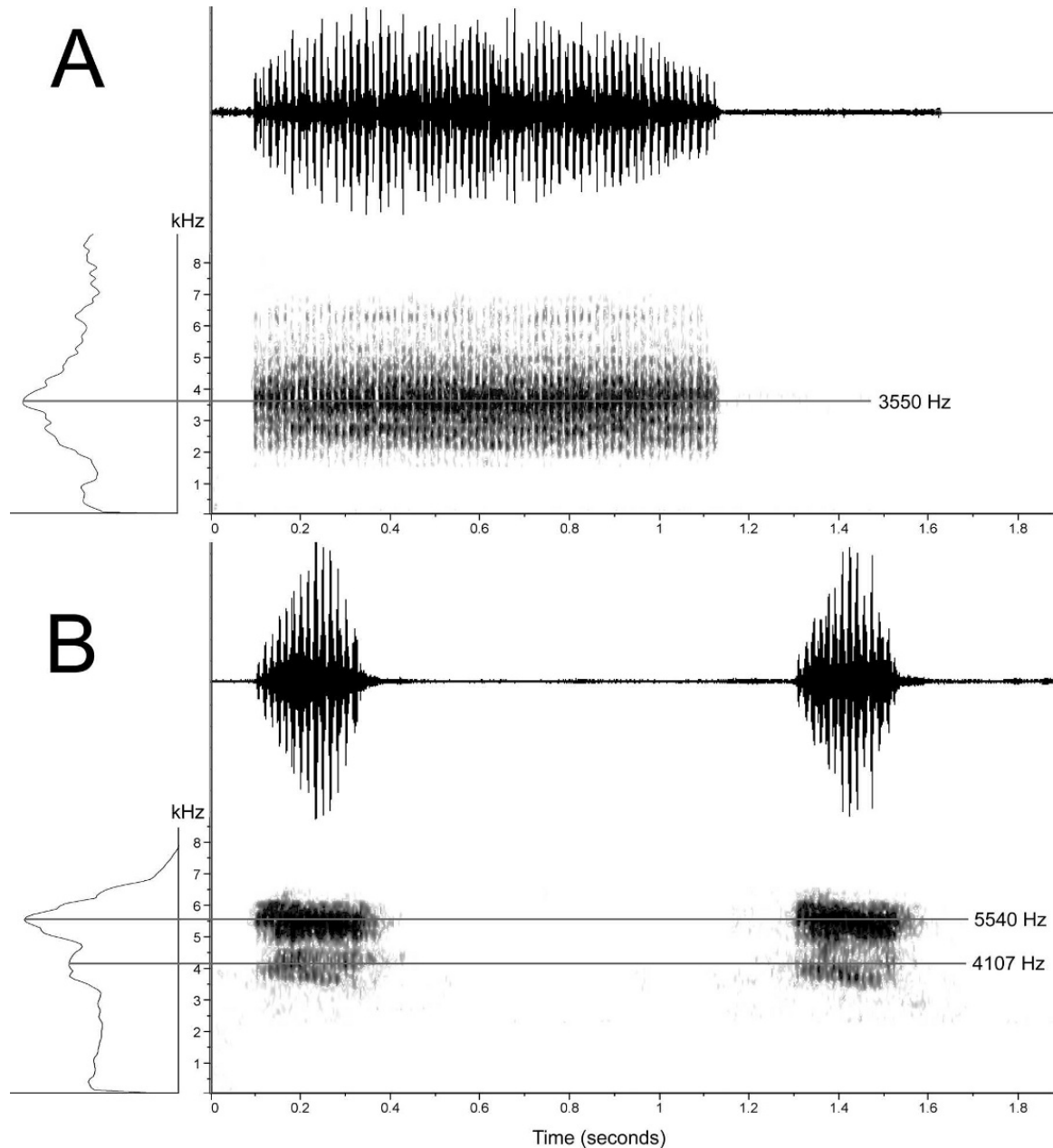


FIG. 3.—Advertisement calls of *Excidobates*, spectrograms for calls with matching time (top) and frequency (left) domain waveforms. (A) *Excidobates mysteriosus*, field recording of male from type locality, 24 C, 27 July 2007. Dominant frequency 3550 Hz. (B) *E. captivus*, field recording of male from type locality, 26 C, 26 June 2006. Dominant frequency 5540 Hz with smaller peak at 4107 Hz. Recordings available online at <http://www.dendrobates.org/calls/Excidobates.zip/>.

anterior and posterior labia forming small flaps free from body wall. Irregular marginal papillae present around posterior labium, double rows in posterior margin. Anterior labium lacking marginal papillae medially, present laterally but not well developed. Jaw sheaths heavily serrated, 0.88 in width, depth is 28% of width, lower jaw sheath slightly

deeper than upper with very slight medial indentation. Lateral processes short, non-serrate. Labial tooth row formula is 2(2)/3(1). A-1 complete, A-2 with medial gap and roughly same length as A-2. P-1 with medial gap. P-2 and P-1 same length, P-3 shorter with smaller labial teeth. Anterior tooth rows 1.48 in width, posterior rows 1.29.

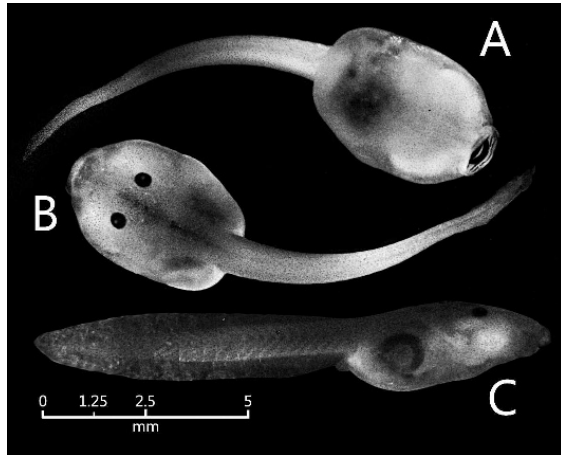


FIG. 4.—Tadpole of *Excidobates captivus*, stage 25, with ventral (A), dorsal (B), and lateral (C) views.

In preservative, the head is translucent white with mouth parts visible from above. Abdomen is mostly transparent and appears light grey except for the intestinal coils which are black. Tail musculature uniform grey, upper and lower fins transparent grey. Life colors of tadpoles are similar but less opaque than preserved specimens.

Phylogenetics.—The final dataset included 2124 base pairs (196 from *cob*, 345 from *rrnS*, 540 from *rrnL*, 550 from *cox1*, and 493 from *RAG-1*), of which 500 were parsimony informative. The topology that resulted from the Bayesian phylogenetic analysis is shown in Fig. 6. Of the 159,600 post burn-in trees, 58,626 (36.7%) match the 80% majority-rule topology (depicted in figure by thick branches, where absent the node was collapsed), of those only 220 exactly matched the consensus topology. Our topology has *Excidobates captivus* as sister to *E. mysteriosus*, forming a clade sister to *Ranitomeya*.

Distribution and Natural History

Excidobates captivus occurs in northwestern Peru, where it is known only from the valley formed between the Cordillera del Condor and the Cerros de Campanquis (Fig. 7). The original description states that Bassler made camps on the west bank of the Santiago near the mouth, but the precise locality where the type series was collected is not specified. It seems probable that the original specimens were collected on the north side of the Marañón, as the south bank

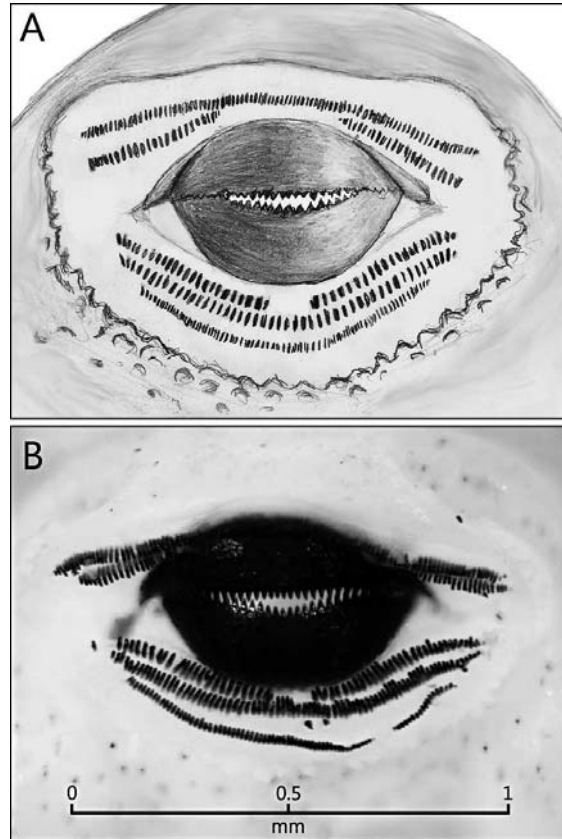


FIG. 5.—Mouth of *Excidobates captivus* tadpole, stage 25, semidiagrammatic (A) and photo (B) taken with dissecting microscope.

is occupied by aggressive Aguaruna natives, making access difficult. Over five days we conducted seven transects covering a total linear distance of ca. 75 km. Transects took place south of the Marañón, and east and west of the Santiago. Our surveys started on the south side of the Marañón, where we observed 16 *E. captivus* over 2 days in the field. We spent equal time on the north bank of the Marañón east of the Santiago, but found only one adult *E. captivus*. We also searched the west bank of the Santiago 8 km from its junction with the Marañón, and though we failed to find *E. captivus*, one of the Huambisa natives familiar with the species confirmed its presence in this site. Three members of our party crossed the Cerros de Campanquis on 28–29 June, walking over 25 km, though were unable to detect *E. captivus*.

In late 2007, we received a report and photographs from a collaborator (M. Pepper) documenting a population of *Excidobates*

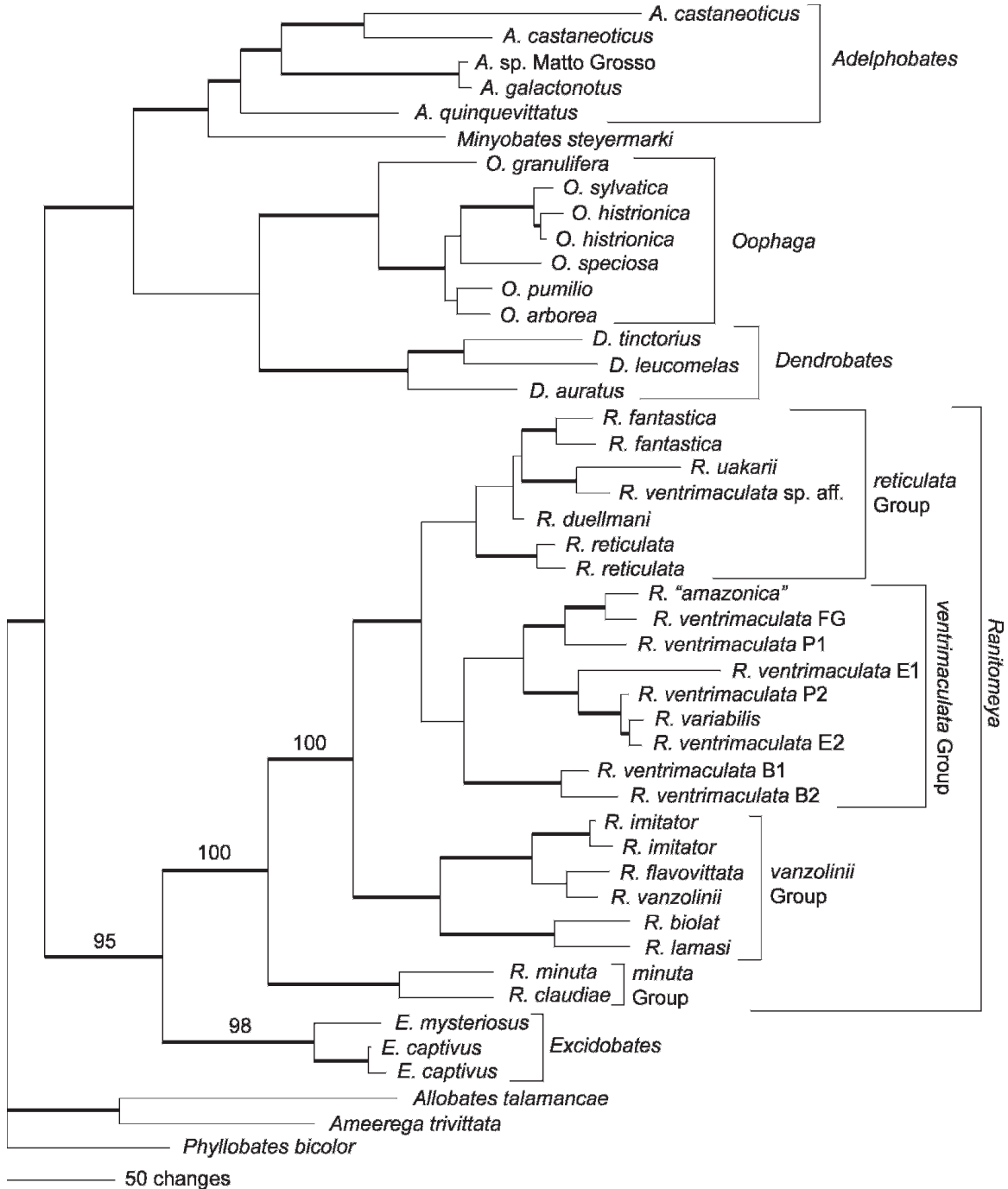


FIG. 6.—Bayesian phylogram. Thickened branches indicate nodes supported by greater than 80% Bayesian posterior probabilities. Numbers above branch indicate Bayesian posterior probabilities of particular interest.

captivus from the Cordillera del Condor approximately 20 km northeast of the type locality of *E. mysteriosus*. However, we do not have precise locality or elevation data for this record, and therefore could not include it in the Maxent niche model. This additional

record does, however, extend the known range of this species at least 130 km to the southwest. Therefore, it appears that *E. captivus* occurs from 200 m to ca. 400 m above sea level (but possibly higher in the Cordillera del Condor), and may be restricted

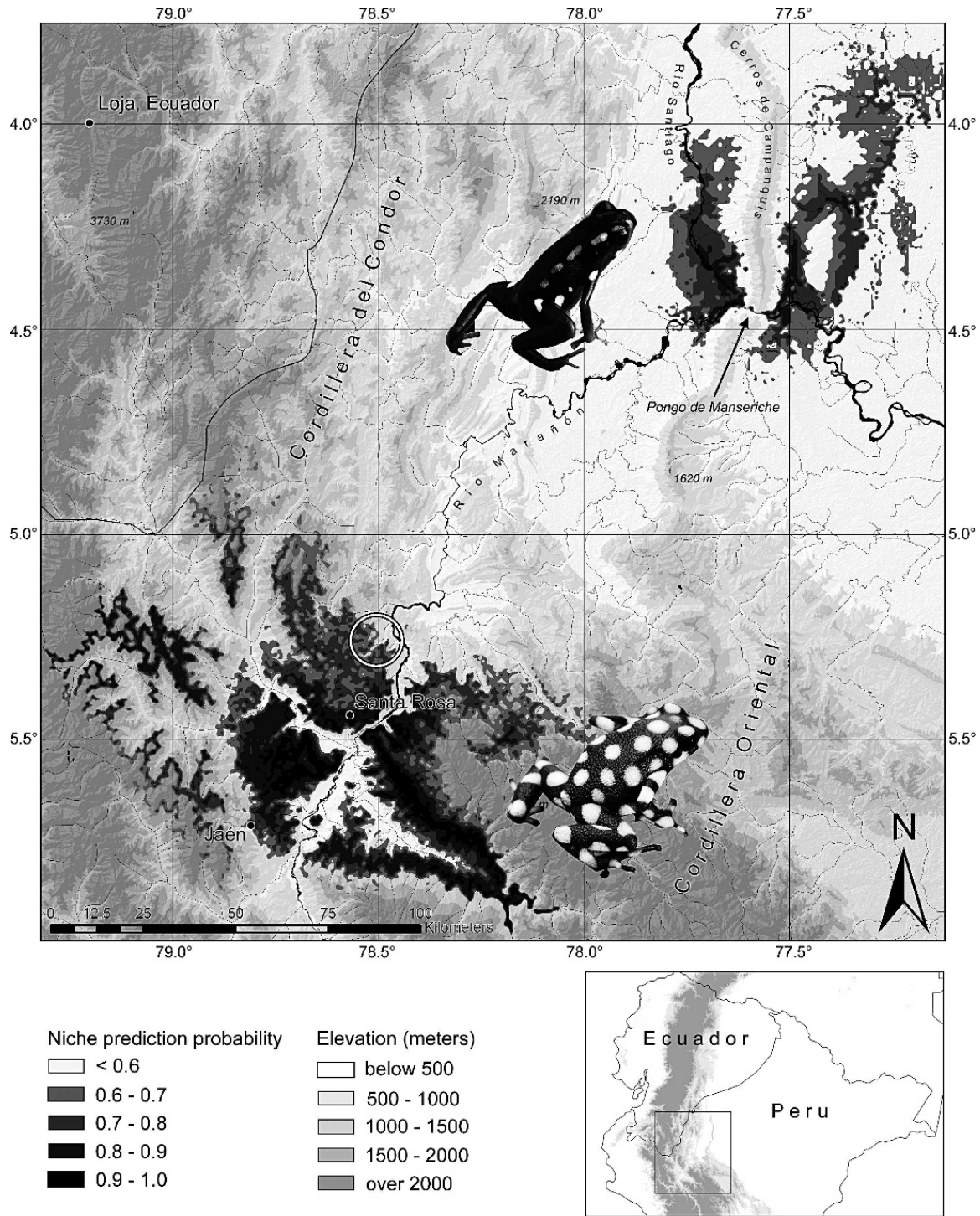


FIG. 7.—Map of northern Peru with Maxent niche prediction models of *Excidobates captivus* and *E. mysteriosus*. AUC values for the two species were both 0.999. Jackknifing the BIOCLIM variables indicated that for *E. captivus*, the precipitation seasonality variable contributed the most information to the model. For *E. mysteriosus*, isothermality, precipitation of wettest period, and precipitation seasonality variables all contributed similar amounts of information to the model. The open circle just north of Santa Rosa marks a population of *E. captivus* discovered in late 2007, and based on the information available to us regarding this population, we can only conclude that this population was found somewhere within this circle. More accurate locality information is not available; therefore, this point was not included in the Maxent analysis.

to the lowland valley between the Cerros de Campanquis and the Cordillera del Condor. *Excidobates captivus* was not observed east of the Cerros de Campanquis, though the Maxent niche-model predicted its presence along a narrow belt at the eastern foothills of these mountains (Fig. 7).

The lowland forest of the Santiago valley is laden with bromeliads and epiphytic moss, suggesting that this area receives more rainfall than the forest east of the Cerros de Campanquis, which is comparatively epiphyte-poor. Dendrobatid species richness is high in the Santiago valley, with *Ranitomeya variabilis*, *Ameerega parvula*, *A. trivittata*, *Hyloxalus nexipus*, *H. bocagei*, and another unidentified dendrobatid all being found in this site. *Excidobates captivus* seems to be more selective of habitat than other local poison frogs, as all 17 were found in wet areas, either in low-lying parts of the forest or in forest adjacent to streams. These streamside habitats are characterized by abundant *Heliconia*, which appears to be an important breeding site for this species. Frogs not found directly in phytotelmata were usually on the ground, suggesting that *E. captivus* is at least partly terrestrial. Egg deposition may also be terrestrial, as an adult pair was observed for several minutes courting in the leaf litter. Although we did not find free-living tadpoles, all four tadpole-carrying males were found in or near *Heliconia*, suggesting these plants are used for tadpole deposition. Heavy reliance on *Heliconia* for reproduction has been observed in *R. imitator* and *R. ventrimaculata* (Summers and Symula, 2001), but the deep divergence between these species and *E. captivus* suggests the capability to reproduce in *Heliconia* evolved independently more than once. Such convergence is not a trivial issue, as *Heliconia* pose harsher conditions than other phytotelmata, and at least in the case of *R. imitator*, require deposition of trophic eggs for tadpole development (J. L. Brown, unpublished data).

DISCUSSION

Excidobates captivus, as suggested by Myers (1982), is sister to *E. mysteriosus* (Fig. 6). Myers predicted this unusual affinity based on (1) shared spotted dorsal and ventral

pattern (particularly on the thighs), (2) elongated first digits of the hands and feet when compared to *Ranitomeya ventrimaculata*, and (3) distribution in the upper Río Marañón drainage. Though Myers (1982) stated the evidence supporting this relationship was meager and possibly a result of both species lacking obvious affinity to other taxonomic groups, this unexpected relationship was recovered in our analysis. The two species are sister to the recently-erected genus *Ranitomeya*.

Prior to evaluating relationships between *Excidobates* and other genera within the Dendrobatinae, we chose to evaluate the validity of dendrobatine genera (excluding *Phyllobates*) proposed by Grant et al., (2006) in a Bayesian framework. Bayes factor analyses (BFA) supported monophyly in *Oophaga* (H₂), *Adelphobates* (containing *Minyobates steyermarki*, H₁), *Dendrobates* (H₃), and *Ranitomeya* (H₄). Therefore, despite methodological differences (parsimony-based vs. Bayesian analysis), our results are consistent with and support the findings of Grant et al., (2006) with the exception of *Minyobates* and *E. captivus* (see below), and their proposed dendrobatine genera remain monophyletic.

The following hypotheses address relationships of *E. captivus* and *E. mysteriosus* to other genera within the subfamily Dendrobatinae. Grant et al., (2006) assigned *Excidobates captivus* to *Adelphobates* based on similarity in pattern to *Adelphobates castaneoticus*. A BFA strongly rejects this relationship (H₅, Table 1). In our Bayesian phylogeny, *E. captivus* is sister to *E. mysteriosus* (supported by BFA H₆), forming a group sister to *Ranitomeya*. Placing *Excidobates* within *Ranitomeya* (H₈) was supported; however, there was similar support for the placement of *Excidobates* in a larger clade containing *Oophaga*, *Dendrobates*, and *Adelphobates* (H₇).

Because of the phylogenetic placement of *Excidobates* as sister to *Ranitomeya*, it would be plausible to simply expand the definition of *Ranitomeya* to encompass *Excidobates*. However, genetic distances between *Excidobates* and *Ranitomeya* (6.94%) meet or exceed most other intergeneric comparisons (e.g., *Dendrobates/Oophaga* = 5.76%, *Dendrobates/Rani-*

tomeya = 6.67%). This tenuous argument could be strengthened by the eventual recognition of the *minuta* group as a genus distinct from *Ranitomeya*, composed entirely of trans-Andean species; however, only 3 of the 13 species in this group have been sequenced, and assumption of monophyly, though suggested by previous authors (e.g., Silverstone, 1975; Myers and Daly, 1980; Myers, 1987), is speculative. This hypothesis would unite *Ranitomeya* as being composed entirely of Amazonian frogs, all sharing the synapomorphy of pale limb-reticulation.

Conservation Status

Niche models generated with Maxent estimate the distribution of *Excidobates captivus* to be between 1836–2525 km² (>60% niche probability, Fig. 7), which is a substantial under-prediction given that this species has been found recently in the Cordillera del Condor well-south of the type locality (Fig. 7). Only in two small areas of the Santiago valley were we able to observe this species in high densities, suggesting that it may be patchily distributed in seemingly homogeneous lowland habitat. Though the extent of our initial survey was limited, the relatively small range and patchy distribution of *E. captivus* could potentially place it at risk if the area were to become well-developed. We did not detect signs of chytrid infection in tadpoles or adults. Mouthparts of the four tadpoles we examined appeared to be normal. PCR tests on swabs from all eight adults tested were consistently negative for Bd. Though we did not detect chytrid infection, due to a small sample size we cannot rule out the possibility of chytrid presence in *E. captivus* or in other species of amphibians in the Santiago valley.

Remarks

The validity of *Minyobates*, as suggested previously (Noonan and Wray, 2006; Roberts et al., 2006a; Vences et al., 1998), is questionable. The topology presented by Grant et al., (2006) placed *M. steyermarki* as sister to all dendrobatines excluding *Phyllobates*; however, an increase from 547 bp from one gene region to 1898 bp from five gene regions moves *M. steyermarki* as sister to *Adelphobates*. Furthermore, the monophyly of *Adel-*

phobates is supported when *M. steyermarki* is contained within (BFA H₁). *Adelphobates* is a genetically diverse genus with a large range, whose ancestors likely inhabited the Guiana Shield. Inclusion of *M. steyermarki*, which occurs on one Venezuelan tepui, would be compatible with this hypothesis.

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