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## SHORT COMMUNICATION

# Confirming the presence of *Clelia equatoriana* Amaral, 1924 (Squamata: Dipsadidae) in Peru

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**Abstract.**—In 2010, Aguilar et al. (2010) reported *Clelia equatoriana* for northern Peru; however, no voucher specimens or any data proving the record were mentioned. Here we confirm the presence of *C. equatoriana* in Peru based on collected specimens from a recent survey conducted in Piura Department, Peru, and provide novel data from the examination of museum specimens. Our findings extend the known distribution of the species ca. 331 km (straight line distance) SE from previous records in central Ecuador.

**Key words.** Latitude effect, subcaudals, Tabaconas Namballe, lizard, geographic distribution, range extension

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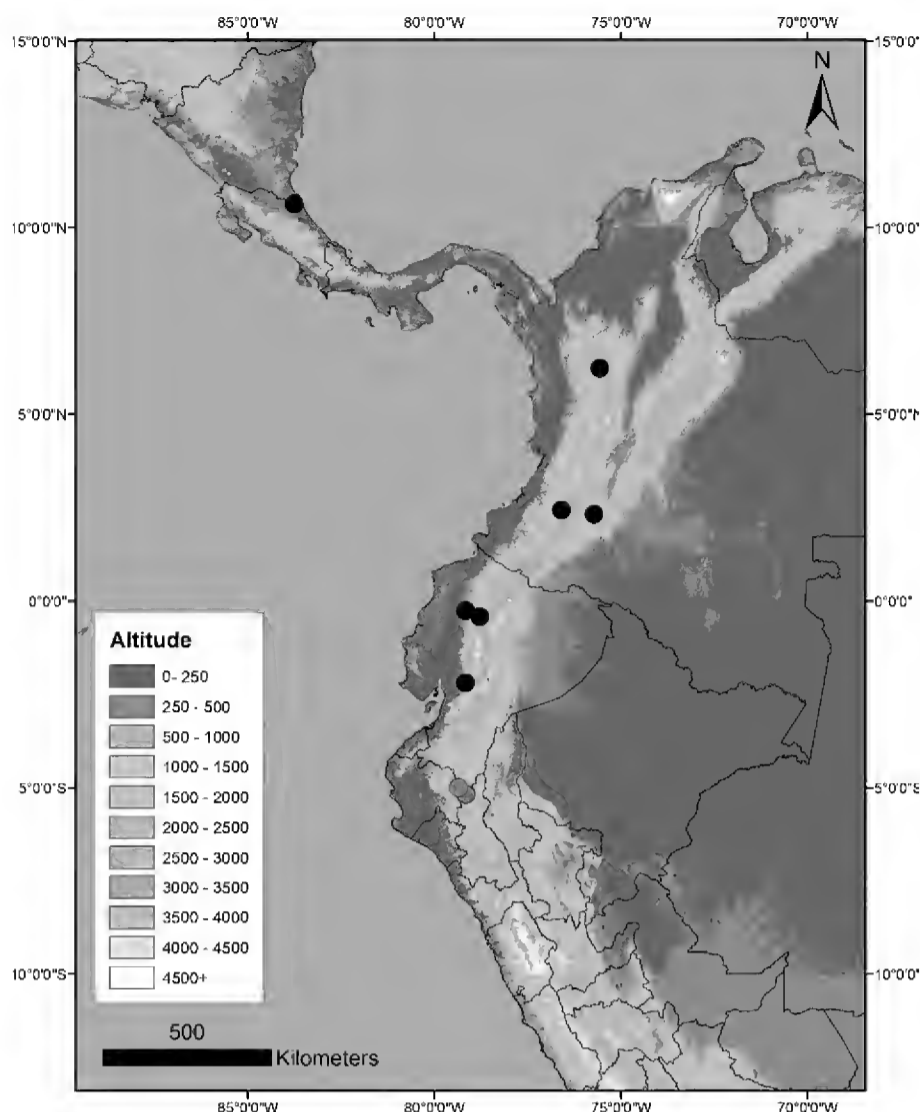
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The neotropical dipsadid snake genus *Clelia* Fitzinger 1826 consists of relatively large snakes (total length > two m in *C. clelia* and *C. plumbea*) that show a striking ontogenetic color change, from orange or red hatchlings to dark gray or black adults (Scott et al. 2006). Currently, the genus contains seven species widely distributed in Central and South America: *C. clelia* distributed from southern Mexico to southwestern Peru; *C. equatoriana* distributed from northern Costa Rica through Panama and Colombia to Amazonian Ecuador; *C. errabunda* in Saint Lucia; *C. hussami* from southern Minas Gerais, Brazil to Uruguay and central Argentina; *C. langeri* in Santa Cruz and Chuquisaca, Bolivia; *C. plumbea* from south of the Amazon river in Brazil to Mato Grosso do Sul and Paraguay, and the Atlantic rainforest of Brazil; and *C. scytalina* from Jalisco and Veracruz in Mexico to Panama, and in South America in Colombia and Ecuador (Zaher 1996; Pizzatto 2005; Cisneros-Heredia et al. 2007; Uetz 2015; Reichle and Embert 2005). These snakes are known by several common names in various countries (e.g., “mus-surana” in Brazil, “zopilota” in Costa Rica, “chonta” in

Ecuador, “aguajemachaco” and “machacuai” in Peru, and “cribo” in some Caribbean islands). Representatives of this genus have the particular habit of preying on other snakes, a behavior that has been reported several times before for *C. clelia*, *C. hussami*, and *C. plumbea* (Vitt and Vangilder 1983; Pinto and Lema 2002), and recently in *C. equatoriana* (Rojas-Morales 2012). Consequently, the genus *Clelia* plays an important role in regulation of populations of other snakes, including large venomous snakes of the *Bothrops* and *Crotalus* genera (Campbell and Lamar 2004).

In Peru there are currently two species of *Clelia* formally reported: *C. clelia* and *C. bicolor* (Dixon and Soini 1986; Carrillo and Icochea 1995), but the latter was re-allocated to the genus *Mussurana* by Zaher et al. (2009). More recently, Aguilar et al. (2010) reported *C. equatoriana* for Tabaconas Namballe National Sanctuary (TNNS), a natural protected area located in the north of Cajamarca department, close to the border between Ecuador and Peru. However, no voucher specimen or any additional information proving the record of *C. equato-*

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**Fig. 1.** Map of Isthmian Central America and northwestern South America showing the locality records of *Clelia equatoriana* (circles). Black circles are records by Zaher (1996), red circle is Quebrada Molletón and blue circle is El Sauce.

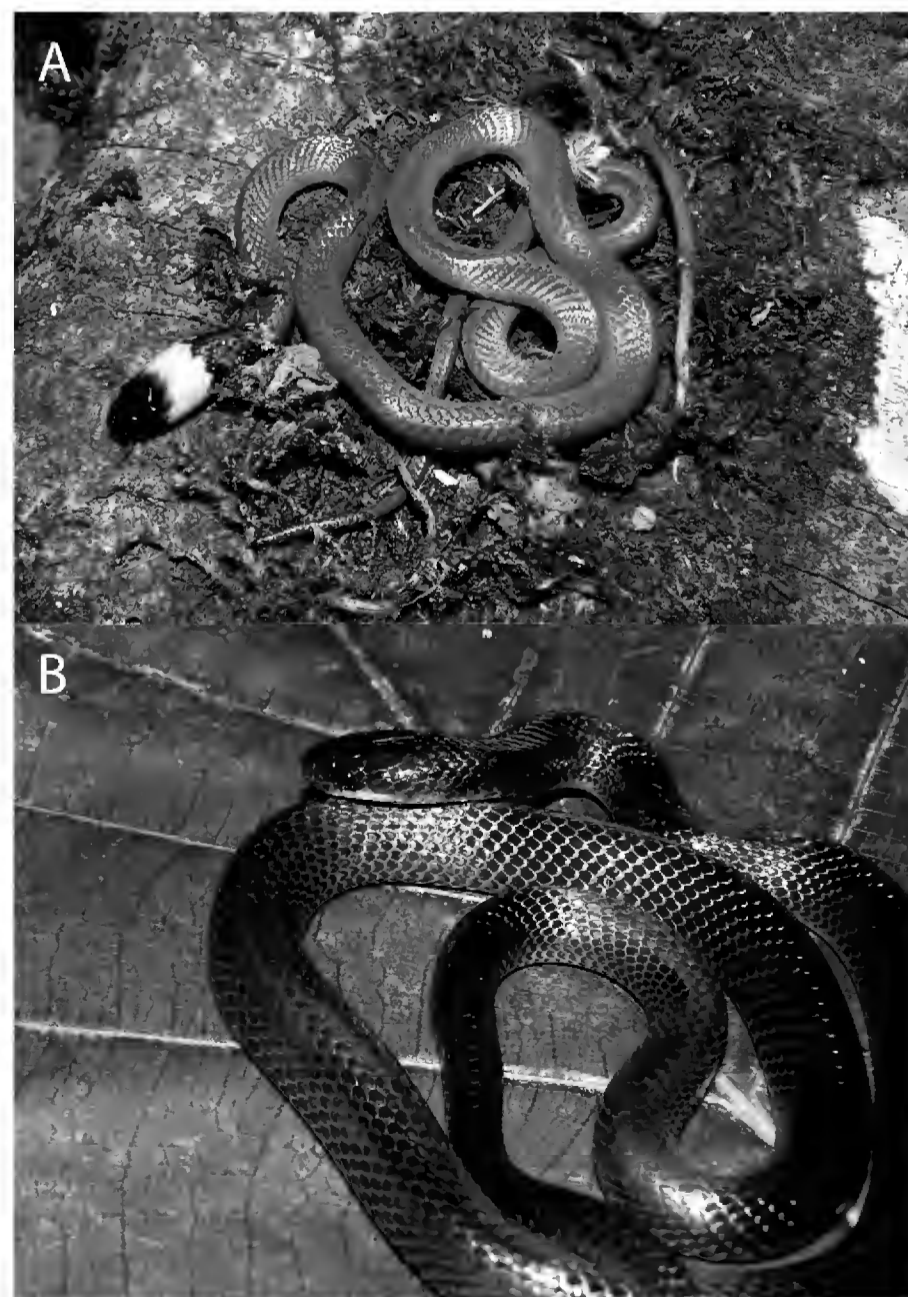
*riana* in Peru was provided. In fact, this record was in a small handbook produced by the WWF, which was intended for public awareness, rather than being a formal scientific report. We examined several specimens of the genus *Clelia* in the Herpetology Collection of Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM). We found a specimen assigned to *C. equatoriana* (MUSM 24981) collected on a survey made in April 2003. Even though not clearly stated, we suspect that this was the specimen in which the Aguilar et al. (2010) record was based. MUSM 24981 is an adult female from El Sauce Forest ( $-5.17^{\circ}\text{S}$ ,  $-79.16^{\circ}\text{W}$ , 1,500 m), Namballe District, San Ignacio Province, Cajamarca Department, Peru (Fig. 1). A recent survey conducted in the montane forests of Piura Department provided us with two additional specimens, which were deposited in the herpetological collection of Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru (CORBIDI 14869 and 14875) (Fig. 2). These specimens were found in August 2014 at Quebrada Molletón ( $-4.99^{\circ}\text{S}$ ,  $-79.37^{\circ}\text{W}$ , 2,222 m), Peña Rica village, in Carmen de la Frontera District, Huancabamba Province, Piura Department, Peru (Fig. 1). Both specimens are juvenile males that were found hiding under a log on the side of a stream in a secondary forest.

All examined specimens agree with the description of *C. equatoriana* by Zaher (1996) in having 17–17–17 dorsal scale rows, as well as the other characters presented in Table 1. However, specimens from Quebrada Molletón show a lower number of subcaudals (60–69)

than the range described for males of *Clelia equatoriana* (75–80 in males) by Zaher (1996). Interestingly, a similar segmental pattern of variation is found in the subcaudals for other Dipsadidae species: *Atractus carrioni* and *A. gigas* (Passos et al. 2010, 2013). Both species have their southernmost records in the same region and similar elevations to the records of *C. equatoriana* reported herein (Piura and Cajamarca departments). In the case of both *Atractus* species, the authors attribute the observed variation to a possible latitude effect in somitogenesis, which leads to the increase of the number of segmental counts in hotter and more humid localities towards the equator. Nevertheless, additional specimens need to be examined to test whether this latitudinal effect holds across different elevational gradients and Dipsadidae genera.

According to Zaher (1996), the southernmost record of *Clelia equatoriana* is in Bucay, Guayas Province, Ecuador. Records from El Sauce Forest and Peña Rica in TNNS extend the known distributional range of *C. equatoriana* by ca. 331 km (straight line distance) SE. These records for Cajamarca and Piura confirm that the distribution of this species can be more austral than previously thought and supports the importance of protected areas such as TNNS in the conservation of this species in Peru.

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**Fig. 2.** Individuals of *Clelia equatoriana* from Quebrada Molletón, Piura, Peru: CORBIDI 14869 (A) and 14875 (B).

ments on a previous version of this manuscript. We are especially grateful to Nature and Culture International, World Land Trust, and the Gerencia de Recursos Naturales del Gobierno Regional de Piura for funding our field work.

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**Table 1.** Morphometric characters (in cm) and scale counts of *Clelia equatoriana* specimens (MUSM 24981, CORBIDI 14869, and CORBIDI 14875) compared to mean measurements and scale counts for *C. equatoriana* and *C. clelia* data from Zaher (1996). (\*) tail incomplete.

Character	MUSM 24981 (female)	CORBIDI 14869 (male)	CORBIDI 14875 (male)	<i>Clelia equatoriana</i>	<i>Clelia clelia</i>
<b>Total length (cm)</b>	136.5	34.2	49	157.5 max	225 max
<b>Tail length (cm)</b>	21	5.7	10	17.5 max	40 max
<b>Dorsal rows</b>	17-17-17	17-17-17	17-17-17	17-17-17	17-19-17 19-19-17
<b>Ventrals</b>	211	220	204	202–207 (male) 200–217 (female)	201–230 (male) 218–244 (female)
<b>Subcaudals</b>	57*	62	72	75–80 (male) 54–64 (female)	81–98 (male) 70–91 (female)
<b>Loreal presence</b>	present	present	present	present	present
<b>Preoculars</b>	1	1	1	1	1
<b>Postoculars</b>	2	2	2	2	2
<b>temporals</b>	2 + 2/2 + 3	2 + 3	2 + 2	2 + 3	2 + 3 1 + 3 rarely 2 + 2 rarely
<b>Supralabials</b>	7	7	7	7	7
<b>Infralabials</b>	7	7	8	8	8



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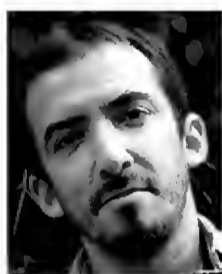
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**Lourdes Y. Echevarría** graduated in biological sciences from Universidad Nacional Agraria La Molina, Lima, Peru, in 2014. As a student, she collaborated constantly in the order and management of the herpetological collections of Centro de Ornitología y Biodiversidad, Lima, developing a great interest in reptiles, especially lizards. For her undergraduate thesis, Lourdes worked on the “Review of the current taxonomic status of *Petracola ventrimaculata* (Cercosaurini: Gymnophthalmidae) using morphological and ecological evidence.” She worked as a researcher of the Museo de Zoología (QCAZ), Pontificia Universidad Católica del Ecuador in Quito during 2015. Lourdes is preparing a monograph on the systematics of the *Petracola ventrimaculata* complex based on the results of her undergraduate thesis, as well as other papers about taxonomy of lizards and snakes.



**Pablo J. Venegas** graduated in Veterinary Medicine from Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru, in 2005. He is currently curator of the Herpetological Collection of Centro de Ornitología y Biodiversidad (CORBIDI). Pablo worked as a researcher of the Museo de Zoología QCAZ, Pontificia Universidad Católica del Ecuador in Quito during 2015. His current research interest is focused on the diversity and conservation of the Neotropical herpetofauna with an emphasis on Peru and Ecuador. He has published more than 40 scientific papers on taxonomy and systematics of Peruvian and Ecuadorian amphibians and reptiles.



# On the distribution and conservation of two “Lost World” tepui summit endemic frogs, *Stefania ginesi* Rivero, 1968 and *S. satelles* Señaris, Ayarzagüena, and Gorzula, 1997

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**Abstract.**—It has been suggested that the inability to migrate in response to climate change is a key threat to tepui summit biota. Tepui summit organisms might thus seriously be threatened by global warming, and there is an urgent need to accurately evaluate their taxonomic status and distributions. We investigated phylogenetic relationships among several populations of *Stefania ginesi* and *S. satelles*, two endemic species reported from some isolated tepui summits, and we examined their IUCN conservation status. Molecular phylogenetic analysis and preliminary morphological assessment indicate that both species are actually restricted to single tepui summits and that five candidate species are involved under these names. We advocate upgrading the conservation status of *S. ginesi* from Least Concern to Endangered, and that of *S. satelles* from Near Threatened to Endangered.

**Key words.** Endangered species, Hemiphractidae, IUCN, molecular phylogenetics, molecular taxonomy, Venezuela

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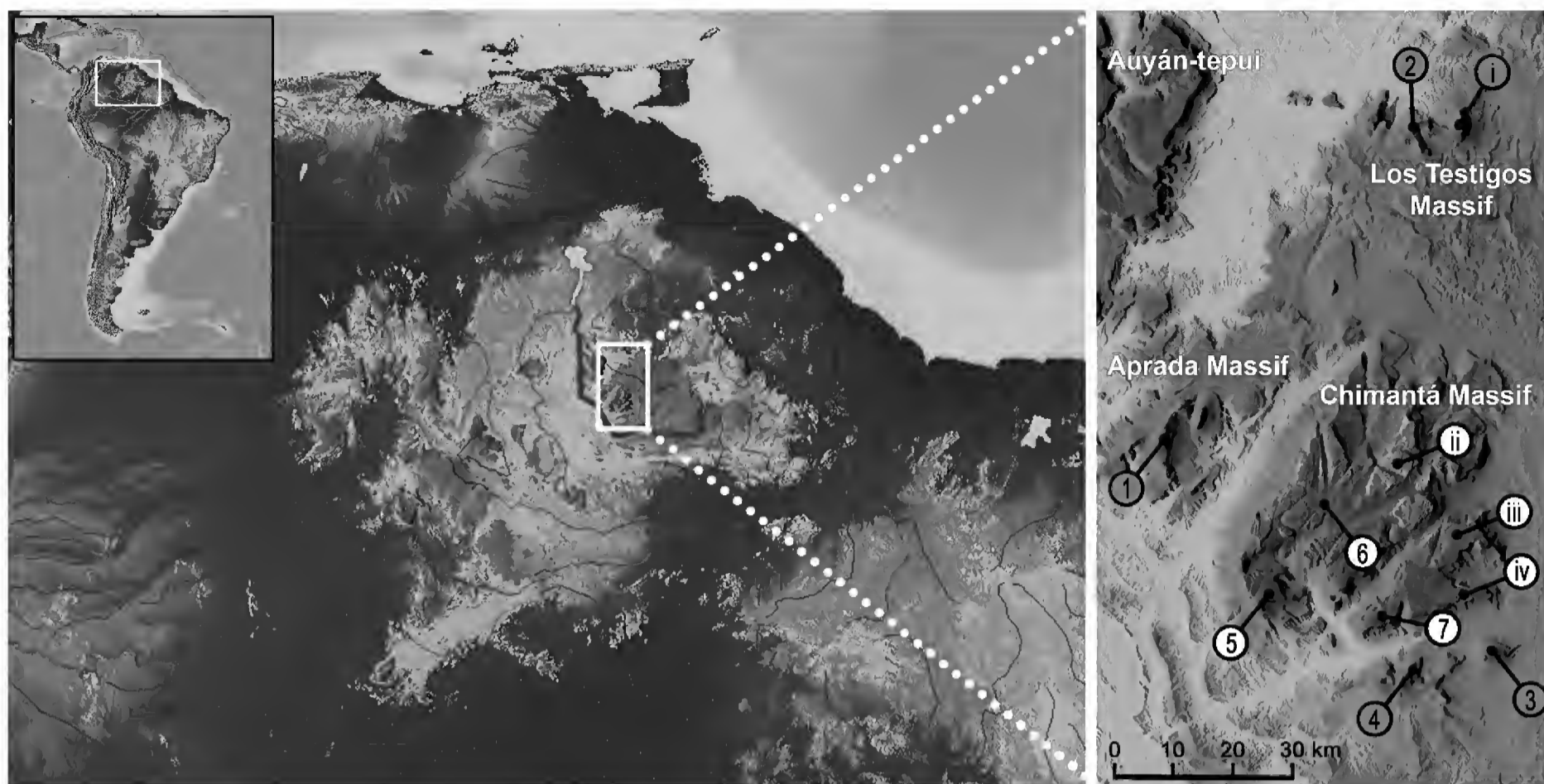
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## Introduction

The frog genus *Stefania* (Hemiphractidae) is endemic to an iconic South American biogeographical region named “Pantepui” (Mayr and Phelps 1967; McDiarmid and Donnelly 2005) (Fig. 1). Pantepui, often referred to as the “Lost World” because of Arthur Conan Doyle’s famous novel (1912), lies in the western Guiana Shield. The region harbors numerous isolated Precambrian sandstone tabletop mountains more formally known as “tepui” (Fig. 2). Although Pantepui was initially restricted to tepui slopes and summits above 1,500 m elevation (Mayr and Phelps 1967; Rull and Nogué 2007), Steyermark (1982), followed by Kok et al. (2012) and Kok (2013a), expanded the original definition of Pantepui to include the intervening Pantepui lowlands (200–400 m asl) and uplands (400–ca. 1,200 m asl) in order to better reflect the biogeography and biotic interactions in the area (Kok 2013a). The genus *Stefania* currently

includes 19 species, 15 of which are restricted to tepui slopes or summits (Duellman 2015; Frost 2015). *Stefania* species are direct-developers (eggs and juveniles carried on the back of the mother) and occupy various types of habitats from lowland rainforest to tepui bogs (Kok 2013a; Schmid et al. 2013; Duellman 2015). The genus *Stefania* was erected by Rivero (1968) to accommodate *Cryptobatrachus evansi* and a few related new species all morphologically divergent from other *Cryptobatrachus*. Shortly later, Rivero (1970) recognized two species-groups within *Stefania*: the *evansi* group including species having the head longer than broad and found in the lowlands and uplands of Pantepui, and the *goini* group including species having the head broader than long and found in the highlands of Pantepui. Kok et al. (2012), followed by Castroviejo et al. (2015), showed that, based on molecular data, these groups are actually not reciprocally monophyletic. A complete molecular phylogenetic analysis of the genus *Stefania* is still lacking, and

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**Fig. 1.** Left: Map of Pantepui and its location within South America (inset); the thick blue line indicates the Río Caroní. Right: Map of the area under study showing localities mentioned in the text (yellow dots represent known localities of occurrence of *Stefania satelles*, white dots represent known localities of occurrence of *Stefania ginesi*). Numbers indicate sampled localities and Roman numerals indicate unsampled localities, as follows: (1) Aprada-tepui, Venezuela; (2) Murisipán-tepui, Venezuela; (3) Upuigma-tepui, Venezuela; (4) Angasima-tepui, Venezuela; (5) Abakapá-tepui, Venezuela; (6) Chimantá-tepui, Venezuela; (7) Amurí-tepui, Venezuela; (i) Kamarkawarai-tepui, Venezuela; (ii) Murei-tepui, Venezuela; (iii) Churí-tepui, Venezuela; (iv) Akopán-tepui, Venezuela.

relationships between many species or populations are unknown. Likewise, the exact distribution of some tepui summit species is uncertain (e.g., Gorzula and Señaris 1999). Among these, two tepui summit endemic *Stefania* species are known from several isolated tepui summits: *Stefania ginesi* Rivero, 1968, which is reported from six tepuis in the Chimantá massif (Chimantá-tepui, Amurí-tepui, Abakapá-tepui, Churí-tepui, Akopán-tepui, and Murei-tepui; Señaris et al. 1997; Gorzula and Señaris 1999; Barrio-Amorós and Fuentes 2012; Fig. 1), and *Stefania satelles* Señaris, Ayarzagüena, and Gorzula, 1997, which has a highly disjunct distribution, being reported from Aprada-tepui (in the Aprada Massif), Angasima-tepui, and Upuigma-tepui (two southern outliers of the Chimantá massif), and from Murisipán-tepui and Kamarkawarai-tepui (in the Los Testigos Massif, north of the Chimantá massif) (Señaris et al. 1997; Gorzula and Señaris 1999; Fig. 1). *Stefania ginesi* is listed as Least Concern (LC) by the International Union for Conservation of Nature (IUCN) (Stuart et al. 2008) and *S. satelles* is listed as Near Threatened (NT) (Stuart et al. 2008). However, preliminary data suggest that their respective distributions could be more restricted than initially thought because more than two species could be involved under these names (the authors, unpublished; see also Señaris et al. 2014 regarding the distribution of *S. ginesi*). Herein we used molecular phylogenetics to investigate the relationships among three populations of *S. ginesi* and four populations of *S. satelles*. We also aim at providing a more precise distribution of these two taxa in order to

refine their conservation status. Indeed, tepui ecosystems are reported as particularly sensitive to global warming (Nogué et al. 2009), and tepui summit organisms might be seriously threatened by habitat loss due to upward displacement (Rull and Vegas-Vilarrúbia 2006; see also below). Likewise, climate envelope distribution models of tepui ecosystems based on future scenarios show that potential distributions become drastically smaller under global warming (Rödder et al. 2010). Species restricted to tepui summits are thus clearly at risk of extinction, and there is an urgent need to evaluate their exact taxonomic status and precise distribution.

## Materials and Methods

### Tissue sampling and molecular data

We combined available GenBank sequences of *Stefania ginesi* and *S. satelles* for fragments of the mitochondrial 16S rRNA gene (16S) and the protein-coding mitochondrial gene NADH hydrogenase subunit 1 (ND1) with 40 novel DNA sequences of *Stefania ginesi* and *S. satelles*: nine of fragments of 16S, five of ND1, 13 of the nuclear recombination activating gene 1 (RAG1), and 13 of the nuclear CXC chemokine receptor type 4 gene (CXCR4). We combined this dataset with DNA sequences of four additional members of the genus *Stefania* from outside the studied area (three species from east of the Río Caroní: *S. scalae*, an upland species, *S. riveroi* and *S. schuberti*, two highland species; and one highland spe-



**Fig. 2.** Typical Pantepui landscape. Photograph taken on 8<sup>th</sup> June 2012 from the summit of Upuigma-tepui, showing Angasima-tepui on the left and Akopán-tepui and Amurí-tepui on the right. Note stretches of savannah mainly caused by anthropogenic fires. *Photo PJRK.*

cies from west of the Río Caroní: *S. riae*; in total 16 novel sequences), and with *Fritziana ohausi*, member of the clade sister to *Stefania* (Castroviejo et al. 2015), which was selected as outgroup (see Table 1). Novel sequences have been catalogued in GenBank under the accession numbers KU958582-958637.

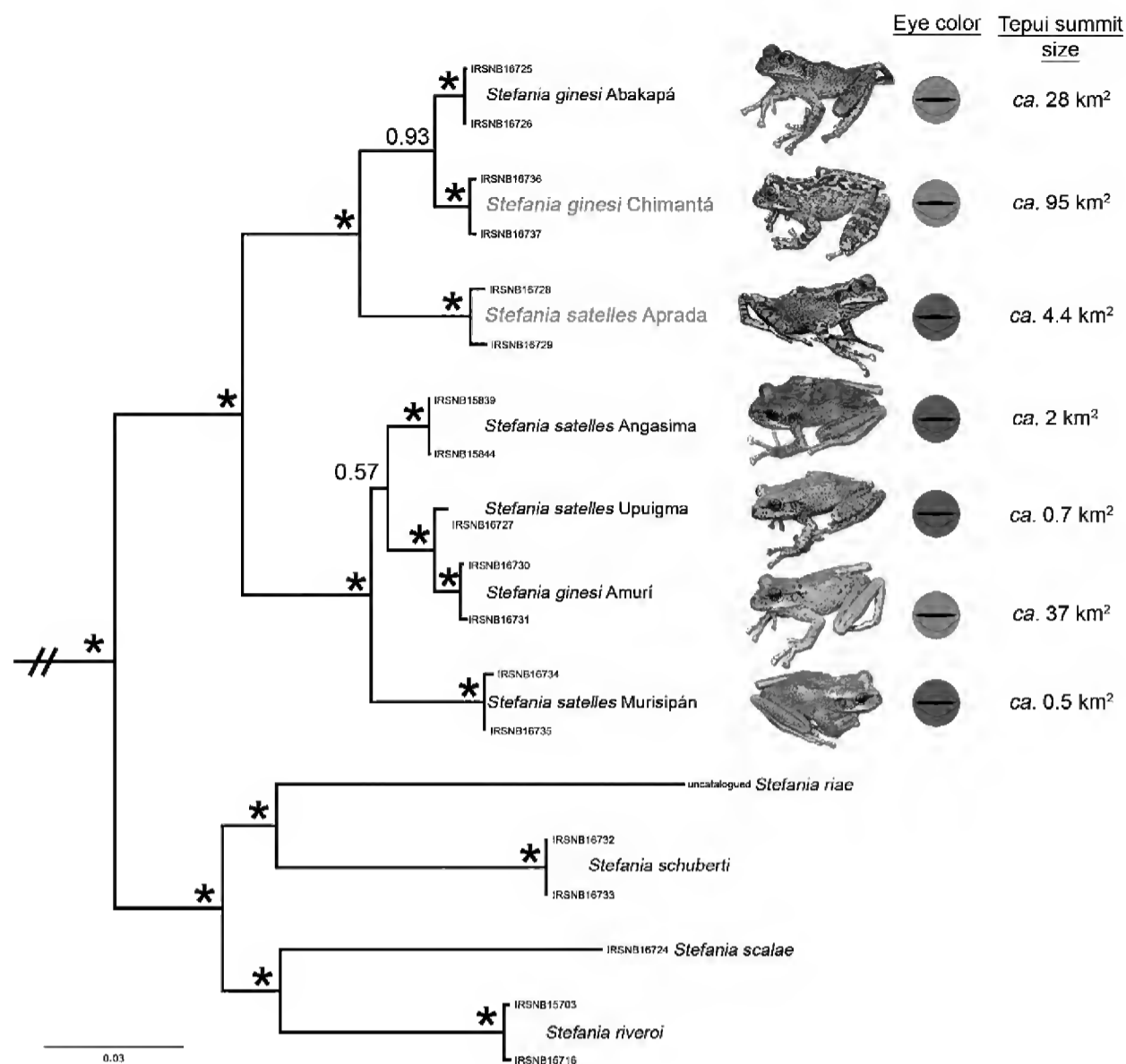
Total genomic DNA was extracted and purified using the Qiagen DNeasy® Tissue Kit following manufacturer’s instructions. Fragments of 16S (ca. 550 base pairs

[bp]), of ND1 (ca. 650 bp), and of RAG1 (ca. 550 bp) and CXCR4 (ca. 625 bp) were amplified and sequenced using the primers listed in Kok et al. (2012) and Biju and Bossuyt (2003) under previously described PCR conditions (Biju and Bossuyt 2003; Roelants et al. 2007; Van Bocxlaer et al. 2010). PCR products were checked on a 1% agarose gel and were sent to BaseClear (Leiden, The Netherlands) for purification and sequencing. Chromatograms were read using CodonCode Aligner 5.0.2

**Table 1.** List of *Stefania* taxa and outgroup used in this study, with localities and GenBank accession numbers. Sequences newly generated are in boldface. IRSNB = Institut Royal des Sciences Naturelles de Belgique, Belgium; MZUSP = Museu de Zoologia, Universidade de São Paulo, Brazil.

Voucher	16S	ND1	RAG1	CXCR4	Genus	Species	Locality	Country	Coordinates	Elevation (m)
IRSNB16724	JQ742191	JQ742362	<b>KU958600</b>	<b>KU958619</b>	<i>Stefania</i>	<i>scalae</i>	Salto El Danto	Venezuela	N 5°57'52" W 61°23'31"	1208
Uncatalogued	JQ742172	JQ742343	<b>KU958601</b>	<b>KU958620</b>	<i>Stefania</i>	<i>riae</i>	Sarisariñama-tepui	Venezuela	N 4°41' W 64°13'	ca. 1100
IRSNB15703	JQ742177	JQ742348	<b>KU958602</b>	<b>KU958621</b>	<i>Stefania</i>	<i>riveroi</i>	Yuruaní-tepui	Venezuela	N 5°18'50" W 60°51'50"	2303
IRSNB15716	JQ742178	JQ742349	<b>KU958603</b>	<b>KU958622</b>	<i>Stefania</i>	<i>riveroi</i>	Yuruaní-tepui	Venezuela	N 5°18'50" W 60°51'50"	2303
IRSNB16725	JQ742173	JQ742344	<b>KU958604</b>	<b>KU958623</b>	<i>Stefania</i>	<i>“ginesi”</i>	Abakapá-tepui	Venezuela	N 5°11'23" W 62°17'52"	2137
IRSNB16726	JQ742174	JQ742345	<b>KU958605</b>	<b>KU958624</b>	<i>“ginesi”</i>	<i>“ginesi”</i>	Abakapá-tepui	Venezuela	N 5°11'07" W 62°17'21"	2209
IRSNB15839	JQ742175	JQ742346	<b>KU958606</b>	<b>KU958625</b>	<i>Stefania</i>	<i>“satelles”</i>	Angasima-tepui	Venezuela	N 5°02'36" W 62°04'51"	2122
IRSNB15844	JQ742176	JQ742347	<b>KU958607</b>	<b>KU958626</b>	<i>Stefania</i>	<i>“satelles”</i>	Angasima-tepui	Venezuela	N 5°02'36" W 62°04'51"	2122
IRSNB16727	<b>KU958582</b>	<b>KU958593</b>	<b>KU958608</b>	<b>KU958627</b>	<i>Stefania</i>	<i>“satelles”</i>	Upuigma-tepui	Venezuela	N 5°05'10" W 61°57'32"	2134
IRSNB16728	<b>KU958583</b>	—	<b>KU958609</b>	<b>KU958628</b>	<i>Stefania</i>	<i>satelles</i>	Aprada-tepui	Venezuela	N 5°24'39" W 62°27'00"	2551
IRSNB16729	<b>KU958584</b>	—	<b>KU958610</b>	<b>KU958629</b>	<i>Stefania</i>	<i>satelles</i>	Aprada-tepui	Venezuela	N 5°24'43" W 62°27'03"	2576
IRSNB16730	<b>KU958585</b>	<b>KU958594</b>	<b>KU958611</b>	<b>KU958630</b>	<i>Stefania</i>	<i>“ginesi”</i>	Amurí-tepui	Venezuela	N 5°08'34" W 62°07'08"	2215
IRSNB16731	<b>KU958586</b>	<b>KU958595</b>	<b>KU958612</b>	<b>KU958631</b>	<i>Stefania</i>	<i>“ginesi”</i>	Amurí-tepui	Venezuela	N 5°08'35" W 62°07'08"	2213
IRSNB16732	<b>KU958587</b>	<b>KU958596</b>	<b>KU958613</b>	<b>KU958632</b>	<i>Stefania</i>	<i>schuberti</i>	Auyán-tepui	Venezuela	N 5°45'56" W 62°32'25"	2279
IRSNB16733	<b>KU958588</b>	<b>KU958597</b>	<b>KU958614</b>	<b>KU958633</b>	<i>Stefania</i>	<i>schuberti</i>	Auyán-tepui	Venezuela	N 5°45'56" W 62°32'25"	2279
IRSNB16734	<b>KU958589</b>	<b>KU958598</b>	<b>KU958615</b>	<b>KU958634</b>	<i>Stefania</i>	<i>“satelles”</i>	Murisipán-tepui	Venezuela	N 5°52'03" W 62°04'30"	2419
IRSNB16735	<b>KU958590</b>	<b>KU958599</b>	<b>KU958616</b>	<b>KU958635</b>	<i>Stefania</i>	<i>“satelles”</i>	Murisipán-tepui	Venezuela	N 5°52'03" W 62°04'30"	2419
IRSNB16736	<b>KU958591</b>	—	<b>KU958617</b>	<b>KU958636</b>	<i>Stefania</i>	<i>ginesi</i>	Chimantá-tepui	Venezuela	N 5°19'12" W 62°12'07"	2180
IRSNB16737	<b>KU958592</b>	—	<b>KU958618</b>	<b>KU958637</b>	<i>Stefania</i>	<i>ginesi</i>	Chimantá-tepui	Venezuela	N 5°19'12" W 62°12'07"	2180
MZUSP139225	JN157635	KC844945	KC844991	—	<i>Fritziana</i>	<i>ohausi</i>	n/a	Brazil	n/a	n/a





**Fig. 3.** Phylogenetic relationships as recovered in the MrBayes analysis (concatenated dataset, 2359 bp), outgroup not shown. Values at each node represent Bayesian posterior probabilities; asterisks indicate values > 95%. *Stefania ginesi* sensu stricto, and *S. satelles* sensu stricto are highlighted in red. Relation between eye color and tepui summit surface is indicated on the right side of the figure. Photos PJRK.

(<http://www.codoncode.com/index.htm>) and a consensus sequence was assembled from the forward and reverse primer sequences. MAFFT version 7 (<http://mafft.cbrc.jp/alignment/server/>) was used to perform preliminary alignment using G-INS-i and default parameters. Minor alignment corrections were made using MacClade 4.08 (Maddison and Maddison 2005). Protein-coding sequences were translated into amino-acid sequences to check for unexpected stop codons. Alignment-ambiguous regions of 16S were excluded from subsequent analyses.

### Molecular phylogenetic analyses

The combined 16S + ND1 + RAG1 + CXCR4 dataset (totalling 2,359 bp after exclusion) was subjected to phylogenetic inference using Bayesian analyses. Optimal partitioning schemes were estimated with PartitionFinder v1.1.1 (Lanfear et al. 2012) using the “greedy” algorithm, the “mrbayes” set of models, and the Bayesian Information Criterion (BIC) to compare the fit of different models. Bayesian posterior probabilities (PP) were used to estimate clade credibility in MrBayes 3.2.2 (Ronquist et al. 2012) on the CIPRES Science Gateway V 3.3 (<https://www.phylo.org/>, Miller et al. 2010). The Bayesian analyses implemented the best substitution models inferred by PartitionFinder v1.1.1 partitioned over the different gene fragments, flat Dirichlet priors for base frequencies and substitution rate matrices and uniform priors for among-

site rate parameters. Four parallel Markov chain Monte Carlo (MCMC) runs of four incrementally heated (temperature parameter = 0.2) chains were performed, with a length of 20,000,000 generations, a sampling frequency of 1 per 1,000 generations, and a burn-in corresponding to the first 1,000,000 generations. Convergence of the parallel runs was confirmed by split frequency SDs (<0.01) and potential scale reduction factors (~1.0) for all model parameters, as reported by MrBayes. All analyses were checked for convergence by plotting the log-likelihood values against generation time for each run, using Tracer 1.5 (Rambaut and Drummond 2009). Effective sample sizes (ESS) largely over 200 were obtained for every parameter. Results were visualized and edited in FigTree 1.4.1 (Rambaut 2014).

### Results

*Stefania ginesi* and *S. satelles* as currently recognized are recovered non-reciprocally monophyletic (Fig. 3). Our molecular phylogeny also reveals the occurrence of five candidate species (sensu Padial et al. 2010) that have been misidentified for more than a decade as *S. ginesi* (two candidate species) or *S. satelles* (three candidate species) (e.g., Señaris et al. 1997; Gorzula and Señaris 1999). Preliminary morphological analyses (in progress) indicate a few, sometimes subtle, morphological characters allowing discrimination among these candidate spe-

cies and *S. ginesi* and *S. satelles*. Our combined results indicate that *S. ginesi* sensu stricto is likely restricted to its type locality, Chimantá-tepui, as we suspect that populations from other tepuis in the Chimantá Massif that were not sampled in this study will prove to be distinct as well. As for *Stefania satelles*, the species is restricted to its type locality, Aprada-tepui.

### Discussion and conservation recommendations

We assumed that misidentifications were likely due to a rather conserved external morphology (e.g., head broader than long, skin strongly granular, absence of prominent cranial crests) of all tepui summit species previously identified as *Stefania ginesi* or *S. satelles*. This conserved morphology appears to be symplesiomorphic, and probably the result of an allopatric non-adaptive radiation (lineage diversification with minimal ecological diversification, see Rundell and Price 2009). It is, however, intriguing that two slightly divergent phenotypes (a “*satelles* phenotype” with brown eyes and a “*ginesi* phenotype” with blue eyes) evolved independently in each subclade (see Fig. 3). Interestingly, selection towards one of these two phenotypes seems closely associated with the size of the summit surface on which the species occur (see Fig. 3). The “*ginesi* phenotype” is found on large tepui summits (surface > 25 km<sup>2</sup>) in the central Chimantá Massif, whereas the “*satelles* phenotype” is found on much smaller tepui summits (surface < 5 km<sup>2</sup>) in the periphery of the core Chimantá Massif. Disentangling this phenomenon and the nature of the ecological constraints possibly involved and their influence on phenotypic trajectories is beyond the scope of this paper and will be treated in a separate study.

Most importantly, our results have direct implications on the conservation status of the populations under study. A complete taxonomic revision of the genus is in progress, but meanwhile we wish to emphasize the restricted distributions of all the populations previously known as *Stefania ginesi* or *S. satelles*. Our results argue for the upgrading of the conservation status of *S. ginesi* from LC to Endangered (EN), and that of *S. satelles* from NT to EN, based on the same argument recently developed for other species restricted to the summit of one or two tepuis, e.g., *Pristimantis imthurni* and *P. jamescameroni* (Kok 2013b), or *P. aureoventris* (IUCN SSC Amphibian Specialist Group 2014), thus in accordance with criteria B1 a-b (iii) and B2 a-b (iii) of the IUCN Red List of Threatened Species (IUCN 2014). We indeed argue that (1) extents of occurrence of *S. ginesi* and *S. satelles* are much less than 5,000 km<sup>2</sup> (less than 100 km<sup>2</sup> and five km<sup>2</sup>, respectively); (2) areas of occupancy of *S. ginesi* and *S. satelles* are much less than 500 km<sup>2</sup> (less than 100 km<sup>2</sup> and five km<sup>2</sup>, respectively); (3) there is an inferred and projected decline in the quality of habitat due to the effects of global warming upon tepui ecosystems, with an expected 2–4 °C increase in temperature

in the region through the next century (IPCC 2007). As stressed by Nogué et al. (2009) and Rödder et al. (2010), this rise in temperature will likely cause a decrease in habitat suitability for tepui biota. In addition, numerous anthropogenic fires in the region (Means 1995; Rull et al. 2013, 2016), coupled with a global rise of temperature, may cause an up to 10% decrease in precipitation (IPCC 2007) instigating an increase in fire range and intensity (Rull et al. 2013, 2016); and (4) the altitudinal range of *Stefania ginesi* and *S. satelles* allows no vertical migration in order to avoid these threats. As mentioned by Rull and Vegas-Vilarrúbia (2006), the inability to migrate to compensate for the climate change is a key threat to tepui summit biota.

There is an urgent need to gain a greater understanding of species boundaries and distributions in Pantepui, especially in Venezuela where the threats are the highest due to ongoing uncontrolled anthropogenic fires (Rull et al. 2013, 2016). However, it is assumed that an even greater threat to Pantepui biota is global climate change. Local actions (such as stopping fires), even if necessary, might only have a limited impact on the long-term survival of Pantepui organisms. Conservation awareness is critically important in the area, notably due to the inaccessibility of tepui ecosystems where an out of sight, out of mind effect may have taken place.

This study adds to the many studies now available demonstrating that estimates of amphibian diversity based on morphology alone are often misleading. Molecular data have indeed been shown to be of great help in detecting cryptic species (e.g., Hebert et al. 2004; Vences et al. 2005; Fouquet et al. 2007; Burns et al. 2008; Fouquet et al. 2016). Unfortunately, while everyone seems to agree that gaining a greater understanding of the world biodiversity is needed in order to prioritize biodiversity conservation (e.g., Wilson 2016), the task turns more and more often into a bureaucratic obstacle course, if not an impossible mission for scientists working with molecular data.

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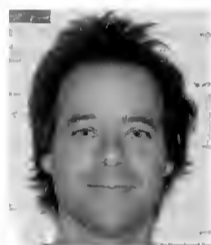
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## SHORT COMMUNICATION

# New records of the Critically Endangered frog *Pristimantis pardalinus* (Craugastoridae) in the eastern Andean slopes of central Peru

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**Key words.** Andes-Amazon, bromeliad, cloud forest, endemic, phytotelmata, Red List

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The eastern slopes of the Andes exhibit high levels of amphibian diversity and endemism coupled with diverse ecosystems and steep elevational gradients (Catenazzi and von May 2014). In central Peru, high-elevation ecosystems such as the Andean grassland, montane scrubland, and the upper cloud forest have experienced high levels of habitat loss and degradation, potentially affecting many amphibian species (Lehr and von May 2004; von May et al. 2008). Conservation of key areas along these Andean slopes is a priority because the type localities of many amphibian species described a long time ago (e.g., Boulenger 1912), or more recently (e.g., Lehr et al. 2006), remain unprotected. Equally important is to resurvey these sites to determine if species that have not been seen in decades are still there (e.g., Lehr and von May 2004; Lehr 2007), to assess their current population status and identify threats to their survival. This is especially critical for endemic and range-restricted species, many of which are vulnerable to local threats such as habitat loss and disease.

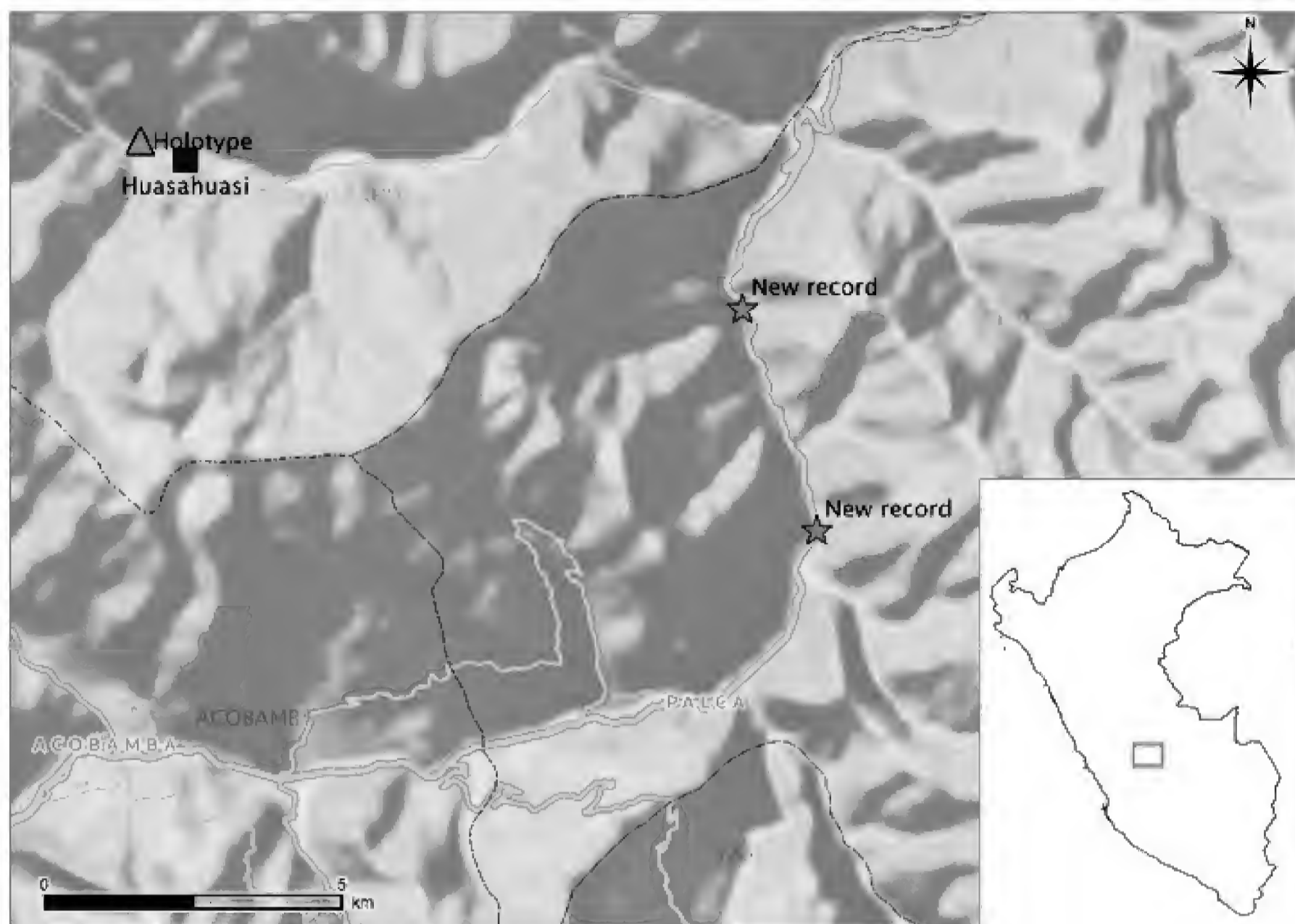
One example of such endemic and range-restricted species is *Pristimantis pardalinus*, a terrestrial breeding frog known from a single locality in central Peru (Lehr et al. 2006). A recent assessment focusing on the extinction risk of 39 potentially threatened amphibian species in Peru (Jarvis et al. 2015) determined that *P. pardalinus*, which was previously categorized as Data Deficient

(DD) according to the International Union for Conservation of Nature Red List (IUCN 2012a), should be categorized as Critically Endangered (CR). Given that the species was known from a single locality, had a small Extent of Occurrence (EOO <100 km<sup>2</sup>), and faced ongoing threats (e.g., agricultural expansion, overgrazing, and human settlement), the status of *P. pardalinus* was “up-listed” from DD to CR B1ab(iii) (IUCN 2014). Though the change in the conservation status of this species, which resulted from the application of the IUCN Red List Categories and Criteria (IUCN 2012b), was an important step, Jarvis et al. (2015) emphasized that additional field assessments are needed in order to understand the geographic distribution, population size, and threats affecting this and many other species.

In this report, I provide new distributional data for *P. pardalinus* based on field observations and the collection of voucher specimens. I used the morphological diagnoses provided by Lehr et al. (2006) to identify specimens and took measurements to the nearest 0.1 mm with calipers under a stereomicroscope. Specimens were deposited in the Herpetological Collection of the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM) and in the Herpetological Collection of the Museum of Vertebrate Zoology, University of California, Berkeley, California, USA (MVZ).

On 14 March 2014, two field assistants and I surveyed five sites located 10–15 km E-SE from Huasahuasi, the

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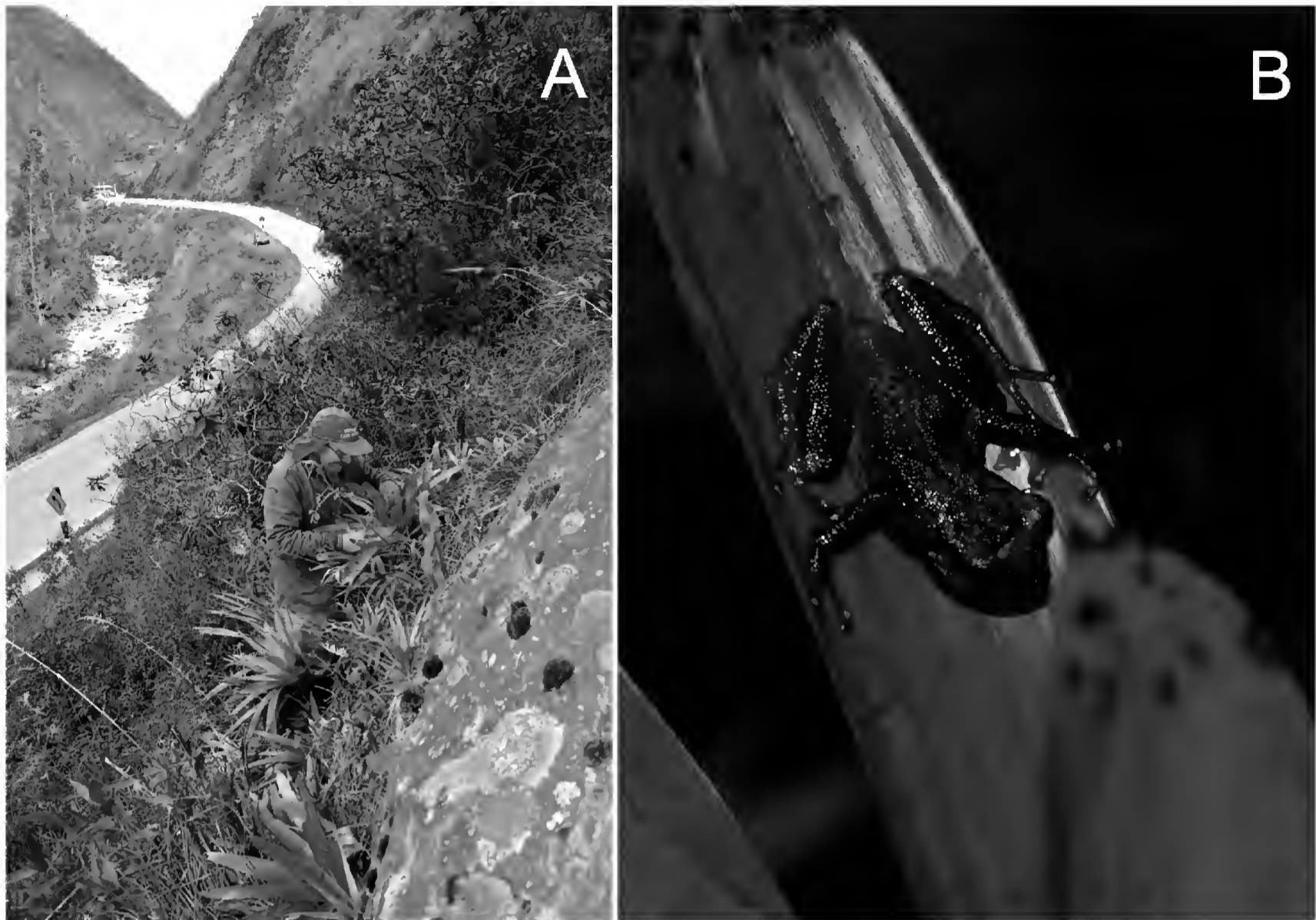
**Fig. 1.** Map showing the currently known distribution of *Pristimantis pardalinus*. The yellow triangle indicates the location of the type locality and the red stars indicate the location of new records reported in this study. The inset shows the location of the study area in Peru (red box).

type locality of *P. pardalinus* (Fig. 1). We focused our search on hillsides located next to the Carretera Central road, Palca District, Tarma Province (Fig. 2). The habitat at the selected sites was a mix of scrubland dominated by terrestrial bromeliads and Peruvian feather grass; two sites also had small patches of cloud forest vegetation. Altogether, we inspected approximately 150 terrestrial bromeliads between 9:00 h and 16:00 h, and found seven individuals of *P. pardalinus* at two sites. All individuals were found inside bromeliads of the genus *Tillandsia*. These bromeliads are commonly distributed along various sections of the road connecting Palca and San Ramón, as well as the road connecting the Carretera Central and Huasahuasi (Fig. 2). One individual (MUSM 33278) was collected from the first site ( $11^{\circ}19'15.78''\text{S}$ ,  $75^{\circ}33'07.81''\text{W}$ ) at 2,702 m elevation and six individuals (MUSM 33279-33281; MVZ 272273-272275) were collected from the second site ( $11^{\circ}17'17.77''\text{S}$ ,  $75^{\circ}33'47.77''\text{W}$ ) at 2,591 m elevation. Morphometric data for all specimens are shown in Table 1. We surveyed three additional sites along the Carretera Central (section connecting Palca and San Ramón) and one site along the road connecting the Carretera Central and Huasahuasi (the type locality), but did not find additional individuals of *P. pardalinus* (Fig. 1).

This report represents an extension of >10 km of the known geographic range of *P. pardalinus*, based on spec-

imens collected 10.56 km and 12.96 km, respectively, from the type locality. Furthermore, I note that the elevation given in the species description, 2,640 m, was in error. A recent inspection of satellite images provided by Google Earth and Fallingrain, a Global Gazetteer, indicate that the holotype and paratopotypes of *P. pardalinus* were actually collected at ca. 2,800 m a.s.l. Therefore, the currently known elevational distribution of *P. pardalinus* ranges from 2,591 to 2,800 m a.s.l. Given that the three known localities of *P. pardalinus* are situated outside protected areas, the long-term conservation of this species will depend on the type of land use at these localities. This is especially relevant considering that large areas of potentially suitable habitat have already been converted to cultivated land (Huasahuasi is one of the main potato production centers in Peru). Thus, *P. pardalinus* should be considered a species of special concern (von May et al. 2008) and the protection of the remaining habitats in the region should be included in future initiatives directed by the Servicio Nacional Forestal y de Fauna Silvestre (SEFOR), Peru's Wildlife Service.

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**Fig. 2.** Local collaborator Elmer Mapelli surveying bromeliads on rocky outcrop along the Carretera Central, Palca District, Tarma Province, 2,591 m elevation (A). Six individuals of *P. pardalinus*, including MVZ 272273 (B), were found at this site.

seum of Vertebrate Zoology, University of California, Berkeley, California, USA, for providing access to the herpetological collections at each institution. I thank Elmer Mapelli and Patricio Valverde for assistance in the field. Research and collecting permits were issued by the Ministry of Agriculture in Peru (Resolución Directoral N° 120-2012-AG-DGFFS-DGEFFS y Resolución Directoral N° 064-2013-AG-DGFFS-DGEFFS). I thank Jes-

sica Deichmann and an anonymous reviewer for providing helpful comments on the manuscript. Fieldwork in central Peru was supported by grants from the National Science Foundation (Postdoctoral Research Fellowship in Biology, DBI-1103087) and the National Geographic Society Committee for Research and Exploration (Grant # 9191-12).

**Table 1.** Measurements (in mm) of individuals of *Pristimantis pardalinus* found in this study. Individual collection number and sex noted for each individual. SVL = snout-vent length, TL = tibia length, FL = foot length, HL = head length, HW = head width, ED = eye diameter, TY = tympanum diameter, IOD = interorbital distance, EW = upper eyelid width, IND = internarial distance, E-N = eye-nostril distance.

	<b>MUSM 33278</b>	<b>MUSM 33279</b>	<b>MUSM 33280</b>	<b>MUSM 33281</b>	<b>MVZ 272273</b>	<b>MVZ 272274</b>	<b>MVZ 272275</b>
<b>Character</b>	<b>male</b>	<b>male</b>	<b>juvenile</b>	<b>male</b>	<b>female</b>	<b>male</b>	<b>juvenile</b>
<b>SVL</b>	25.00	24.60	20.82	24.84	30.03	25.28	20.69
<b>TL</b>	12.61	12.49	10.21	11.96	16.99	12.17	10.29
<b>FL</b>	10.53	10.88	8.68	10.79	14.97	10.67	8.13
<b>HL</b>	9.28	8.33	7.02	8.58	10.80	8.53	6.84
<b>HW</b>	9.15	9.30	7.44	9.29	11.49	8.89	7.45
<b>ED</b>	3.13	3.20	2.24	3.45	3.50	3.63	2.43
<b>TY</b>	1.27	1.24	0.97	1.45	1.61	1.71	0.97
<b>IOD</b>	3.36	2.92	2.62	3.34	4.36	3.26	2.66
<b>EW</b>	2.05	2.22	1.94	2.20	2.50	2.31	1.93
<b>IND</b>	2.04	2.05	1.56	2.04	2.41	2.11	1.36
<b>E-N</b>	2.82	2.64	2.09	2.80	3.64	2.70	2.16



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**Rudolf von May** is a postdoctoral research fellow at the Department of Ecology and Evolutionary Biology at the University of Michigan. His current research seeks to understand how amphibian and reptile communities are structured across habitats and elevations, taking into account the phylogenetic relatedness among species present in those communities.



## SHORT COMMUNICATION

# New distributional records of the Amazon River Frog *Lithobates palmipes* (Spix, 1824) in Peru

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**Key words.** Amazonia, lowland rainforest, *Rana*, Ranidae, true frogs

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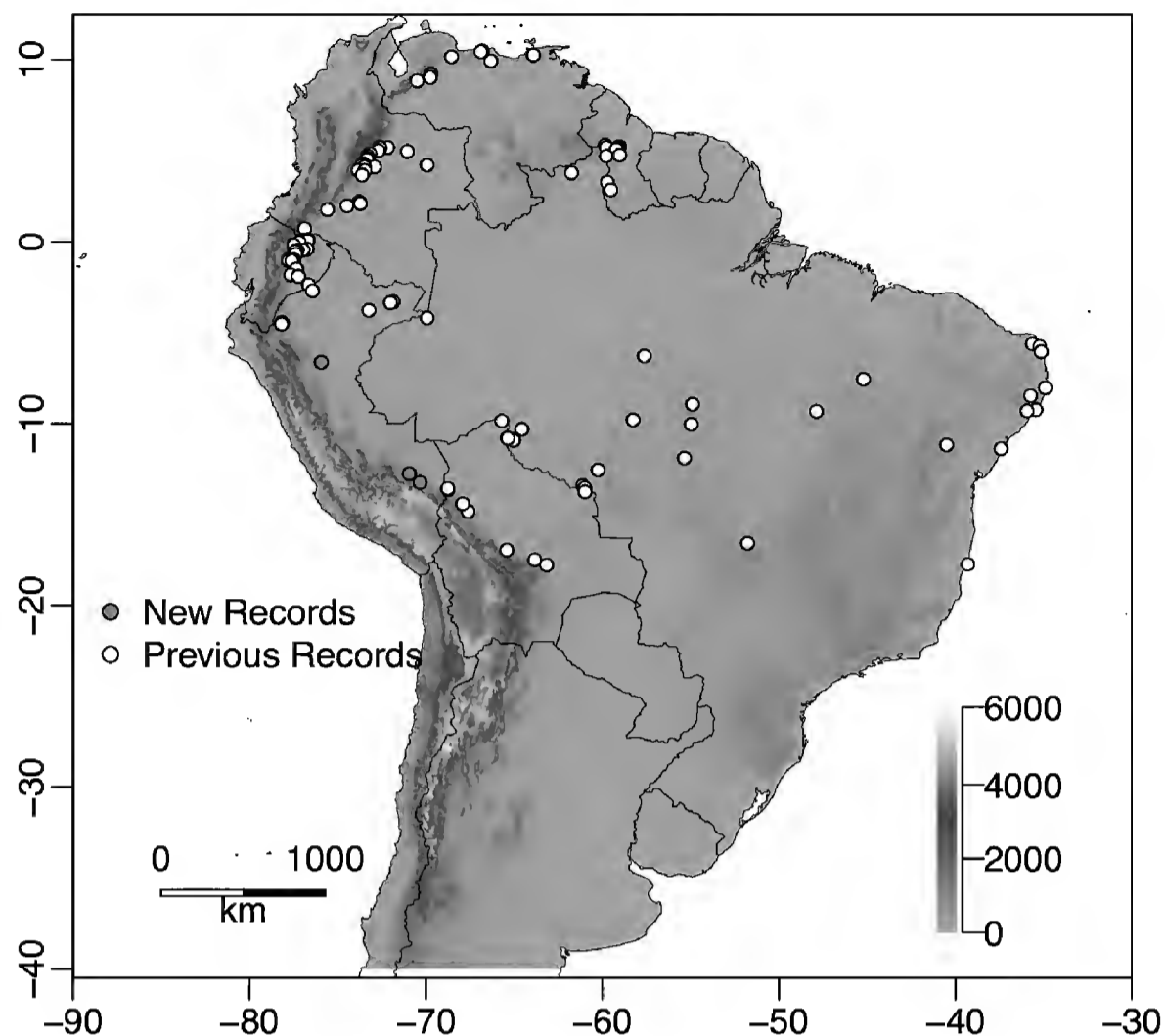
The Amazon River Frog *Lithobates palmipes* (Spix, 1824) is an aquatic-breeding species that inhabits various types of rainforest throughout the lowlands of northern South America, including both the Amazon and Orinoco basins, part of the Guyana Shield, the Atlantic Forest, the Cerrado and neighboring areas in Brazil (Hillis and De Sá 1988; Acosta-Galvis 1999; Canedo and Bilate 2005; Oliveira et al. 2010; Ferreira and Faria 2011; Ramalho et al. 2011; Santos and Vaz-Silva 2012; Rodrigues et al. 2013; Frost 2015). According to Hillis and De Sá (1988), this species belongs to the complex *Rana palmipes*. Frost et al. (2006) placed this and other closely related species in the genus *Lithobates*, a name originally proposed by Fitzinger in 1843. Differences in the recommended species name vary according to different classification criteria (e.g., *Rana palmipes* vs. *Lithobates palmipes*), and were thoroughly discussed by Hillis (2007). However, this species may contain cryptic species (Hillis and Wilcox 2005). In this report, we use the binomen *Lithobates palmipes* because it is still widely accepted, though we recognize that an equally valid alternative would be to treat *Lithobates* as a subgenus of *Rana* in order to preserve a long-standing taxonomy (Hillis and Wilcox 2005; AmphibiaWeb 2015).

Previous studies documenting the distribution of *L. palmipes* in South America (e.g., Hillis and De Sá 1988; Canedo and Bilate 2005; Rodrigues et al. 2013)

provided records from Loreto Region, northern Peru (a Región in Peru is equivalent to a federal state; it was formerly known as Departamento), but its distribution along the Peruvian Amazon remains poorly known. It is notable that *L. palmipes* had not been detected in other well-studied lowland sites in Peru, such as Panguana Biological Station (Schlüter et al. 2004), Cuzco Amazónico (Duellman 2005), Los Amigos Biological Station (von May et al. 2009, 2010), and Cocha Cashu Biological Station in Manu National Park (Catenazzi et al. 2013), despite intensive surveys conducted at those sites. In this report, we provide new distributional data for *L. palmipes* in Peru and update the map of its known distribution in South America. We used the morphological diagnoses provided by Hillis and De Sá (1988) to identify specimens and took measurements to the nearest 0.1 mm with calipers under a stereomicroscope.

Our report is based on field observations and the collection of voucher specimens from two localities in southern Peru, and an additional observation (with a photographic voucher) from northern Peru (Fig. 1). On 09 April 2009, a juvenile individual of *L. palmipes* was collected at Lechemayo, Carabaya Province, Puno Region (13°15'7.39"S, 70°20'18.44"O, 390 m elevation). This specimen was deposited in the Herpetological Collection of the Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru, with voucher number

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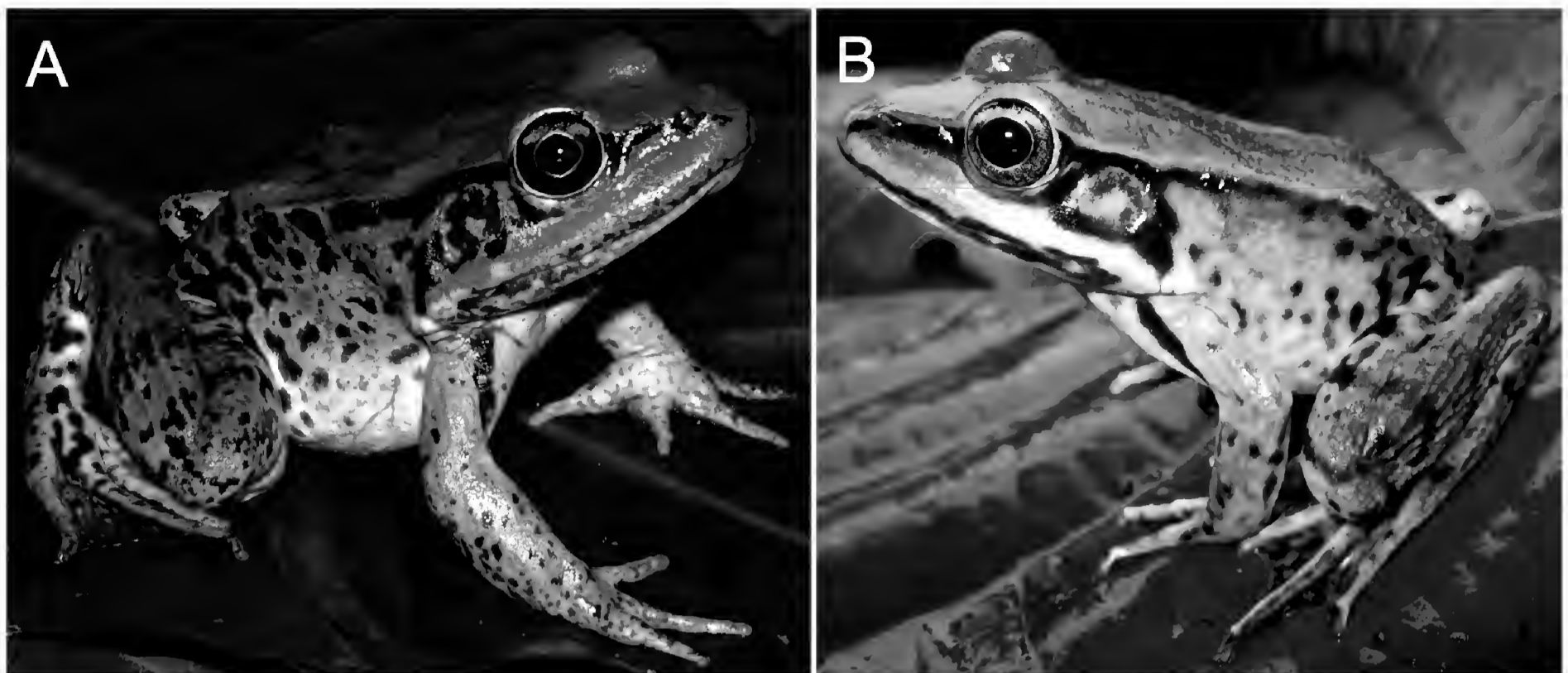
**Fig. 1.** Known distribution of *Lithobates palmipes* in South America and location of new records in Peru. White circles represent literature data and red circles indicate the new records in San Martín (<http://www.inaturalist.org/observations/2384262>), Madre de Dios (MUSA-3722, MUSA-3723) and Puno (MHNC-7864) regions.

MHNC-7864 (snout-vent length 57.07 mm). On 28 May 2011, two individuals of *L. palmipes* were collected at the Reserva Comunal Amaraeri, Manu Province, Madre de Dios Region ( $12^{\circ}46'20.26''S$ ,  $70^{\circ}56'44.56''O$ , 367 m elevation). Both specimens were found on the ground at a slow-moving stream dissecting a middle floodplain forest scattered with bamboo. These specimens were deposited in the Herpetological Collection of the Museo de Historia Natural (MUSA), Universidad Nacional de San Agustín de Arequipa, Peru, with voucher numbers MUSA-3722 and MUSA-3723 (snout-vent length 119.30 mm and 118.10 mm, respectively; see Table 1 for additional morphometric data). The third locality record is supported by a field observation made by Alessandro Catenazzi on 15 July 2002 at Callanayacu, at the border of the Cordillera Azul National Park, San Martín Region, 320 m (photographic voucher: <http://www.inaturalist.org/observations/2384262>). In addition to the new records reported here, we updated the known distribution of *L. palmipes* in Bolivia using georeferenced data published by Reichle (2007).

This report represents an extension of >175 km of the known geographic range *L. palmipes* in southwestern Amazonia. Furthermore, it is worth noting that two other species of *Lithobates* have been recorded in Peru: *L. bwana* and *L. catesbeianus* (Catenazzi and von May 2014). One of these, the American Bullfrog, *L. catesbeianus*, is an exotic species that has invaded various

South American ecosystems and its presence in northern Peru was confirmed recently (Cossios 2010). As such, this exotic species could pose a threat to many native aquatic-breeding frogs including *L. palmipes*. Given that both *L. palmipes* and *L. catesbeianus* may inhabit similar types of water bodies such as slow-moving streams, seasonal ponds, swamps, and flooded forests (Duellman 1978; La Marca et al. 2010), continuous field assessments in areas where these species have been sighted is a priority (Catenazzi and von May 2014).

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**Fig. 2.** Individuals of *Lithobates palmipes* recorded in this study. (A) Adult, female (MUSA-3722) from Reserva Comunal Amara-kaeri, Manu Province, Madre de Dios Region, Peru. (B) Juvenile MHNC-7864 from Lechemayo, Carabaya Province, Puno Region, Perú. Photographs by Roy Santa Cruz (A) and Amanda Delgado (B).

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**Table 1.** Measurements (in mm) of two adult female individuals of *Lithobates palmipes*. SVL = snout–vent length, TL = tibia length, FL = foot length, HL = head length, HW = head width, ED = eye diameter, TY = tympanum diameter, IOD = interorbital distance, EW = upper eyelid width, IND = internarial distance, E–N = eye–nostril distance.

Character	MUSA-3722	MUSA-3723
SVL	114.2	112.57
TL	57.97	57.72
FL	57.7	56.61
HL	47.13	46.53
HW	46.22	46.4
ED	13.27	12.67
TY	10.37	10.93
IOD	10.78	10.65
EW	9.77	8.8
IND	9.83	9.78
E–N	11.41	11.39

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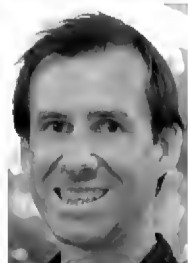
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**Rudolf von May** is a postdoctoral research fellow at the Department of Ecology and Evolutionary Biology at the University of Michigan. His current research seeks to understand how amphibian and reptile communities are structured across habitats and elevations, taking into account the phylogenetic relatedness among species present in those communities.



## A new species of Andean microteiid lizard (Gymnophthalmidae: Cercosaurinae: *Pholidobolus*) from Peru, with comments on *P. vertebralis*

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**Abstract.**—Based on morphological and molecular evidence, herein is reported the discovery of a new species of *Pholidobolus* from the Andes of northwestern Peru. The new species is known from the montane forests of Cajamarca and Lambayeque departments, at elevations of 1,800–2,300 m. It differs from other species of *Pholidobolus* in lacking prefrontal scales and having both strongly keeled dorsal scales and a diagonal white bar in the rictal region. Additionally, it is shown that records of *P. vertebralis* from Peru are based on misidentified specimens. The southernmost distribution records of *P. vertebralis* are from northwestern Ecuador. Also, an updated identification key for species of *Pholidobolus* is provided.

**Key words.** Andes, hemipenial morphology, lizards, *Pholidobolus vertebralis*, systematics

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### Introduction

Lizards in the New World family Gymnophthalmidae Merrem 1820 are small, with elongate bodies and relatively short limbs, which are reduced in various degrees in some species and nearly absent in others (Pianka and Vitt 2003). Gymnophthalmidae comprises 47 taxa traditionally ranked as genera with 253 species (Uetz and Hošek 2016). The diversity of gymnophthalmid lizards is high in both the Amazonian rainforests and the Andes (Presch 1980). Some genera like *Euspondylus*, *Gelanesaurus*, *Macropholidus*, *Pholidobolus*, *Petracola*, *Proctoporus*, and *Riama* are restricted to the Andes and reach high elevations. For example, *Proctoporus bolivianus* can be found at 4,080 m in Peru (Duellman 1979).

Species of *Pholidobolus* occur between 1,800 and 4,000 m along the northern Andes from northern Peru in the Huancabamba Depression to extreme southern Colombia (Torres-Carvajal and Mafla-Endara 2013). Montanucci (1973) defined *Pholidobolus* using morphological characters and recognized five species: *P. affinis* (Peters 1863), *P. annectens* (Parker 1930), *P. macbrydei* Montanucci 1973, *P. montium* (Peters 1863), and *P. prefrontalis* Montanucci 1973. Twenty-three years later Reeder (1996) described *P. huancabambae*. However, recent taxonomic changes have been proposed based on molecular phylogenetic evidence. Two species of *Pholidobolus*, *P. annectens*, and *P. huancabambae*, were allocated in its sister clade, *Macropholidus* (Torres-Carvajal and Mafla-Endara 2013). More recently, “*Cercosaura*” *dicra* (Uzzell, 1973) and “*C.*” *vertebralis*

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O'Shaughnessy 1879 were found to be members of *Pholidobolus* (Torres-Carvajal et al. 2015), increasing the number of species in this genus to seven, including the recently described *P. hillisi* (Torres-Carvajal et al. 2014).

Morphologically, members of *Macropholidus* and *Pholidobolus* can be distinguished from each other by the presence of a single palpebral disk in the lower eyelid in *Macropholidus* (divided in *Pholidobolus*), and the lack of a lateral fold in *Macropholidus* (present in *Pholidobolus*). Nonetheless, the phylogenetic position of *P. anomalus* Müller 1923, a geographically disjunct species from southern Peru, is still uncertain (Montanucci 1973; Reeder 1996; Torres-Carvajal and Mafla-Endara 2013).

Herein, based on morphological and previously published molecular evidence (Torres-Carvajal et al. 2015 and 2016), we report the discovery of a new species of *Pholidobolus* collected in different field trips to montane forests in the Andes of northwestern Peru. This discovery increases the number of species of *Pholidobolus* to eight.

## Materials and Methods

All type specimens of the new species described in this paper were deposited in the herpetological collection of Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru. Specimens used for comparisons are housed at Museo de Zoología, Pontificia Universidad Católica de Ecuador, Quito (QCAZ) (Appendix I). The following measurements were taken with digital calipers and recorded to the nearest 0.1 mm, except for tail length (TL), which was taken with a ruler and recorded to the nearest millimeter: head length (HL), head width (HW), shank length (ShL), axilla-groin distance (AGD), and snout-vent length (SVL). Sex was determined either by dissection or by noting the presence of everted hemipenes. We follow the terminology of Reeder (1996) for the description of the holotype and scale counts, and Montanucci (1973) for the diagnosis. Morphological data from other species of *Pholidobolus* were taken from the literature (Montanucci 1973; Reeder 1996; Torres-Carvajal et al. 2014).

The left hemipenis of the holotype (CORBIDI 12734) was prepared following the procedures described by Manzani and Abe (1988), modified by Pesantes (1994) and Zaher (1999). The retractor muscle was manually separated and the everted organ filled with stained petroleum jelly. The organs were immersed in an alcoholic solution of Alizarin Red for 24 hours in order to stain eventual calcified structures (e.g., spines or spicules), in an adaptation proposed by Nunes et al. (2012) on the procedures described by Uzzell (1973) and Harvey and Embert (2008). The terminology of hemipenial structures follows previous literature (Dowling and Savage 1960; Savage 1997; Myers and Donnelly 2001, 2008; Nunes et al. 2012).

## Results

**Systematics:** The taxonomic conclusions of this study are based on the observation of morphological features and color pattern, as well as on previously inferred phylogenetic relationships based on molecular data (Torres-Carvajal et al. 2015). We consider this information as species delimitation criteria following a general lineage or unified species concept (de Queiroz 1998, 2007).

### *Pholidobolus ulisesi* sp. nov.

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Figs. 1–3.

*Cercosaura vertebralis*—Doan and Cusi 2014 (part): 1,195–1,200.

*Pholidobolus* sp.—Torres-Carvajal et al. 2015: 286.

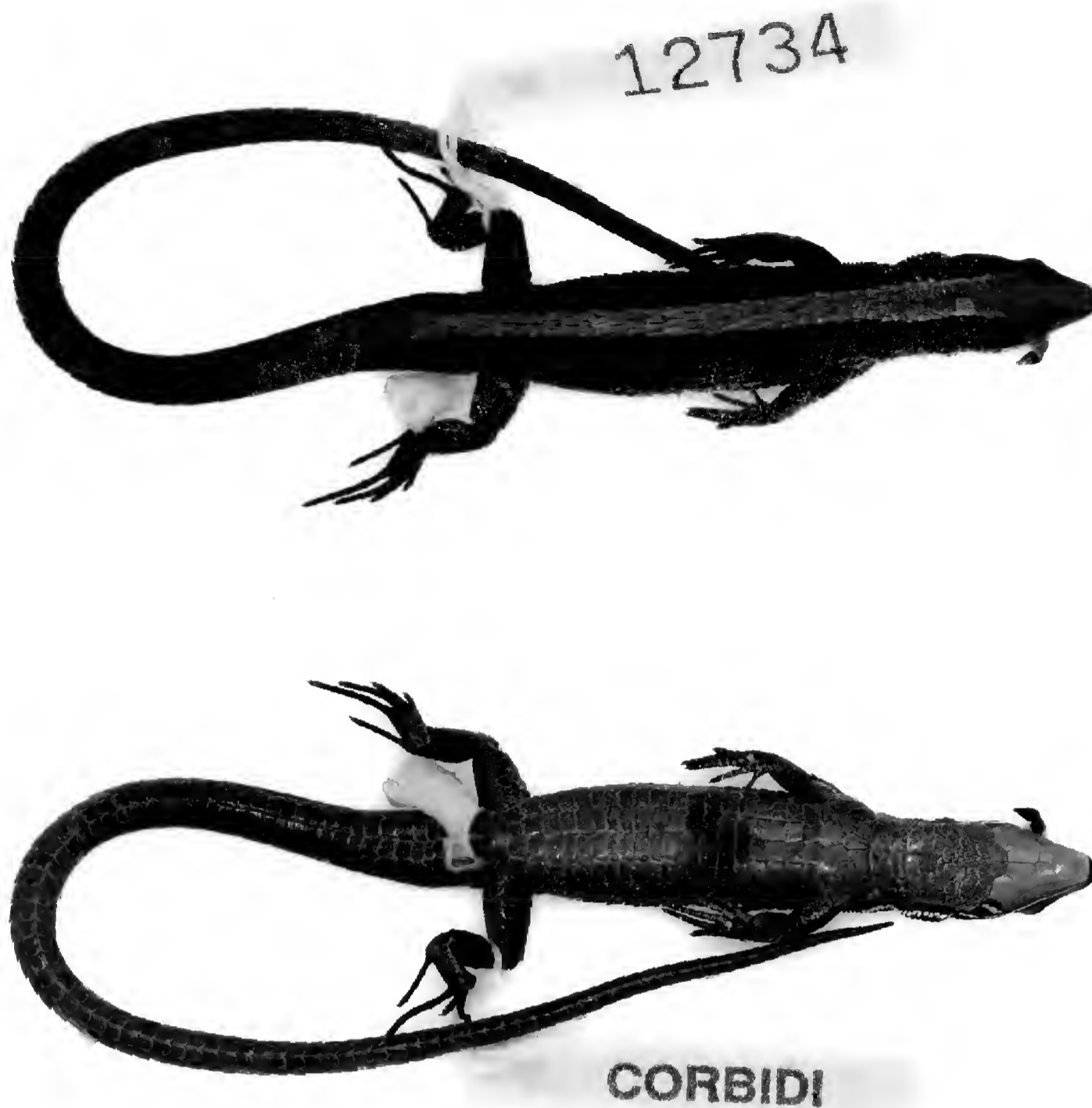
*Pholidobolus* sp.—Torres-Carvajal et al. 2016: 70 (Fig. 2).

**Holotype:** CORBIDI 12734, an adult male from Bosque de Huamantanga (5°39'48.09" S, 78°56'35.8" W), at 2,211 m elevation, Huabal district, Jaén province, Cajamarca department, Peru, collected on 7 March 2013 by P.J. Venegas.

**Paratypes (17):** CORBIDI 12740–46 juveniles, CORBIDI 12735–36, 12739 adult males, CORBIDI 12737–38 adult females, all collected with the holotype; CORBIDI 00871–73, an adult female, an adult male and a juvenile, respectively, from El Chaupe (5°14'8.16" S, 79°5'56.58" W), at 2,016 m elevation, Namballe district, San Ignacio province, Cajamarca department, Peru, collected by M. Doherty on 24 August 2008; CORBIDI 14889, an adult female, and CORBIDI 14896, a juvenile, from San Felipe de Jaén (5°45'10.854" S, 79°14'19.881" W), at 2,641 m elevation, Jaén province, Cajamarca department, Peru collected by K. Garcia on 26 September 2014.

**Photo voucher specimen:** Cañaris (6°03'26.18" S, 79°16'00.35" W), at 2,318 m elevation, Ferreñafe province, Lambayeque department, Peru, captured and released by P.J. Venegas on 25 May 2007 (Fig. 3D).

**Diagnosis:** *Pholidobolus affinis*, *P. dicrus* (Fig. 4A), *P. hillisi* (Fig. 4B), *P. prefrontalis*, and *P. vertebralis* (Fig. 4C) differ from the new species in having prefrontal scales. *Pholidobolus montium* and *P. macbrydei* have striated and quadrangular dorsal scales (strongly keeled and hexagonal in *P. ulisesi*), and lack the conspicuous narrow, pale brown, vertebral stripe present in *P. ulisesi*. In addition, the new species has fewer dorsal scales (28–31,  $\bar{x}$



**Fig. 1.** Holotype of *Pholidobolus ulisesi* sp. nov. (CORBIDI 12734; male, SVL = 45.5 mm) in dorsal (**top**) and ventral (**bottom**) views. Photographs by OTC.

= 29.75) than *P. affinis* (45–55), *P. montium* (35–50), *P. prefrontalis* (37–46), and *P. macbrydei* (31–43).

**Characterization:** (1) Two or three supraoculars, anteriormost larger than others; (2) prefrontals absent; (3) femoral pores absent in both sexes; (4) two to six opaque lower eyelid scales; (5) scales on dorsal surface of neck striated, becoming strongly keeled between forelimbs and tail; (6) two or three rows of lateral granules at midbody; (7) lateral body fold present; (8) usually two rows of keeled ventrolateral scales on each side; (9) dorsum dark brown with a distinct pale brown middorsal stripe, slender at midbody, becoming grayish brown towards the tail; (10) labial stripe white becoming cream or pale brown along ventrolateral region; (11) sides of body dark brown; (12) cream stripe along forearm; (13) a distinct diagonal white bar with dark brown edges on each side of the mandible, extending from sixth infralabial to proximal preangular; (14) orange spots on sides of body, usually above forelimb and the base of tail in adult males.

**Description of holotype:** Adult male (CORBIDI 12734; Fig. 1–3A); SVL 45.5 mm; TL 104 mm; dorsal

and lateral head scales juxtaposed, finely wrinkled; rostral hexagonal, 2.03 times as wide as high; frontonasal quadrangular, wider than long, longer than frontal, laterally in contact with nasal, loreal, and first superciliary; prefrontals absent; frontal pentagonal, longer than wide, slightly wider anteriorly, in contact with frontonasal and supraocular I on each side; frontoparietals hexagonal, longer than wide, with medial suture, each in contact laterally with supraoculars I and II; interparietal roughly heptagonal, its lateral borders parallel to each other; parietals slightly smaller than interparietal, pentagonal and positioned anterolaterally to interparietal, each in contact anteriorly with supraocular II and dorsalmost postocular; postparietals three, medial scale smaller than laterals; supralabials seven, fourth longest and below the center of eye; infralabials five, fourth below the center of eye; temporals enlarged, irregularly pentagonal or hexagonal, juxtaposed, finely wrinkled; two finely wrinkled supratemporals, dorsal conspicuously larger than ventral one; nasal divided, irregularly tetragonal, longer than wide, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally, loreal posterodorsally and frenocular posteroventrally; nostril on ventral aspect





**Fig. 2.** Head of the holotype of *Pholidobolus ulisesi* sp. nov. (CORBIDI 12734) in dorsal (A), ventral (B), and lateral (C) views. Photographs by OTC.

of nasal, directed lateroposteriorly, piercing nasal suture; loreal rectangular; frenocular enlarged, in contact with nasal, separating loreal from supralabials; supraoculars two, with the first being the largest; four elongate superciliaries, first one enlarged, in contact with loreal; palpebral disk divided into two pigmented scales; suboculars three, elongated and similar in size; three postoculars, ventral one smaller than the others; ear opening vertically oval, without denticulate margins; tympanum recessed into a shallow auditory meatus; mental semicircular, wider than long; postmental pentagonal, slightly wider than long, followed posteriorly by three pairs of genials, the anterior two in contact medially and the posterior one separated by postgenials; all genials in contact with infralabials; gulars imbricate, smooth, widened in two longitudinal rows; gular fold incomplete; posterior row of gulars (collar) with two enlarged scales medially, larger than the anterior gulars.

Scales on nape similar in size to dorsals, except for the anteriormost that are widened; scales on sides of neck small and granular; dorsal scales elongated, imbricate,

arranged in transverse rows; dorsal scales on nape striated, becoming progressively keeled from forelimbs to tail; number of dorsal scales between occipital and posterior margin of hind limbs 30; dorsal scale rows in a transverse line at midbody 19; dorsals separated from ventrals by two longitudinal rows of large keeled scales on each side; longitudinal fold between fore and hind limbs present; ventrals smooth, wider than long, arranged in 21 transverse rows between collar fold and preanals; six ventral scales in a transverse row at midbody; subcaudals smooth; limbs overlap when adpressed against body; axillary region composed of granular scales; scales on dorsal surface of forelimb striated, imbricate; scales on ventral surface of forearm small and imbricate, those on ventral surface of arm granular; two thick, smooth thenar scales; supradigitals (left/right) 3/3 on finger I, 6/6 on II, 8/8 on III, 9/9 on IV, 6/6 on V; supradigitals 3/3 on toe I, 6/6 on II, 10/9 on III, 12/11 on IV, 8/8 on V; subdigital lamellae of forelimb single, 6/6 on finger I, 11/12 on II, 15/16 on III, 16/16 on IV, 9/8 on V; subdigital lamellae on toes I and II single, on toe III paired on the middle, on toe IV paired except for a few ones, on toe V paired at the base; number of subdigital lamellae (pairs when applicable) 6/6 on toe I, 10/11 on II, 16/17 on III, 21/21 on IV, 12/12 on V; groin region with small keeled, imbricate scales; scales on dorsal surface of hind limbs keeled and imbricate; scales on ventral surface of hind limbs smooth; scales on posterior surface of thighs granular and on shanks striated and imbricate; femoral pores absent; preanal pores absent; cloacal plate paired, bordered by two scales anteriorly, smaller than cloacal scales.

Additional measurements (mm) and proportions of the holotype: HL 9.91; HW 6.95; ShL 3.9; AGD 25.6; TL/SVL 2.05; HL/SVL 0.21; HW/SVL 0.15; ShL/SVL 0.08; AGD/SVL 0.56.

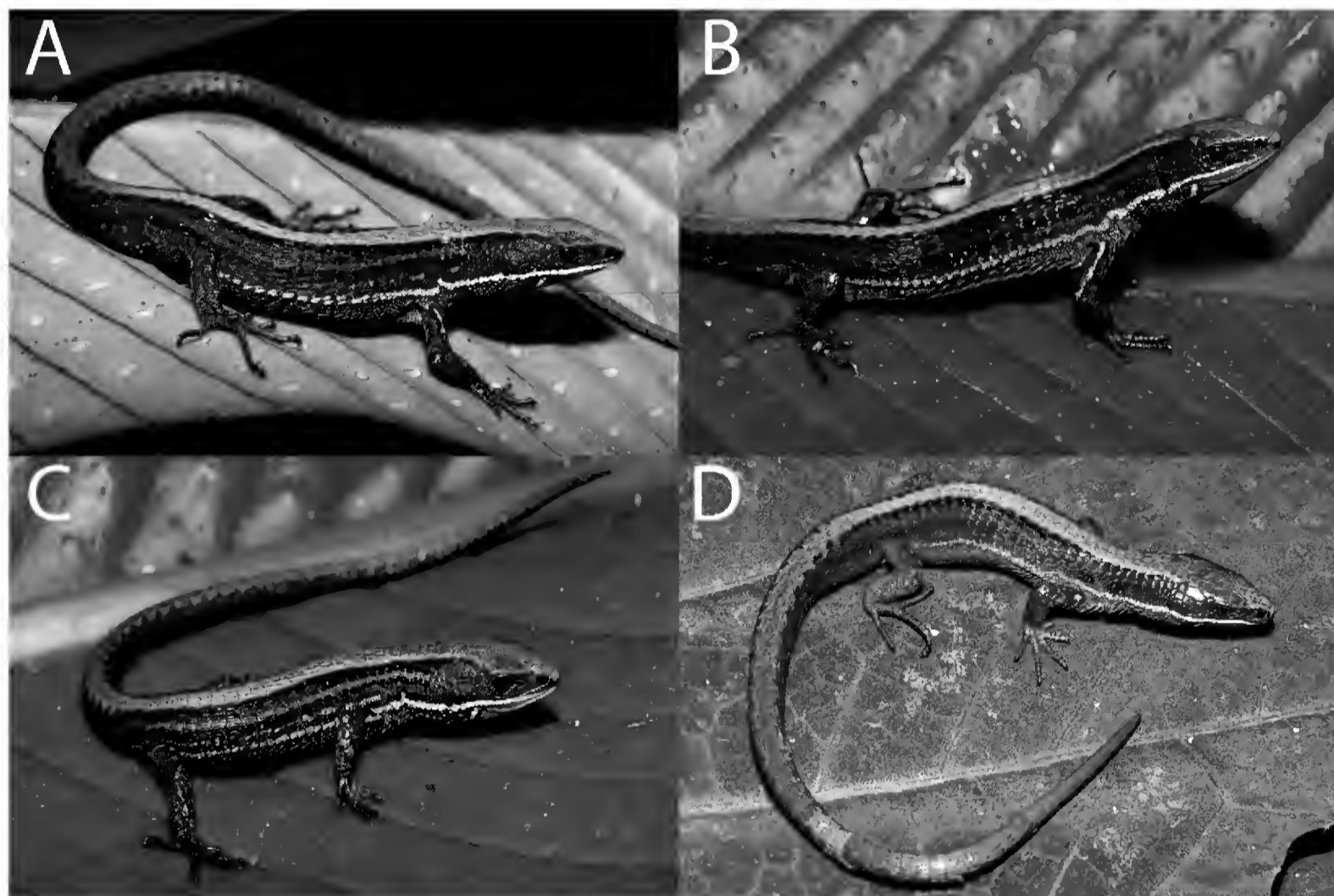
**Coloration in preservative (Figs. 1 and 2):** Dorsum dark brown with a grayish brown vertebral stripe that is four scales broad at midbody, and extends from occiput onto tail; vertebral stripe wide anteriorly becoming slightly slender at midbody; dorsal surface of head brown, sides of head and body dark brown; two bright cream spots on each side above insertion of forelimbs; light stripe extending ventrolaterally from lips to insertion of hind limbs, white on lips and grayish brown along the body; a distinct diagonal white bar with dark edges on each side of the mandible, extending from the sixth infralabial onto the proximal pregular; dorsal surface of limbs dark brown with a cream stripe along the arms; gular region pale gray, chest and venter dark gray; ventral surface of tail dark gray.

**Coloration of holotype in life (Fig. 3A):** Similar to that in preservative, but the bright cream spots on each side above forelimbs are replaced by two black ocelli with red centers, and the sides of the base of the tail have scattered red flecks. The iris is light brown.

## A new species of Andean microteiid lizard

**Table 1.** Squamation characters of *Pholidobolus ulisesi*. Range, followed by mean  $\pm$  standard deviation, is given for quantitative characters (if applicable). \*Includes adults of both sexes and 10 juvenile specimens of undetermined sex.

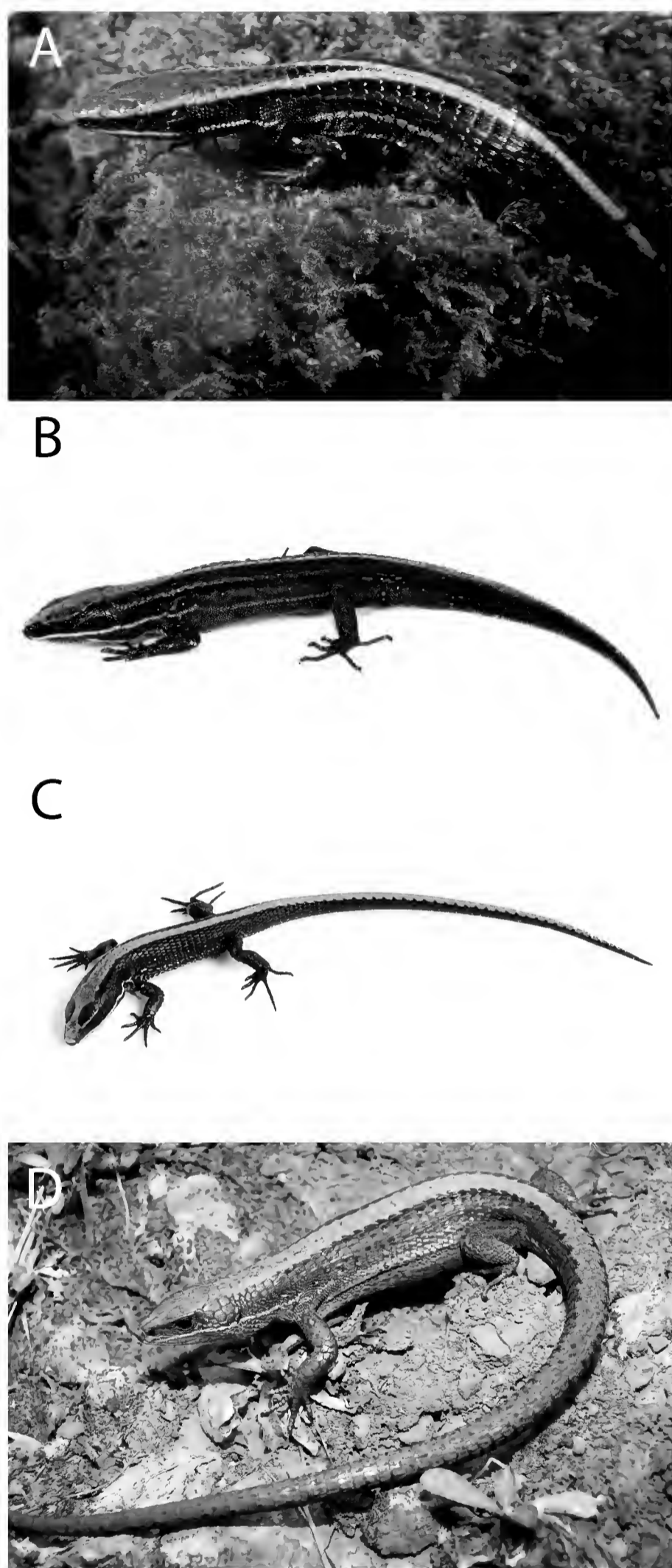
Characters	<i>Pholidobolus ulisesi</i>		
	Males <i>n</i> = 5	Females <i>n</i> = 4	All specimens* <i>n</i> = 19
Dorsal scales between occipital and posterior margin of hind limb	28–31 29.6 $\pm$ 1.14	29–32 30.6 $\pm$ 1.14	28–32 30.05 $\pm$ 1.13
Dorsal scale rows in a transverse line at midbody	19–22 20.4 $\pm$ 1.14	18–21 19.6 $\pm$ 1.34	17–22 20.05 $\pm$ 1.43
Ventral scales between collar fold and preanals	20–21 20.75 $\pm$ 0.5	20–23 20.8 $\pm$ 1.3	20–23 21.06 $\pm$ 0.87
Ventral scale rows in a transverse line at midbody	6	6–8 6.8 $\pm$ 1.1	6–8 6.56 $\pm$ 0.92
Subdigital lamellae on Finger IV	15–16 15.6 $\pm$ 0.55	15–17 15.8 $\pm$ 0.84	11–18 15.05 $\pm$ 1.65
Subdigital lamellae on Toe IV	20–21 20.4 $\pm$ 0.55	18–22 20.6 $\pm$ 1.52	15–22 19.32 $\pm$ 1.95
Maximum SVL	45.52	57.46	57.46
TL/SVL	1.92–2.28 2.12 $\pm$ 0.18 ( <i>n</i> = 3)	1.83–2.17 2.05 $\pm$ 0.19 ( <i>n</i> = 3)	1.83–2.28 2.05 $\pm$ 0.18 ( <i>n</i> = 7)



**Fig. 3.** Four individuals of *Pholidobolus ulisesi* sp. nov. in life. (A) holotype (CORBIDI 12734); (B) adult female (CORBIDI 12737); (C) juvenile (CORBIDI 12744); (D) adult male from Cañaris (photo voucher). Photographs by PJV.

**Variation:** Variation in measurements and scutellation of *Pholidobolus ulisesi* is presented in Table 1. Usually two supraoculars, 2/3 (left/right) in specimen CORBIDI 12742; superciliaries usually four, 3/4 in CORBIDI 12749, 6/5 in CORBIDI 00873, and 5/5 in CORBIDI 00872; little intrusive scales present on each side, in the posterior angle of frontonasal in three specimens (CORBIDI 12735, 12741, 12744); usually seven supralabials, 7/6 in CORBIDI 00871, 12738 and 6/6 in CORBIDI 12742–43; infralabials usually six, 5/5 in CORBIDI 12738, 12740, 12742, 6/5 in CORBIDI 00873, 12744

and 5/6 in CORBIDI 12735, 12743. Rows of ventrolateral keeled scales vary from two rows in nine specimens (56% of the type series), one row on each side in three specimens (CORBIDI 00872, 12741, and 12745), three rows on each side in one specimen (CORBIDI 12739), and absent in two adult specimens (CORBIDI 00871 and CORBIDI 00873). Usually two scales on posterior cloacal plate, only two specimens (CORBIDI 12737–38) have three scales, and two other specimens (CORBIDI 00871 and 00873) have four scales.



**Fig. 4.** Four species of *Pholidobolus*. (A) adult female of *P. dicrus* (QCAZ 5304); (B) adult male of *P. hillisi* (QCAZ 4999); (C) a juvenile of *P. vertebralis* (QCAZ 5082); (D) adult female of *P. sp.* from La Granja (CORBIDI 1678). Photographs by: (A) and (B) Santiago R. Ron, (C) OTC, and (D) PJV.

Males can be distinguished from females by having the contacted margins of rostral and mental distinctly dark brown or black (indistinct or not contrasting in females), and by the presence of red or orange spots above the insertion of forelimbs and on the sides of the base of tail (absent in females; Fig. 3B). Females are longer

(maximum SVL 57.4 mm,  $n = 4$ ) than males (maximum SVL 45.5 mm,  $n = 5$ ). Juvenile CORBIDI 12743 (Fig. 3C) differs from adults in having a fragmented dirty cream stripe along the flanks above the ventrolateral stripe.

**Hemipenial morphology:** The left hemipenis of the holotype of *Pholidobolus ulisesi* (Fig. 5) was everted during preservation and prepared posteriorly. The organ extends along approximately eight millimeters in length. The lobes of the organ are partially everted and the hemipenis is fully expanded. The hemipenial body is roughly conical in shape, with the basis distinctly thinner than the rest of the organ, and bears two small lobes with apical folds in the apex. The sulcus spermaticus is central in position, originating at the base of the organ, and proceeding in a straight line towards the lobes. The sulcus is broader in the region of the lobular crotch, where it is divided by a small fleshy fold; its branches lie on the medial region of the lobes, and end in their tips among folds. The sulcate face of the hemipenial body presents two nude areas parallel to the sulcus spermaticus that run along the entire hemipenial body.

The lateral and asulcate faces of the hemipenis are ornamented with a series of roughly equidistant flounces with calcareous spinules. Twenty-three rows of flounces extend along the body of the organ. There are four proximal rows restricted to a central position on the basal asulcate face of the hemipenis, all of them are roughly chevron-shaped. The four proximal flounces on the sides are diagonally positioned; the third to fifth flounces are separated from a complementary flounce positioned on the asulcate face and oriented in an inverse diagonal. The subsequent flounces towards the lobes cross the sides of the organ from the sulcate to the asulcate face, forming chevrons with vertices in the central region of each side pointing towards the basis of the organ. These chevron-shaped rows become reduced in size progressively towards the hemipenial apex. Similar to the description of the hemipenis of *Cercosaura vertebralis* by Uzzell (1973), the five distalmost lateral flounces of the hemipenis have an enlarged tooth in the vertex of the chevrons.

The lateral flounces are separated in two groups by a nude area in the central asulcate face that increases in size in the apical region, becoming Y-shaped. The region between the asulcate and lateral sides are marked by a conspicuous unevenness forming a distinctive bulge, which is also present in other species of the *Macropholidus* + *Pholidobolus* clade (*Macropholidus annectens*, *M. huancabambae*, *M. ruthveni*, *Pholidobolus affinis*, *P. hillisi*, *P. macbrydei*, *P. montium*, *P. prefrontalis*, *P. vertebralis*; Nunes, 2011; Torres-Carvajal et al. 2014).

The hemipenis of the holotype of *P. ulisesi* described herein (Fig. 5) is broadly congruent with the illustrated by Doan and Cusi (2014) for a specimen of *P. ulisesi*, considered by them as *P. vertebralis* (see “Discussion”

hereafter). Although Doan and Cusi (2014) reported a reduced count of flouces ornamenting the organ (14 versus 23 in the holotype of *P. ulisesi*), their Fig. 5B clearly shows at least 18 visible flouces ornamenting the hemipenis sides, plus other flouces not countable due the positioning of the organ and the lack of focus in some areas of the hemipenis photograph. Similar to the hemipenis of *P. ulisesi* described by Doan and Cusi (2014), but contrasting with the hemipenis of *P. vertebralis* illustrated by Hernández-Ruz and Bernal-González (2011) for a specimen from Nariño, Colombia, the hemipenis of the holotype of *P. ulisesi* presents the four flouces in basal position at the asulcate face separated from the other flouces ornamenting the hemipenis laterally. In the drawing presented by Hernandez-Ruz (2005) for *Cercosaura ampuedai* (synonym of *P. vertebralis* according to Doan and Cusi [2014]) such flouces are not visible, probably due the distally misplaced tie made during hemipenial preparation.

**Distribution and natural history observations:**

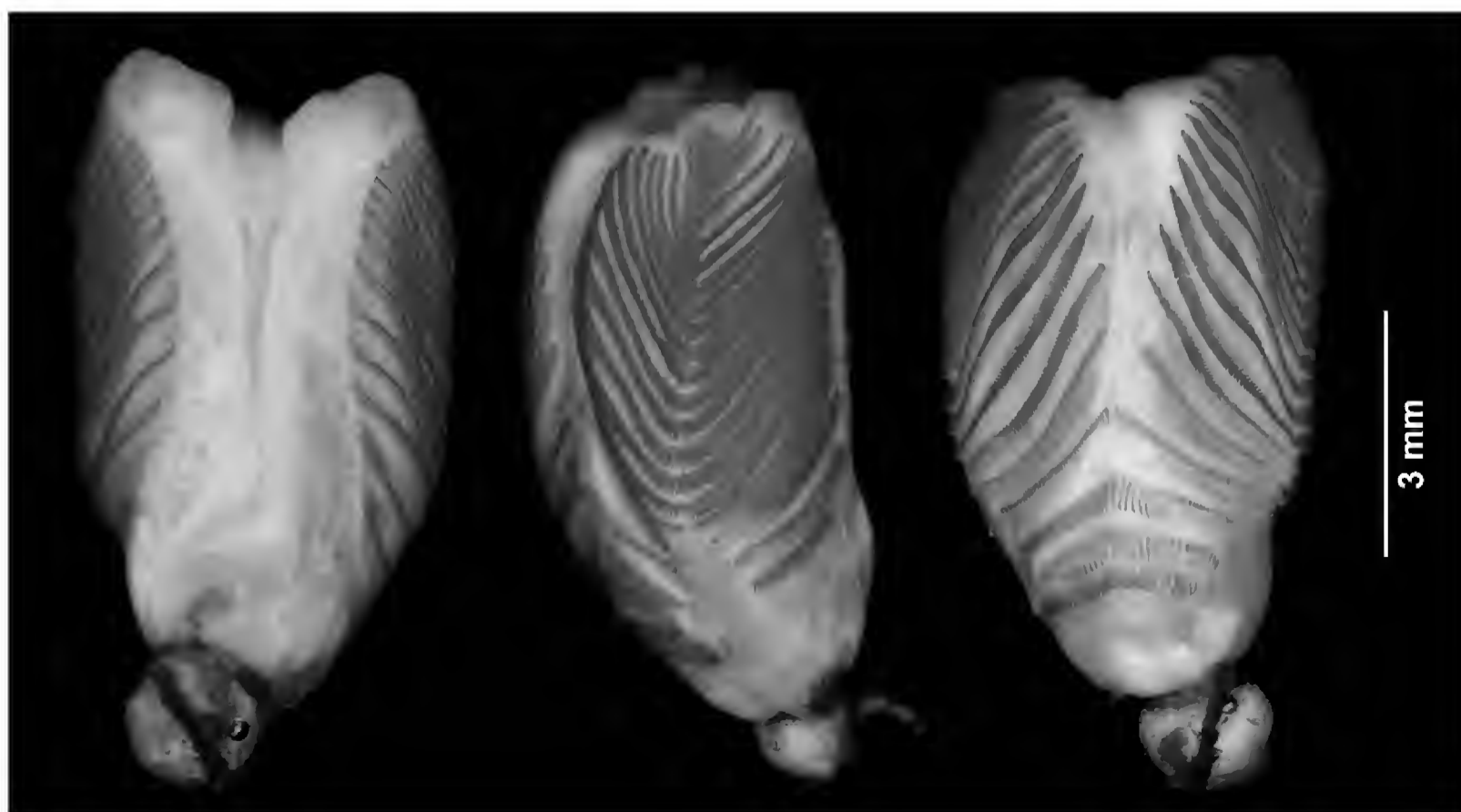
*Pholidobolus ulisesi* is known from five localities at elevations of 1,900–2,300 m in Cajamarca and Lambayeque departments, northern Peru (Fig. 6). All recorded localities lie within the Huancabamba depression, a region where the relatively low altitude of the Andean mountains causes fragmentation of montane habitats, and the northern extreme of the Central Andes at Cordillera Occidental in northern Peru. According to the terrestrial ecoregions of the world by Olson et al. (2001), *P. ulisesi* occurs within Eastern Cordillera real montane forest and Marañón dry forest.

*Pholidobolus ulisesi* was found during the day in sunny and cloudy conditions in secondary montane forest, in the edges of primary montane forest and recently opened areas for cattle ranching, as well as in small plantations

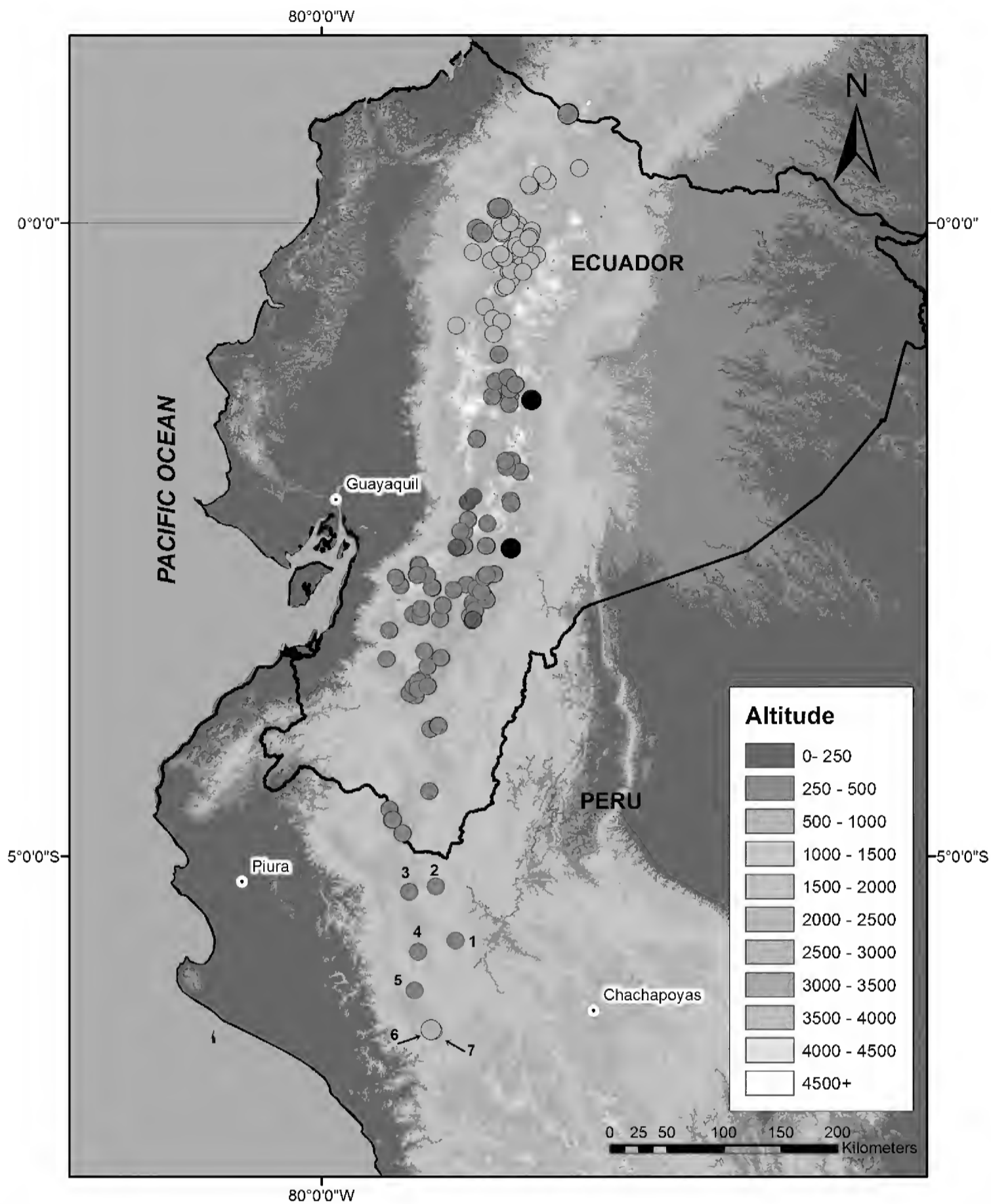
of bean and coffee. In the open cattle-ranching areas, *P. ulisesi* was found moving on fallen trees or hiding under trunks; in secondary montane forest, the lizards were found foraging within herbaceous vegetation and running through the patches of grass. They were especially abundant in coffee and bean plantations, where they were observed running through the herbaceous vegetation and hiding in leaf litter. Sympatric squamate reptiles collected with *P. ulisesi* were *Chironius monticola* and *Dipsas peruana* at El Chaupe and Huamantanga, and *Chironius monticola*, *Epictia teaguei*, *Erythrolamprus taeniurus*, *Micrurus peruvianus*, *Stenocercus arndti*, *S. huancabambae*, and *S. stigmatosus* at Quebrada La Iraca.

**Etymology:** The specific epithet “*ulisesi*” is a noun in the genitive case and a patronym for Ulises Gamonal Guevara, for his significant contribution to the archaeology of Cajamarca in northwestern Peru. One of his major contributions is the discovery of the >6,000-year-old Fajal cave paintings in San Ignacio, declared as Cultural Patrimony of the Nation.

**Remarks:** In a molecular phylogeny of *Cercosaura* and related taxa, Torres-Carvajal et al. (2015) showed, with high support, that *Pholidobolus ulisesi* (*Pholidobolus* sp. in their paper) and *P. hillisi* are sister species. Together they form a clade sister to all other species of *Pholidobolus*. In addition, these authors found that both “*Cercosaura*” *vertebralis* and “*Cercosaura*” *dicra* were nested within *Pholidobolus*, and were therefore referred to this genus (Torres-Carvajal et al. 2015). An identical topology can be observed in a recent molecular phylogeny of the clade Cercosaurinae by Torres-Carvajal et al. (2016). Therefore, we adopt this taxonomic change in the discussion below.



**Fig. 5.** Left hemipenis of *Pholidobolus ulisesi* sp. nov. (CORBIDI 12734 - holotype) in sulcate (**left**), lateral (**middle**), and asulcate (**right**) views. Photographs by PMSN.



**Fig. 6.** Distribution of *Pholidobolus* in Ecuador and Peru (circles): *P. affinis* (green); *P. dicrus* (black); *P. hillisi* (purple); *P. macbrydei* (blue); *P. montium* (gray); *P. prefrontalis* (brown); *P. ulisesi* sp. nov. (red); *P. vertebralis* (orange); and *P. sp.* (pink). Localities for *P. ulisesi* are: (1) Bosque de Huamantanga (type locality); (2) El Chaue; (3) Estación Biológica Chichilapa in the Santuario Nacional Tabaconas Namballe, taken from Doan and Cusi (2014); (4) San Felipe de Jaén; (5) Cañarís; (6) Quebrada La Iraca (near La Granja village); and (7) Quebrada Checos (near La Granja village) taken from Doan and Cusi (2014).

## Discussion

*Pholidobolus vertebralis* has been repeatedly reported for Peru based on misidentified specimens. Uzzell (1973) reported one specimen (LACM 58811) of this species (as *Prionodactylus vertebralis*) from Piura, 11 miles E of Canchaque, on the western slope of the Huancabamba Mountains. He noted, however, that this specimen was different morphologically from other specimens of *P. vertebralis*. Doan and Cusi (2014) confirmed this specimen as *P. vertebralis* even though they also noted important morphological differences with other specimens,

such as the absence of prefrontal scales, an undivided palpebral disk, and the absence of a light vertebral stripe. After reviewing several specimens of *C. vertebralis* from Ecuador ( $n = 22$ ; see Appendix 1), we found that all have prefrontal scales, a divided palpebral disk, and a light vertebral stripe (“*vertebralis*” refers to that stripe). Based on photographs of specimen LACM 58811, as well as its examination by staff of the herpetological collection at the Natural History Museum of Los Angeles County, we were able to identify it as *Macropholidus huancabambae* Reeder 1996. Besides the differences between this specimen and other specimens of *P. vertebralis* noted by

**Key to species of *Pholidobolus***

- 1a. Loreal scale usually present and frequently in contact with supralabials; dorsals striated; conspicuous light vertebral stripe absent..... 2
- 1b. Loreal scale present, not in contact with supralabials; dorsals keeled; conspicuous light vertebral stripe present..... 5
  
- 2a. Prefrontal scales present..... 3
- 2b. Prefrontal scales absent..... 4
  
- 3a. Ocelli on flanks present, supraoculars three..... *P. affinis*
- 3b. Ocelli on flanks absent, supraoculars two..... *P. prefrontalis*
  
- 4a. Sexual dimorphism strong, with males having distinctly broader heads and colorful flanks (red stripes and white flecks)..... *P. macbrydei*\*
- 4b. Sexual dimorphism not very marked, with males having slightly broader heads and inconspicuously colored flanks (different tones of brown stripes)..... *P. montium*
  
- 5a. Prefrontal scales absent..... 6
- 5b. Prefrontal scales present..... 7
  
- 6a. Diagonal white bar along rictal region, extending from the posteriormost infralabial to the proximal preangular..... *P. ulisesi*
- 6b. Diagonal white bar in the rictal region absent..... *P. sp.*
  
- 7a. Vertebral stripe bifurcates anteriorly at midbody..... *P. dicrus*
- 7b. Vertebral stripe straight, not bifurcated..... 8
  
- 8a. Diagonal white bar in the rictal region, extending from the proximal preangular to the forelimb..... *P. hillisi*
- 8b. Diagonal white bar in the rictal region absent..... *P. vertebralis*

\*We observed some specimens of *Pholidobolus macbrydei* with small loreal scales, not contacting supralabials, as well as specimens lacking a loreal scale.

Uzzell (1973) and Doan and Cusi (2014), the dorsal and flank scales are similar in size, whereas in *P. vertebralis* flank scales are noticeably smaller than dorsals.

Doan and Cusi (2014) also reported two new localities for *Pholidobolus vertebralis* in Peru based on misidentified specimens of *P. ulisesi* and an undescribed species of *Pholidobolus*. These localities lie in the Cajamarca department, one in the Tabaconas Namballe Natural Sanctuary (*P. ulisesi*) and the other in Quebrada Checos, approximately one km away from La Granja village (*P. sp.*) (see Fig. 6). Although *P. ulisesi* is similar to *P. vertebralis* (Fig. 4C) in having a dark brown dorsum with a conspicuous narrow middorsal pale stripe, and a white labial stripe that extends posteriorly as a cream or pale brown stripe along the ventrolateral region, it differs from *P. vertebralis* (character states in parenthesis) in lacking prefrontal scales (prefrontals present), and in having a diagonal white bar in the rictal region (rictal bar absent); ocelli above forelimbs and along the sides of the base of tail (ocelli also present along the flanks); a cream stripe along the forearm (stripe absent, one or two ocelli along the forearm); a gray venter in adults of both sexes in preservative (creamy gray with dark gray reticulations

or dark gray with pale marks); middorsal stripe between 3–4 scales wide at midbody (only two scales wide); and slender hemipenial body (robust). In addition, *P. ulisesi* is smaller than *P. vertebralis*, with a maximum SVL of 45.5 mm in males ( $n = 5$ ) and 57.4 mm in females,  $n = 4$  (males 58.9 mm,  $n = 5$ , and females 68.4 mm,  $n = 5$ ). The specimens of *Pholidobolus sp.* from Quebrada Checos reported by Doan and Cusi (2014), and a specimen examined by us from Quebrada la Iraca, both localities approximately two km apart, can be easily distinguished from *P. vertebralis* by lacking prefrontal scales, and from *P. ulisesi* by lacking the rictal diagonal white bar and a white stripe on the forearm. We acknowledge that the differences in color pattern between *P. ulisesi* and *P. sp.* might only represent interpopulational variation within *P. ulisesi*, which should be addressed with the examination of further specimens, as well as phylogenetic analyses of molecular data.

In conclusion, there are no voucher specimens of *Pholidobolus vertebralis* from Peru, and its presence in this country has been based on misidentified specimens of *Macropholidus huancabambae*, *P. ulisesi*, and an undescribed species of *Pholidobolus*. Furthermore, we also

examined the single specimen of *P. vertebralis* reported by Uzzell (1973) from southwestern Ecuador (AMNH 18312) and conclude that it represents another undescribed species of *Pholidobolus*. Thus, the southernmost records of *P. vertebralis* are from northwestern Ecuador around its type locality (Intag, Imbabura province). Finally, as noted by Uzzell (1973), the few records of *P. vertebralis* east of the Andes in Ecuador are most likely based on erroneous locality data, as has been noted for other species of amphibians and reptiles from the same localities (e.g., Uzzell 1973).

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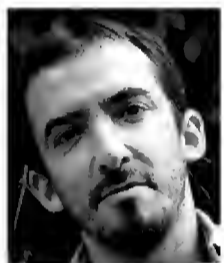
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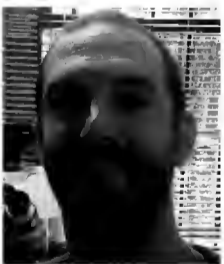
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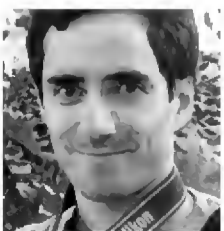
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## Appendix 1

### Additional specimens examined

*Macropholidus huancabambae*.—PERU: Piura: 11 miles E of Canchaque, on the western slope of the Huancabamba Mountains, LACM 58811.

*Pholidobolus affinis*.—ECUADOR: Provincia Chimborazo: Colta, 1°41'56"S, 78°46'25"W, 3,215 m, QCAZ 9899–01; Sicalpa, 1°42'18"S, 78°46'32"W, 3,212 m, QCAZ 11887. Provincia Cotopaxi: Cutuchi river, San Miguel de Salcedo, 1°2'9"S, 78°35'53"W, 2,640 m, QCAZ 9641. Provincia Tungurahua: six km N Mocha to 400 m Panamerican Highway, 1°22'1"S, 78°39'16"W, 3,205 m, QCAZ 9895–97; Ambato surroundings, 1°14'59.8"S, 78°37'33"W, QCAZ 9340–73, 9375–9443; Chamisa on road to Guadalupe, 1°21'44"S, 78°30'39"W, 2,348 m, QCAZ 7266; Cotaló on path to Mucubi Community, 1°25'46"S, 78°31'3"W, 2,626 m, QCAZ 9839; Patate, 1°18'42"S, 78°30'36"W, 2,199 m, QCAZ 9847–50; Poatug Hamlet, Aya Samana, 1°16'58"S, 78°29'29"W, 2,573 m, QCAZ 10005, 10008, 10011–13, 10016, 10018; Poatug Hamlet, Terremoto, 1°16'23"S, 78°29'29"W, 2,547 m QCAZ 9997–10000, 10002–10004; San Miguelito on path to Pillaro, 1°13'12"S, 78°31'31"W, 2,689 m, QCAZ 9844; San Miguelito on path to Terán, 1°12'58"S, 78°31'42"W, 2,741 m, QCAZ 9843.

*Pholidobolus dicrus*.—ECUADOR: Provincia Morona Santiago: Guarumales, 2°34'0.0006"S, -78°30'0"W, 1,700 m, QCAZ 5292, 5304. Provincia Tungurahua: Río Blanco, Vía Baños-Puyo, 1°23'55.6434"S, 78°20'24"W, 1,600 m, QCAZ 6936, no locality data QCAZ 8015.

*Pholidobolus hillisi*.—ECUADOR: Provincia Zamora-Chinchipec: near San Francisco Research Station on Loja-Zamora road, 3°57'57"S, 79°4'45"W, WGS84, 1,840 m, QCAZ 4998–99, 5000; San Francisco Research Station, 3°58'14"S, 79°4'41"W, 1,840 m, QCAZ 6840, 6842, 6844.

*Pholidobolus macbrydei*.—ECUADOR: Provincia Azuay: 10 km S Cutchil, 3°8'22"S, 78°48'47"W, 2,900 m, QCAZ 823–24; 1.2 km E Osoranco, 2°46'8"S, 78°38'10"W, 2,390 m, QCAZ 826; 6.2 km S Cutchil, 3°6'32"S, 78°48'4"W, 2,800 m, QCAZ 827; 20 km NE Cuenca, 2°51'0"S, 78°51'14"W, QCAZ 1359; seven km Sigsig, 2°59'56"S, 78°48'25"W, 2,890 m, QCAZ 1537; 6 km S Oña, 3°29'49"S, 79°9'47"W, QCAZ 3658; 20 km Cuenca-El Cajas, 2°46'39"S, 79°10'12"W, 3,508 m, QCAZ 9932–34, 9936–38, 10020; Cochapamba, 2°47'50"S, 79°24'56"W, 3,548 m, QCAZ 10133–35; Cochapata, 3°25'47"S, 79°3'35"W, 3,074 m, QCAZ 12605–07; Cuenca, Cuenca-Azoguez Panamerican Highway 2°53'43"S, 78°57'30"W, 2,486 m, QCAZ 6985; El Cajas National Park, path to Patul Community, 2°44'28"S, 79°14'5"W, 4,092 m, QCAZ 8010–11; El Cajas National Park, Patul river, 2°41'37"S, 79°13'56"W, 3,610 m, QCAZ 8893; El Cajas National Park, Zhurcay river, 3°2'30"S, 79°12'56"W, 3,766 m, QCAZ 8900–01; El Cajas National Park, 2°42'21"S, 79°13'32"W, 3,600 m, QCAZ 8946; El Capo, 2°46'43"S, 79°14'43"W, 4,100 m, QCAZ 4997; Girón, San Gregorio Community, Quinsacocha paramo, 3°6'22"S, 79°13'4"W, 3,242 m, QCAZ 8510–11; Girón, San Gregorio Community, Quinsacocha paramo, 3°2'30"S, 79°12'56"W, 3,766 m, QCAZ 8894–99, 8902–05, 8907; Girón, San Gregorio Community, Quinsacocha paramo, 3°2'30"S, 79°12'57"W, 3,766 m, QCAZ 8906; Guablid, 2°46'30"S, 78°41'51"W, 2,453 m, QCAZ 9913–17, 9919–20, 9940–41; Gualaceo-Limón road, 2°56'53"S, 78°42'43"W, 3,110 m, QCAZ 819–22; Gualaceo-Limón road, 8.1 km O Azuay-Morona Santiago border, 2°57'50"S, 78°42'7"W, 3,140 m, QCAZ 825; Gualaceo, 2°52'56"S, 78°46'31"W, 2,298 m, QCAZ 9606; Gualaceo-Plan de Milagro road, 2°54'35"S, 78°44'4"W, 2,624 m, QCAZ 10875; Las Tres Cruces, 2°46'30"S, 79°14'53"W, QCAZ 4136; Maylas, Gualaceo-Macas road, 2°58'25"S, 78°41'41"W, 3,100 m, QCAZ 7269; Mazán Protected Forest, 2°52'29"S, 79°7'26"W, 2,700 m, QCAZ 1296–97; Mazán Protected Forest, 2°52'31"S, 79°7'45"W, 3,189 m, QCAZ 8008, 8013; Oña-La Paz road, 3°22'42"S, 79°11'20"W, 2,969 m, QCAZ 6031; Patacocha hill, 3°7'16"S, 79°3'54"W, 3,340 m, QCAZ 6144; Pucara, Tres Chorreras, 3°12'49"S, 79°28'3"W, QCAZ 11038; Quinoas river, 3°5'14"S, 79°16'40"W, 3,200 m, QCAZ 1564–66; San Antonio, 2°51'40"S, 79°22'43"W, 2,943 m, QCAZ 9668; San Vicente-Cruz path, 2°47'43"S, 78°42'53"W, 3,044 m, QCAZ 11416–17, 11420; Sigsig, 3°7'46"S, 78°48'14"W, 2,969 m, QCAZ 5605–08; Sigsig road, 3°3'17"S, 78°47'19"W, 2,574 m, QCAZ 9605; Tarqui, 3°0'57"S, 79°2'40"W, 2,627 m, QCAZ 8512. Provincia Cañar: Cañar, 2°33'39"S, 78°55'51"W, QCAZ 9947; Culebrillas, 2°25'35"S, 78°52'12"W, 4,000 m, QCAZ 1349; Guallicanga ravine, 2°25'56"S, 78°54'8"W, 3,960 m, QCAZ 10048–49; Guallicanga river, 2°28'24"S, 78°58'22"W, 3,048 m, QCAZ 10051–52; Ingapirca, 2°32'43"S, 78°52'28"W, 3,400 m, QCAZ 1551; Juncal, 2°28'24"S, 78°58'22"W, 3,048 m, QCAZ 10050; Mazar Protected Forest, 2°32'48"S, 78°41'54"W, QCAZ 7376–84, 7883; Mazar Reserve, La Libertad, 2°32'45"S, 78°41'46"W, 2,842 m, QCAZ 10970–72. Provincia Chimborazo: Alao, 10 km Huamboya, 1°52'22"S, 78°29'51"W, 3,200 m, QCAZ 1567–68; Atillo Grande, Magdalena lake, 2°11'15"S, 78°30'25"W, 3,556 m, QCAZ 9214; Atillo Grande, Frutatián lake, 2°12'57"S, 78°30'5"W, 3,700 m, QCAZ 9216–18; Culebrillas, Sangay National Park, 1°57'39"S, 78°25'55"W, 3,345 m, QCAZ 9612; Pungalá, Etén Community, Timbo, 1°55'45"S, 78°32'14"W, 3,408 m, QCAZ 9616–21; Pungalá, Melán Community, 1°52'30"S, 78°32'52"W, 3,564 m, QCAZ 9626–29, 9631; Ozogoché, 2°22'7"S, 78°41'20"W, 4,040 m, QCAZ 6006–07; Shulata, 2°20'22"S, 78°50'36"W, 3,228 m, QCAZ 5597–98. Provincia El Oro: Guanazán, 3°26'24"S, 79°29'13"W, 2,638 m, QCAZ 7891, 7894. Provincia Loja: 17.1 km S Saraguro, 3°43'45"S, 79°15'53"W, 3,150 m, QCAZ 828; 26 km N Loja, Huashapamba Native Forest, 3°39'30"S, 79°16'20"W, 2,894 m, QCAZ 8651; Cordillera of Lagunillas, Jimbura, 4°49'1"S, 79°21'43"W, 3,600 m, QCAZ 3785; Cordillera of Lagunillas, Jimbura, 4°37'42"S, 79°27'49"W, 3,450 m, QCAZ 6145–47; Fierro Urco, 3°42'38"S, 79°18'18"W, 3,439 m, QCAZ 6949–50; Gurudel, 3°39'22"S, 79°9'47"W, 3,100 m, QCAZ 5078–79; Jimbura, Jimbura lake, 4°42'32"S, 79°26'48"W, 3,036 m, QCAZ 6945–48; Jimbura, path to Jimbura lake, 4°42'34"S, 79°26'8"W, 3,348 m, QCAZ 10054–62; Military antenna, Saraguro, 3°40'46"S, 79°14'16"W, 3,190 m, QCAZ 3673–75, 9632; San Lucas, 3°43'55"S, 79°15'38"W, 2,470 m, QCAZ 2861; Saraguro, 3°37'13"S, 79°14'9"W, 3,100 m, QCAZ 3606, 3754; Urdaneta, 3°36'6"S, 79°12'31"W, QCAZ 2019. Provincia Tungurahua: Poatug Hamlet, El Corral, 1°16'21"S, 78°28'5"W, 3,468 m, QCAZ 8047, 9995–96. Provincia Zamora-Chinchipec: Loja-Podocarpus National Park road, 3°59'44"S, 79°8'28"W, 2,776 m, QCAZ 10870–71; Valladolid, Podocarpus National Park, 4°29'3"S, 79°8'56"W, 1,800 m, QCAZ 3743.

*Pholidobolus montium*.—ECUADOR: Provincia Cotopaxi: two km S Chugchilán on road to Quilotoa, 0°48'24"S, 78°56'11"W, 2,917 m, QCAZ 8056–58; Latacunga, 0°52'27"S, 78°38'26"W, 2,857 m, QCAZ 873–74, 1411–12, 9642; Mulaló, 0°46'35"S, 78°34'40"W, 3,030 m, QCAZ 9639; San Juan de Pasto Calle, 0°45'4"S, 78°38'51"W, 1,956 m, QCAZ 8053–54; South Illiniza, 0°39'43"S, 78°42'40"W, 3,400 m, QCAZ 858–59, 1454. Provincia Imbabura: Atuntaqui, 0°19'59"N, 78°12'50"W, QCAZ 855; Cotacachi, Peribuela, Cuicocha Lake, Cotacachi-Cayapas Reserve, 0°17'34"N, 78°21'5"W, 3,082 m, QCAZ 9683, 9685–86; 0°23'4"N, 78°15'25"W, 2,900 m, QCAZ 6137, 6139; Cotacachi-Cayapas Reserve, José María Yerovi Islets, 0°18'20"N, 78°21'41"W, 3,093 m, QCAZ 10959–60; El Juncal, 0°26'6"N, 77°57'58"W, QCAZ 6451. Provincia Pichincha: 16 km W Chillogallo, Quito-Chiriboga road, 0°17'46"S, 78°39'30"W, 3,100 m, QCAZ 797; five km E Pifo-Papallacta road, 0°15'3"S, 78°17'58"W, 2,800 m, QCAZ 1107–08; Alambi, 0°1'59"S, 78°34'26"W, 2,727–3,800 m, QCAZ 9691; Alangasí, 0°18'24"S, 78°24'40"W, QCAZ 1453, 1469; Amaguaña, Hacienda San Ignacio, 0°22'22"S, 78°30'14"W, QCAZ 1463–64, 5275; Calacalí, Simón Bolívar Street, uphill through secondary road, 0°1'1"N, 78°30'49"W, 3,001 m, QCAZ 11674, 11676, 11678–79; Calacalí Stadium, 0°0'0.3"S, 78°30'38"W, 2,833 m, QCAZ 11682; Carretas, 0°6'25"S, 78°26'46"W, QCAZ 875; Chillogallo, 0°16'48"S, 78°33'25"W, QCAZ 840–43; Cumbayá, La Primavera, 0°12'6"S, 78°25'40"W, QCAZ 7248; Guayllabamba, 0°3'23"S, 78°20'26"W, QCAZ 7905; Inga, 5.5 km SE La Merced, 0°17'51"S, 78°20'52"W, 2,798 m, QCAZ 5278; Lloa, 0°14'52"S, 78°34'33"W, QCAZ 4109; Lloa Stadium, 0°14'39"S, 78°35'12"W, 3,059 m, QCAZ 11661; Loreto, road to Molinuco, Central Stadium,

## Appendix 1 (continued) Additional specimens examined

0°23'4"S, 78°24'30"W, 2,844 m, QCAZ 11663; Machachi, 0°29'50"S, 78°32'25"W, QCAZ 844–48, 1374–77, 1462; Machachi, The Tesalia Springs Company S.A. surroundings, 0°30'27"S, 78°33'57"W, 2,900 m, QCAZ 1465–67, 830–31, 833, 860–61, 1459–61; Nono, 0°4'42"S, 78°34'24"W, 2,843 m, QCAZ 11653–55; Nono School, 0°4'4"S, 78°34'35"W, 2,754 m, QCAZ 11656–58; Pasochoa, 0°26'24"S, 78°30'15"W, 2,850 m, QCAZ 1451–52; Pomasqui, 0°3'3"S, 78°27'21"W, QCAZ 862–68; Pululahua Volcano, 0°2'34"N, 78°30'15"W, QCAZ 1450, 1520; Quito, Bellavista, 0°11'21"S, 78°28'35"W, QCAZ 1099; Quito, Chillogallo, 0°16'26"S, 78°33'23"W, QCAZ 8967; Quito, Itchimbía, 0°13'21"S, 78°29'56"W, QCAZ 834, 1455–58, 1643, 2843; Quito, Garden of the Pontificia Universidad Católica del Ecuador (PUCE), 0°12'33"S, 78°29'28"W, 2,800 m, QCAZ 856–57, 7032, 1295, 2853; Quito, Parque Metropolitano, 0°10'35"S, 78°27'40"W, QCAZ 4051; Quito, Universidad Central del Ecuador, 0°11'59"S, 78°30'19"W, 2,800 m, QCAZ 3727; Río Guajalito Protected Forest, 0°13'44"S, 78°48'22"W, QCAZ 1338–39; San Antonio de Pichincha, 0°0'33"S, 78°26'45"W, QCAZ 580–81, 790–92, 849, 1119–20, 1368, 1393, 2220, 2223, 2653; Tababela, International Airport, 0°6'21"S, 78°21'4"W, QCAZ 8046, 9044, 10064, 10974–76; Quito, Tumbaco, 0°12'34"S, 78°24'2"W, QCAZ 1113–14; Uyumbicho, 0°22'59"S, 78°31'6"W, QCAZ 870.

*Pholidobolus prefrontalis*.—ECUADOR: Provincia Azuay: Sigsig, 3°7'46"S, 78°48'14"W, 2,480 m, QCAZ 1553; Provincia Cañar: Cañar, 2°33'29"S, 78°56'4"W, QCAZ 1410; Provincia Chimborazo: Alausí, 2°11'54"S, 78°50'42"W, 2,359 m, QCAZ 9907–9911; Tixán, 2°9'22"S, 78°48'3"W, 2,908 m, QCAZ 9951–54; Tixán, 2°9'22"S, 78°48'3"W, 2,908 m, QCAZ 9951–54.

*Pholidobolus vertebralis*.—ECUADOR: Provincia Carchi: Chilma Bajo, 0°51'53.83" N, 78°2'59.26" W, 2,071 m, QCAZ 5057, 8671–8673, 8678, 8679, 8717, 8724, 0°51'50.31" N, 78°2'50.05" W, 2,022 m, QCAZ 8684–8689. Provincia Pichincha: Mindo, 0°3'2.41" S, 78°46'18.77" W, 1,700 m, QCAZ 2911, 2912, 2915, 0°4'40.98"S, 78°43'55.02"W, 1,601 m, QCAZ 7528; Cooperativa El Porvenir, El Cedral 0°6'50.40" N, 78°34'11.75" W, 2,297 m, QCAZ 5081, 5082; Santa Lucía de Nanegal, 0°6'48.70"N, 78°36'48.60"W, 1,742 m, QCAZ 10667, 0°7'8.51"N, 78°35'58.70"W, 1,900 m, QCAZ 10750. LOCALITY IN ERROR: ECUADOR: Provincia Pastaza: Mera, AMNH 60586–97.

*Pholidobolus* sp.—ECUADOR: Provincia El Oro: El Chiral, 1,350 m, AMNH 18312.

*Pholidobolus* sp.—PERU: Cajamarca: Provincia de Chota: Quebrada La Iraca (near to La Granja village), 6°22'09.9"S, 79°08'04.61"W, 2,213 m, CORBIDI 1679.

In accordance with the International Code of Zoological Nomenclature new rules and regulations (ICZN 2012), we have deposited this paper in publicly accessible institutional libraries. The new species described herein has been registered in ZooBank (Polaszek 2005a, b), the official online registration system for the ICZN. The ZooBank publication LSID (Life Science Identifier) for the new species described here can be viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/." The LSID for this publication is: urn:lsid:zoobank.org:pub:CAB026AC-B1CE-4F43-B0C3-AB908645159F.

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### Citations

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SHORT COMMUNICATION

## New records, range extension and call description for the stream-breeding frog *Hyloscirtus lascinius* (Rivero, 1970) in Venezuela

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**Abstract.**—The stream-breeding frog *Hyloscirtus lascinius* is known from a few localities on the Eastern versant of the Cordillera Oriental of Colombia and Tamá massif in Colombia and Venezuela. It has also been reported from Cordillera de Mérida in Venezuela but without precise localities or voucher specimens. Herein we report *H. lascinius* from the Sierra de Perijá in Venezuela, and provide four locality records in Cordillera de Mérida. The record of Perijá extends the known species' distribution ca. 213 km NW from the northernmost locality previously recorded. We also describe the advertisement call of this species for the first time, and provide some notes on its natural history.

**Keywords.** Amphibia, Anura, Biogeography, Bioacoustics, Sierra de Perijá, Andes

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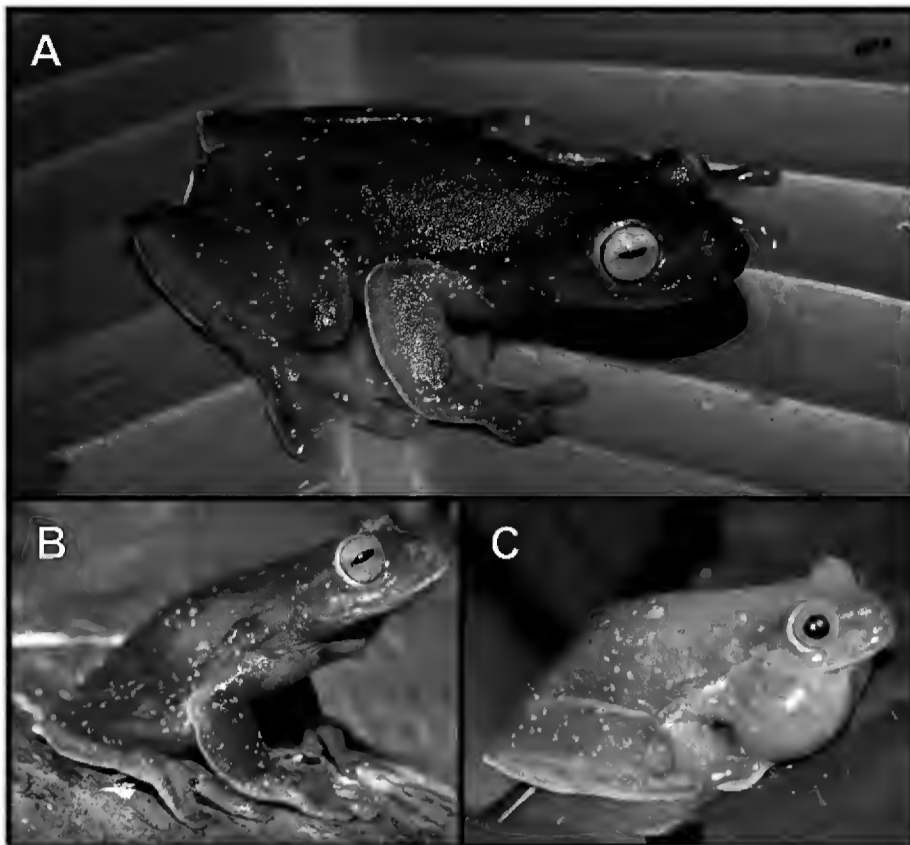
The hylid genus *Hyloscirtus* Peters, 1882 was resurrected by Faivovich et al. (2005) to accommodate all the stream-breeding frogs previously included in the *Hyla armata*, *H. bogotensis*, and *H. larinopygion* species groups. This genus was supported by 56 molecular synapomorphies and by having wide dermal fringes on fingers and toes (the only putative morphological synapomorphy). Recently, Duellman et al. (2016) described *Colomascirtus* for the clade formed by the *H. armatus* and *H. larinopygion* species groups, restricting *Hyloscirtus* to the *H. bogotensis* species group.

*Hyloscirtus* as currently defined (Duellman et al. 2016), contains 17 species (Guayasamin et al. 2015) and is distributed from Costa Rica and Panama in Central America, through the Andes of Colombia, Venezuela, Ecuador, Peru, and Bolivia in South America (Frost 2016). In Venezuela, only three species of *Hyloscirtus* are currently known: *H. jahni* (Rivero, 1961), *H. lascinius* (Rivero, 1970), and *H. platydactylus* (Boulenger, 1905).

*Hyloscirtus lascinius* was originally described by Rivero (1970) as a member of *Hyla Laurenti*, 1768 (as *Hyla lascinia*). Its relationships with other stream-breeding frogs were earlier presumed by Rivero (1970), Goin (*in* Rivero 1970) and Duellman (1972), but only after two decades was this recognized as part of the *Hyla bogotensis* group (Ruiz-Carranza and Ardila-Robayo 1991; Duellman et al. 1997). Faivovich *et al.* (2005) transferred it to *Hyloscirtus* based only on morphological evidence. However, molecular corroboration of his taxonomic position was presented more recently by Wiens et al. (2010), Pyron and Wiens (2011), Faivovich et al. (2013), Almendariz et al. (2014), Guayasamin et al. (2015), and Duellman et al. (2016).

*Hyloscirtus lascinius* is distributed on the eastern versant of the Andean Cordillera Oriental and Tamá massif in Colombia at Norte de Santander department, between 1,730–1,960 m asl (Ruiz-Carranza et al. 1996; Bernal and Lynch 2008; Sánchez 2010), and in the Venezuelan

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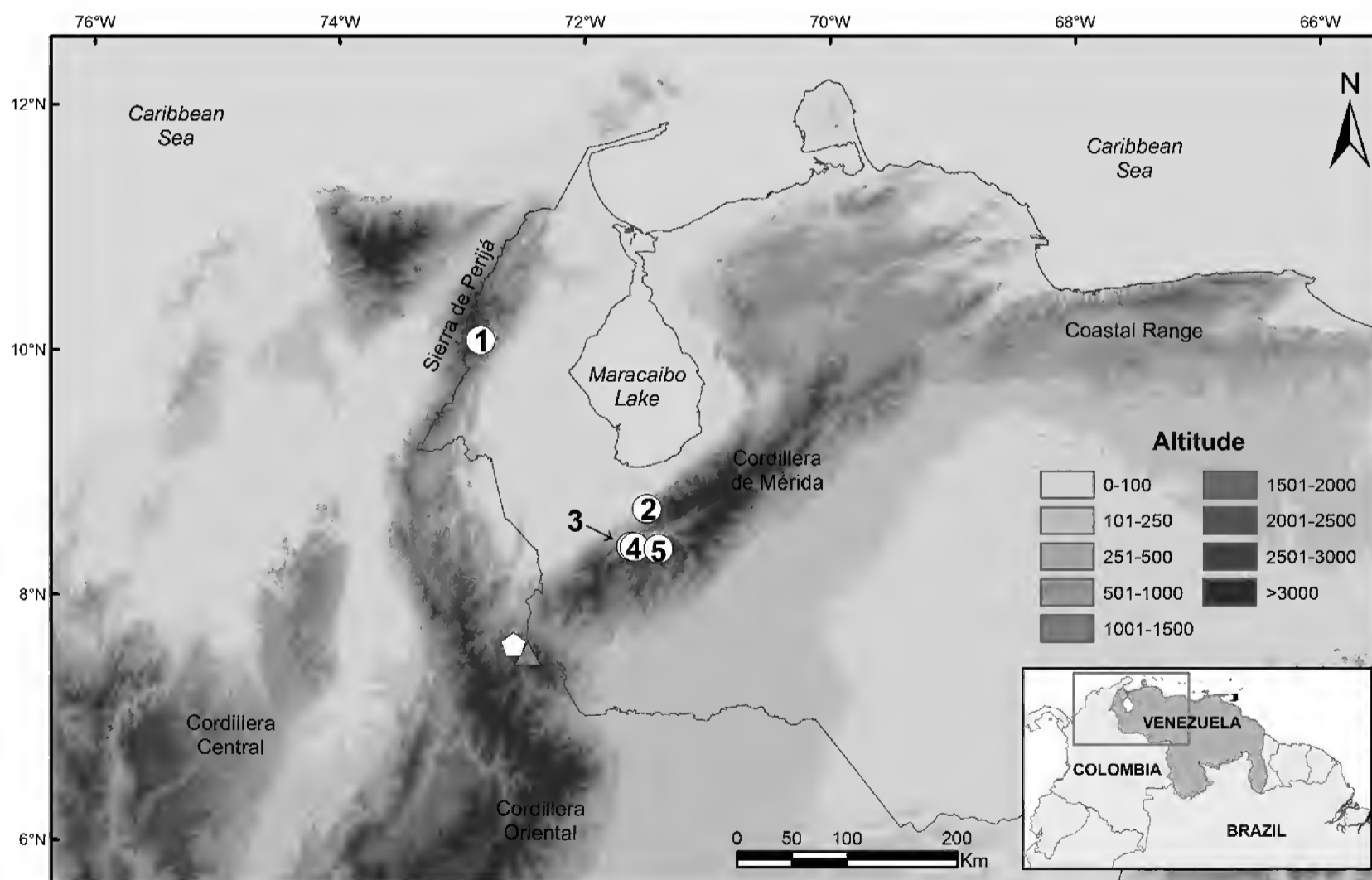


**Fig. 1.** *Hyloscirtus lascinius* from Venezuela. (A) Campamento Guacharaca, Sierra de Perijá, Zulia state; (B) Near La Macana, Mérida state; (C) Quebrada La Rana, Mérida state. Photos: F.J.M. Rojas-Runjaic (A) and C.L. Barrio-Amorós (B and C).

portion of Tamá, Táchira state, between 1,250–1,700 m asl (Rivero 1970; Mijares-Urrutia 1992). La Marca et al. (2004) and Barrio-Amorós (2004) mentioned its presence on the western versant of Cordillera de Mérida, in Mérida state, but without referring any precise locality or voucher specimens.

Herein, we report the first records of *Hyloscirtus lascinius* for Zulia state, northwestern Venezuela, and formally documented its presence in several localities of Mérida state, based on museum specimens deposited at Museo de Historia Natural La Salle, Caracas, Venezuela (MHNLS), and uncollected specimens photographed by CLBA. Also we describe for the first time the call of this species. Call description is based on a 73 s digital recording of the advertisement call of a single male at Quebrada La Rana, in Santa Cruz de Mora, Mérida state, Venezuela, on 13 August 2006. Air temperature was 17 °C. Calls were analyzed using Raven Pro 1.3 (Bioacoustics Research Program 2008).

Specimens from Zulia state (Fig. 1A) were found during a survey of the inventory of amphibian and reptiles of the Venezuelan side of Sierra de Perijá, conducted by Museo de Historia Natural La Salle (MHNLS). They are five adult males (MHNLS 19163–19165, 19237–19238; SVL: 41.6–44.0 mm) collected at Campamento Guacharaca, Caño Tétari Kopejoacha, Río Negro upper basin, Machiques de Perijá municipality, Sierra de Perijá (10°04'22"N, 72°51'16"W, 1,661 m; Fig. 2) on 21–27 May 2009. The creek where the specimens were collected (Fig. 3A) was surrounded by a primary ombrofilous submontane/montane evergreen forest (Huber and Alarcón 1988), with abundant ferns, Heliconiaceae, Araceae, and Cyclanthaceae plants. All specimens were found between 20:00–22:00 h, calling from branches of



**Fig. 2.** Distribution of *Hyloscirtus lascinius* in Venezuela and Colombia. **1:** Campamento Guacharaca, Sierra de Perijá, Zulia state, Venezuela. **2:** San Luis, Mérida state, Venezuela. **3:** Road Santa Cruz de Mora-La Macana, Mérida state, Venezuela. **4:** Quebrada Ovalles, Mérida state, Venezuela. **5:** Quebrada De La Rana, Mérida state, Venezuela. **Yellow triangle:** Tabor, Tamá massif, Táchira state, Venezuela (type locality); **White pentagon:** Chinácota, Norte de Santander department, Colombia (Sánchez 2010); The record of headwaters of Río Táchira, Norte de Santander, Colombia (Ruiz-Carranza et al. 1996) and additional localities between Delicias and Tabor (Rivero 1970) are included in the yellow triangle that indicates the type locality.



**Fig. 3.** Habitat of *Hyloscirtus lascinius* at Campamento Guacharaca, Sierra de Perijá, Zulia state (A). Males calling from a branch; (B) and from a rocky wall; (C) at the edge of the creek in Campamento Guacharaca. Photos: F.J.M. Rojas-Runjaic.

bushes (Fig. 3B) or rocky walls (Fig. 3C) on the sides of the creek and between 50–300 cm above ground.

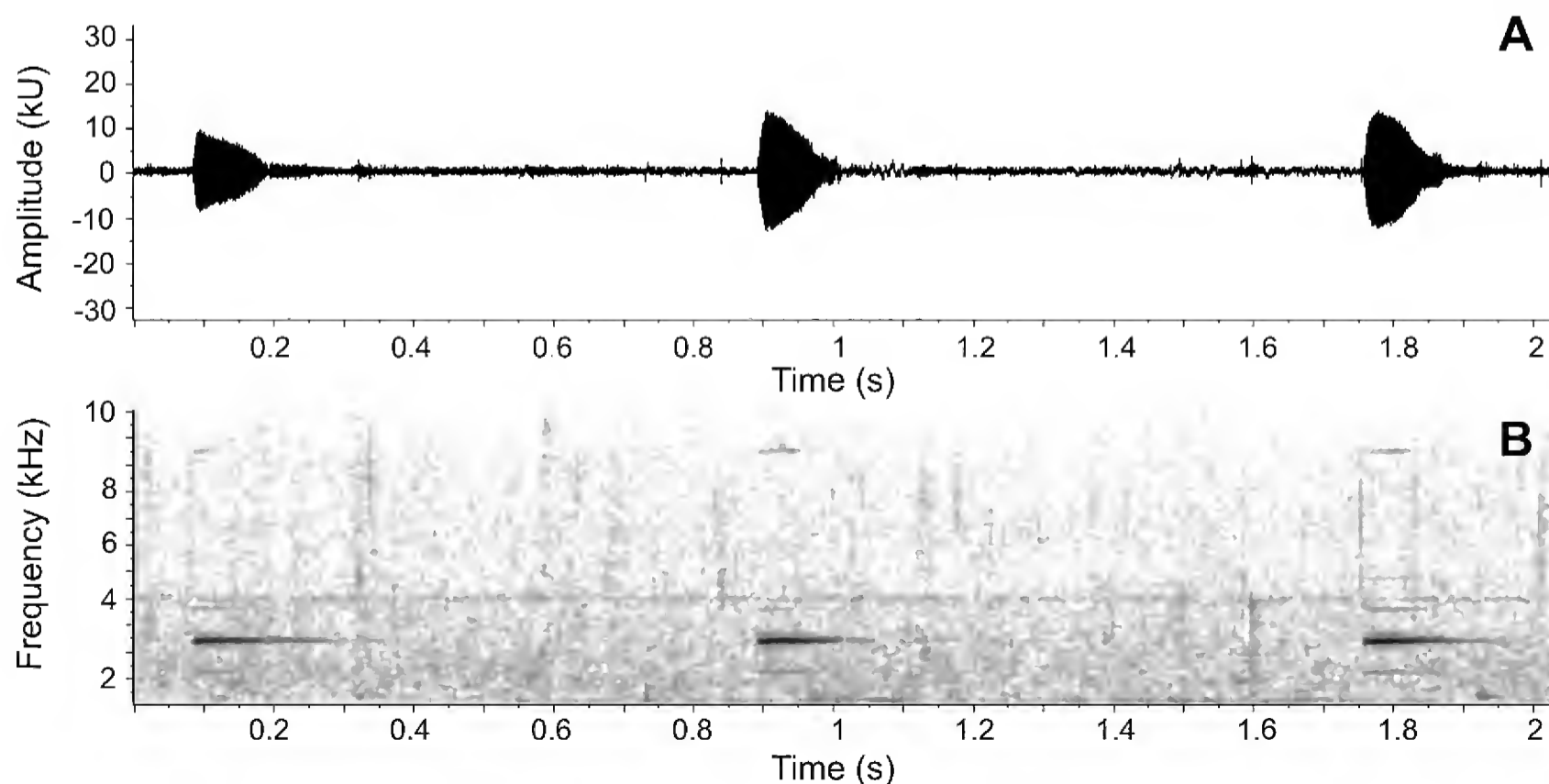
When captured the frogs released a strong citrus smell. At this locality *Hyloscirtus lascinius* was sympatric with *Hyloscirtus* sp., *Cryptobatrachus remotus* Infante-Rivero, Rojas-Runjaic and Barrio-Amorós, 2009, *Centrolene daidaleum* (Ruiz-Carranza and Lynch, 1991), *Centrolene notostictum* Ruiz-Carranza and Lynch, 1991, *Hyalinobatrachium pallidum* (Rivero, 1985),

*Pristimantis rivasi* Barrio-Amorós, Rojas-Runjaic, and Barros, 2010, and *Pristimantis* sp.

This new record of *Hyloscirtus lascinius* for the Venezuelan Sierra de Perijá in Zulia state (Fig. 2) extend the species' distribution by ca. 276 km north (straight-line) from Chinacota, Norte de Santander, Colombia, the northeasternmost locality previously documented (Sánchez 2010), and ca. 213 km NW (straight-line) from San Luis, Mérida state, Venezuela, the northernmost locality known in the Cordillera de Mérida (locality record also in this work).

Four more localities are reported herein (Fig. 2), all from Cordillera de Mérida, to confirm previous statements (Barrio-Amorós 2004; La Marca et al., 2004). These are: 1) San Luis, La Azulita Andrés Bello municipality, Mérida state (08°41'27"N, 71°29'44"W; ca. 1,614 m; CLBA personal observation); 2) creek on the road Santa Cruz de Mora-La Macana, Pinto Salinas municipality, Mérida state (08°23'13"N, 71°37'35"W, 1,130 m; photographic record; Fig. 1B); 3) Quebrada Ovalles, above La Macana, Pinto Salinas municipality, Mérida state (08°22'56"N, 71°35'51"W, 1,478 m; MHNLS 17913); and 4) Quebrada de la Rana, Pinto Salinas municipality, Mérida state (08°22'N, 71°24'W, ca. 1,200 m; photographic record; Fig. 1C). In San Luis, *Hyloscirtus lascinius* is sympatric with *Scinax manriquei* Barrio-Amorós, Orellana and Chacón-Ortiz, 2004, *Dendropsophus* aff. *minutus* (Peters, 1872) and *Espadarana andina* (Rivero, 1968). At the creek between Santa Cruz de Mora and La Macana with *Flectonotus pygmaeus* (Boettger, 1893), and *Tachiramantis lentiginosus* (Rivero, 1984); and at Quebrada de la Rana with *Hyalinobatrachium pallidum*, and *Pristimantis* cf. *vanadise* (La Marca, 1984).

Based on all localities previously documented (Rivero 1970; Mijares-Urrutia 1992; Ruiz-Carranza et al. 1996; Bernal and Lynch 2008; Sánchez 2010) and the referred in this note, the species' altitudinal range is extended to



**Fig. 4.** Oscillogram (A) and spectrogram (B) of the advertisement call of *Hyloscirtus lascinius*.

ca. 1,130–1,960 m.

The advertisement call of *Hyloscirtus lascinius* is a single tonal note, emitted as single and sporadically (6 of 22 notes), or in groups of two (one group; 2 of 22 notes), three (two groups; 6 of 22 notes) or four notes (two groups; 8 of 22 notes) (Fig. 4). These notes (calls) sound as metallic whistles. Note duration is 91–164 ms ( $123 \pm 16.2$ ;  $n = 19$ ), with a note interval (only among notes emitted in groups) of 700–1,075 ms ( $888.1 \pm 121.9$ ;  $n = 10$ ). Fundamental frequency is at 1.077–1.227 kHz ( $1.160 \pm 0.040$ ;  $n = 17$ ) and the dominant frequency (=peak frequency) is at 2.132–2.369 kHz ( $2.330 \pm 0.074$ ;  $n = 19$ ), with modulation frequency throughout the calls.

The Sierra de Perijá, and extension of the Andean Cordillera Oriental and natural border between northeastern Colombia and northwestern Venezuela, remains poorly explored and its anuran fauna is still not well known. However, the finding of *Hyloscirtus lascinius* in this mountain system, as well other amphibian discoveries documented in the last decade (Infante-Rivero et al. 2006a, b, 2009; Castroviejo-Fisher et al. 2007; Barrio-Amorós et al. 2008, 2010; Rojas-Runjaic et al. 2010, 2011, 2012) show that this region harbors a diverse amphibian fauna closely related to the amphibian faunas of the Andean Cordillera Oriental and Cordillera de Mérida. We predict that new expeditions to Perijá will result in the discovery of numerous additional species. These future findings will improve the knowledge of the amphibian diversity of the Sierra de Perijá, and its biogeographical affinities with neighboring bioregions.

*Hyloscirtus lascinius* was classified as Least Concern (LC) in the IUCN Red List of Threatened Species because, although its extent of occurrence was estimated less than 5,000 km<sup>2</sup>, it is considered an adaptable species, and does not appear to be in decline (La Marca et al. 2004). At the western versant of the Cordillera de Mérida, the localities where we documented this species are currently being modified for agricultural purposes, and the habitat is declining in extent and quality, by which these populations may be being affected. However, the new locality in the Venezuelan Sierra de Perijá is within Parque Nacional Sierra de Perijá and significantly increase its species' distribution, indicating that it has an extent of occurrence much wider than previously known and that, at least the populations of Perijá are apparently protected. Thus, we consider that the conservation status of LC is adequate for *H. lascinius*.

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## SHORT COMMUNICATION

# Monitoring a population of *Cruziohyla craspedopus* (Funkhouser, 1957) using an artificial breeding habitat

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**Abstract.**—We report the detection of *Cruziohyla craspedopus* in Madre de Dios, Peru via use of an artificial breeding habitat: 1) giving us crucial information about the population, 2) contributing to the population by providing habitat, and 3) emphasizing the value of this method in detecting elusive species.

**Key words.** Oviposition, primary forest, tree hole breeding, phytotelm breeding, canopy, frog, Peru, rare

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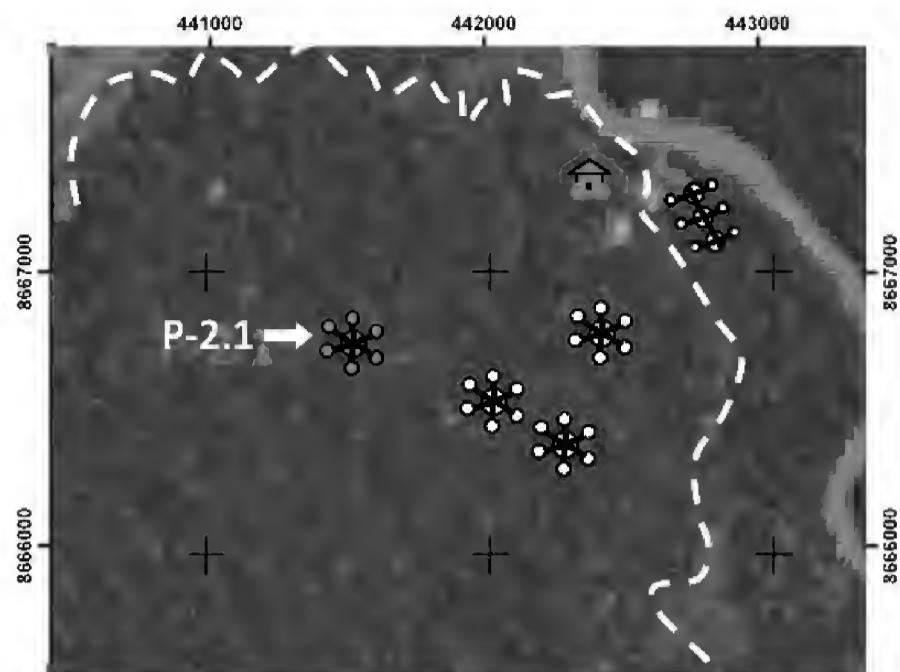
For the more elusive and endangered anuran species in South America it can be difficult to get accurate representations of abundance and population size. Implementing different sampling techniques, including those focusing on aquatic larval stages, may bring population levels into clearer focus. Tadpoles are often restricted to well-defined water bodies where they can be easily caught for identification. Reproduction may depend on the availability and quality of these water bodies. The sparse literature addressing Amazonian tadpole identification is underdeveloped and often conflicting. Proper identification therefore represents a major hurdle in this type of investigation. We aimed to address problems with tadpole identification while measuring the effect of breeding habitat location via use of artificial breeding habitats (ABHabs) (Gascon 1994).

Adult amphibians with aquatic larvae often occupy drastically different environments from their tadpoles (Werner and Gilliam 1984). *Cruziohyla craspedopus* (Funkhouser, 1957) inhabit the high xeric environments of forest canopy, but are known to descend to the forest floor to breed (Rodriguez and Duellman 1994). Ranging from northern Ecuador to southern Peru (Faivovich et al. 2005), *C. craspedopus* has since been found throughout the Brazilian Amazon (Lima et al. 2003; Meneghelli

et al. 2011; Venancio et al. 2014). While classified as Least Concern by the IUCN (Angulo et al. 2004), it is elusive and existing data does not adequately represent the population. In a comprehensive article, Hoogmoed and Cadle 1991 established the reproductive mode of *C. craspedopus*, with important updates by Block et al. (2003) and Rodrigues et al. (2011): phytotelm “tree hole” breeding involving logs, hollows, or depressions at ground level (pitfall buckets).

Resetarits and Wilbur (1989) mentioned the following factors influencing breeding habitat choice and oviposition site: species present at the pond, vegetation structure, pond age, temperature, and degree of permanence. Following Pearman (1993), who found varied responses to ABHabs across species in terms of area and depth, we used a variety of sizes, ranging from 0.5 L to as much as 400 L, to account for differing preferences. This was the approach of our pilot study at the Las Piedras Biodiversity Station (LPS), Tambopata Province, Madre De Dios Department, Peru (UTM 19 L 442465 8667117, 265 m asl, see Crnobrna et al., in prep. for site description). We placed five clusters of five plastic containers evenly across the primary terra firma forest landscape (Fig. 1). Clearing overhanging vegetation was often required, so we built makeshift oviposition sites

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**Fig. 1.** Site map for ABHabs points at LPS: dashed line is approximate separation of terra firma and flood plain forest.

from sticks and situated them over the larger ABHabs. We used tadpole descriptions by both Duellman (2005) and Hero (1990) to aid us in identification. Where the literature did not provide, or where the descriptions were not adequate, we kept individuals until metamorphosis and/or preserved specimens in 10% formalin (CORBIDI field # FF2222).

Our most successful ABHabs (unique code P-2.1) was a blue cylindrical kiddie pool with a 25 cm plastic rim surrounding a 150 cm diameter plastic sheet. We left this particular ABHabs in the “driest” forest available as expressed by its vicinity to water: all known permanent and ephemeral water sources were more than 250 m away. We drew water from a palm swamp 360 m away until the pool was approximately one half full (200 L), but subsequent rain filled it to its maximum capacity (more than 400 L). After set up on 28 August 2013 successive checks during the end of the dry season returned no observations of anuran breeding or tadpoles (Table 1). We left the site unattended between November 2013 and January 2014, during which time rain intensity was highest (160 cm, SENAMHI 2014). On 26 January 2014 we found approximately 20 *Cruziohyla craspedopus* tadpoles. *Phyllomedusa tomopterna* and *Dendropsophus* sp. tadpoles were simultaneously using the ABHabs. This

was the first positive check of P-2.1. In addition to the tadpoles found, there were also multiple aquatic insect species, and the remaining jelly of two empty egg masses present on our makeshift overhanging oviposition sites (Fig. 2A). While we cannot rule out *P. tomopterna* as the source, repetition of this protocol at other sites returned *Phyllomedusa* species. We could not, however, observe such persistent, low-viscosity masses at any other site ( $n = 25$ )—implying the original egg masses seen at P-2.1 were indeed laid by *C. craspedopus*.

On 26 January, we found live tadpoles in two Gosner stages: stage 28 and stage 45 (Gosner 1960). At stage 28, the *Cruziohyla craspedopus* tadpole (Fig. 2B) had a total length of 32 mm, a body length of 13 mm, width of six mm and height of two mm; the interorbital distance was five mm. The dorsum and tail were black. In some individuals there was a slight yellow tint to the tail, and in later stages small yellow specks on the dorsum. The venter was a translucent purple, then white at very late stages. The tail was pointed with a ventral fin equal in size to the caudal musculature, and a slightly smaller dorsal fin originating on the body. The greatest body width was halfway down the body, and largest body height was where the caudal musculature and the body meet. The nostrils were a quarter of the distance from the snout to the eyes. The eyes were yellow, situated dorsolaterally, and visible from below. The spiracle was ventrolateral and the oral disc bore a single row of marginal papillae ventrally and laterally. The labial tooth row formula (LTRF) observed was 2/2. However, both Duellman (2005) and Hoogmoed and Cadle (1991) recorded the LTRF to be 2/3, underscoring the difficulties in tadpole identification.

We continued to monitor the site throughout 2014, and in March another Gosner stage was observed (27). Throughout April and May *Cruziohyla craspedopus* tadpoles were present in the ABHabs, presumably from at least one more breeding event, although specimens were not retrieved and “staged.” However, we saw no diminishment in their numbers during this time, and no newly metamorphosed or adult individuals were seen. Delayed metamorphosis, to 100 days, could explain



**Fig. 2.** A) ABHabs P-2.1 with empty egg mass; B) Tadpole of *Cruziohyla craspedopus* (preserved).

<b>Table 1. Timeline: P-2.1 ABHab at Las Piedras Biodiversity Station</b>		# coexistent size classes of <i>C. craspedopus</i> larva	Gosner stages observed
<b>Date</b>	<b>Activity</b>		
August 2013	Fauna Forever ABHab project starts at Piedras Station		
28 August 2013	P-2.1 set up	0	n/a
September 2013	P-2.1 checked weekly	0	n/a
October 2013	final negative check, Fauna Forever relocates to other field sites	0	n/a
December 2013	heavy rains begin, ABHabs at max. volume	0	n/a
26 January 2014	First positive check, upwards of 20 tadpoles of 2 species, 2 emptied egg masses	2	28, 45
30 January 2014	Most advanced stage tadpole collected from P-2.1 metamorphoses in captivity	2	52
March 2014	positive check	2	27
May 2014	Fauna Forever relocates to Piedras Station, begins to monitor P-2.1 nightly	1	not collected
June 2014	Fauna Forever AHB project shuts down, maintenance and checking of ABHabs ceases		

these observations, but all documented metamorphosis has been less than 100 days (Hoogmoed and Cadle 1991), making it a distant possibility in a typically water-stressed species.

At P-2.1 we observed one breeding pair of *Cruziohyla craspedopus* actively breeding in December and January. Two details indicate that the number of individuals involved was greater than the minimum two: 1) Multiple egg masses as well as two distinct developmental and size classes persisted at once, indicating staggered, non-synchronous laying/hatching—a feat more easily achieved by more than one female (Yeager and Gibbons 2013); 2) In past observations of *C. craspedopus* each breeding habitat was attended by multiple individuals including multiple males (Block et al. 2003; Hoogmoed and Cadle 1991), which tended to congregate in typical amphibian style. Considering that smaller, earlier stage individuals were present in the ABHab into May of 2014, the evidence suggests that at least three independent breeding events took place involving many productive females. We collected one individual in Gosner stage 45 on 26 January 2014, and it metamorphosed in our captivity within four days (Fig. 3). Due to logistical constraints we were unable to monitor the site during this time, but according to the developmental scheme demonstrated, a subset of 10–20 individuals would

have metamorphosed in late January. Asynchronous metamorphosis remains a possibility, but undoubtedly one well-placed artificial habitat contributed to the local population of *C. craspedopus*. In such an understudied species there is no way to know how much population growth this represents, i.e., the individuals surviving past metamorphosis weighed against naturally occurring habitats' size, hydroperiod, and predation.

Lamentably, even after repeated visits to P-2.1 at night, these tadpole observations represent the only evidence of a breeding *Cruziohyla craspedopus* population at LPS, regardless its productivity. Extensive effort to find amphibians at LPS included, but was not limited to, three years of coordinated surveys utilizing 100 m transects, call surveys, long opportunistic walks, and time constrained searches of breeding habitats (Crnobrna et al., in prep.). Despite this fact, only one adult individual of *C. craspedopus* has ever been seen at LPS, which was outside of herpetological fieldwork (a solo night hike of devoted enthusiast Paul Rosolie, pers. comm.). The well-documented difficulty in detecting the species, in Madre De Dios and elsewhere, is reflected in Duellman (2005), where almost 20 years of searching found no *C. craspedopus* until a suitable breeding habitat was discovered in 2000 (Block et al. 2003). Our results indicate that while artificial habitats contribute to local



**Fig. 3.** Newly metamorphosed juvenile *Cruziohyla craspedopus*.

populations of *C. craspedopus*, they also appear to be the only way to detect populations within logistical time constraints and with any degree of certainty. The dual advantage of this approach could be widely beneficial to future surveys attempting to find this charismatic and photogenic species.

It bears repeating that no natural habitat has been encountered at LPS, which implies that the majority of *Cruziohyla craspedopus* phytotelm breeding pools are themselves in the canopy (Crnobrna and Turrell, pers. obs.), and therefore can only be surveyed via canopy access—a costly, training intensive, and at times dangerous method. Similarly costly in training is recognizing the advertisement call, which could confirm the species on event of finding one near ground level. Call surveys for *C. craspedopus* would need to be extremely sensitive in picking up the soft and sporadic call (see Read and Ron 2011), which would often be relegated to anecdotal evidence.

The success of this ABHab was consistent with Marsh et al. (1999) that degree of pond isolation was more important than habitat quality. Although we spaced ABHab points evenly throughout the forest, they only numbered five and most were associated with available aquatic habitats that the ABHabs would share species with. P-2.1, however, was the only pool in unbroken terra firma with no direct influence from any known water body. We expected little to no amphibian occupancy on the assumption that the pool was not in the vicinity of any breeding sites visible from the ground. Yet even still the pool harboured both common and elusive species. This brings focus to the link between larval and adult environments, which when broken can contribute to the loss of amphibian diversity in imperilled ecoregions (Becker et al. 2007). Although *Cruziohyla craspedopus* populations are likely more abundant than indicated by published records (Faivovich et al. 2005; Lima et al. 2003; Meneghelli et al. 2011; Venancio et al. 2014), they are still subject to the specificity of their breeding habitats—their availability limiting population growth. It is worth noting that detection of *C. craspedopus* in this way confirms its presence in primary forest, and one could argue that this species can be used as a primary

forest indicator (Gardner et al. 2007; von May et al. 2010). Other “tree hole” breeders have been implicated in deforestation and fragmentation studies (Zimmerman and Bierregaard 1986; Ernst and Rödel 2008) because of their perceived reliance on features of primary forests: phytotelm structures of large trees. Future studies should take into account oviposition parameters of *C. craspedopus* in the event that the species’ absence in ideally placed artificial habitats (Tocher et al. 1997; Gagliardi 2008) and/or known naturally occurring habitats does in fact indicate degraded forests.

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# Epidemiological surveillance and amphibian assemblage status at the Estación Experimental de San Lorenzo, Sierra Nevada de Santa Marta, Colombia

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**Abstract.**—Amphibian population declines and extinctions have occurred in conserved sites or protected areas far from anthropogenic activities as a result of emerging infectious diseases such as chytridiomycosis. Regular epidemiological surveillance, monitoring of key species, and the implementation of biosecurity protocols are fundamental actions for the *in-situ* conservation of amphibian fauna. Since 2008 biosecurity protocols have been implemented for all personnel that enter the Estación Experimental de San Lorenzo, a partly mountainous protected and conserved area of the Sierra Nevada de Santa Marta with a high diversity of endemic and endangered amphibians. Semiannual disease screenings of amphibians were carried out, as well as an amphibian inventory and a survey of species of the genus *Atelopus*. To-date no mass mortality events have been reported and *Bd* has not been detected. Nevertheless, some individuals of *Ikakogi tayrona* and *Pristimantis megalops* showed symptoms of disease, the latter of which included individuals affected with skin tumors. Deformities in individuals of *Atelopus* were also observed. The implementation of epidemiological surveillance, monitoring of key amphibian species, and biosecurity protocols are important strategies for the conservation management of the endemic amphibians within the protected area of the Sierra Nevada of Santa Marta.

**Key words.** Anura, *Atelopus*, *Pristimantis*, tumors, chytridiomycosis, disease screening, mortality events, health, disease, *Batrachochytrium dendrobatidis*, *Bd*

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## Introduction

During the last decades amphibian population declines and extinctions have been observed around the world, causing concern from academic, scientific, and governmental entities (Gascon et al. 2007; Mendelson et al. 2006; Stuart et al. 2008). Close to 41% of amphibian species worldwide are categorized under

some level of threat (Baillie et al. 2010; IUCN 2014) and additionally about a quarter of amphibian species are classified as Data Deficient (DD, IUCN 2014), which makes it more difficult to determine the actual status of populations. Colombia harbors approximately 215 threatened amphibian species, which represent slightly more than a fourth of its entire amphibian fauna (Acosta-Galvis 2014), making Colombia the country with the

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greatest number of threatened amphibian species in the world (IUCN 2014). Nevertheless, some species have experienced serious declines while others remain stable. In addition entire lineages have been affected almost to the brink of extinction, as reported for the species of the genus *Atelopus* (La Marca et al. 2005; Lötters 2007), which in Colombia 76% (33 of 43) of the species are categorized as Endangered and Critically Endangered (IUCN 2014; Acosta-Galvis 2014).

Although habitat destruction continues to be the main cause of population declines and extinctions worldwide (IUCN 2014), it is puzzling that many species have disappeared in well conserved, remote areas such as primary forests in protected refuges (Crawford et al. 2010; Crump et al. 1992; Lips et al. 2003; Pounds et al. 2006). We now have a better understanding of the pathogenic microscopic fungus *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999), which causes the disease known as chytridiomycosis, one of the leading factors behind mass mortality events previously considered enigmatic. This fungus interferes with the process of osmoregulation and affects electrolyte balance, which often leads to the death of susceptible individuals (Voyles et al. 2009). Furthermore, *Bd* seems to inhibit the immune response of its hosts (Fites et al. 2013) which may present symptoms such as lethargy, abnormal postures, and hyperemia (Berger et al. 2000; Daszak et al. 1999).

In Colombia, there are about 565 protected areas (RUNAP 2011), of these, 58 are administered by the Parques Nacionales Naturales de Colombia, and constitute approximately 11% of the continental territory (PNNC 2015). These areas safeguard the country's biodiversity and represent a refuge for amphibian assemblages, including endemic species or species with narrow distributions, which may be susceptible to the loss and fragmentation of their habitat. However, these areas

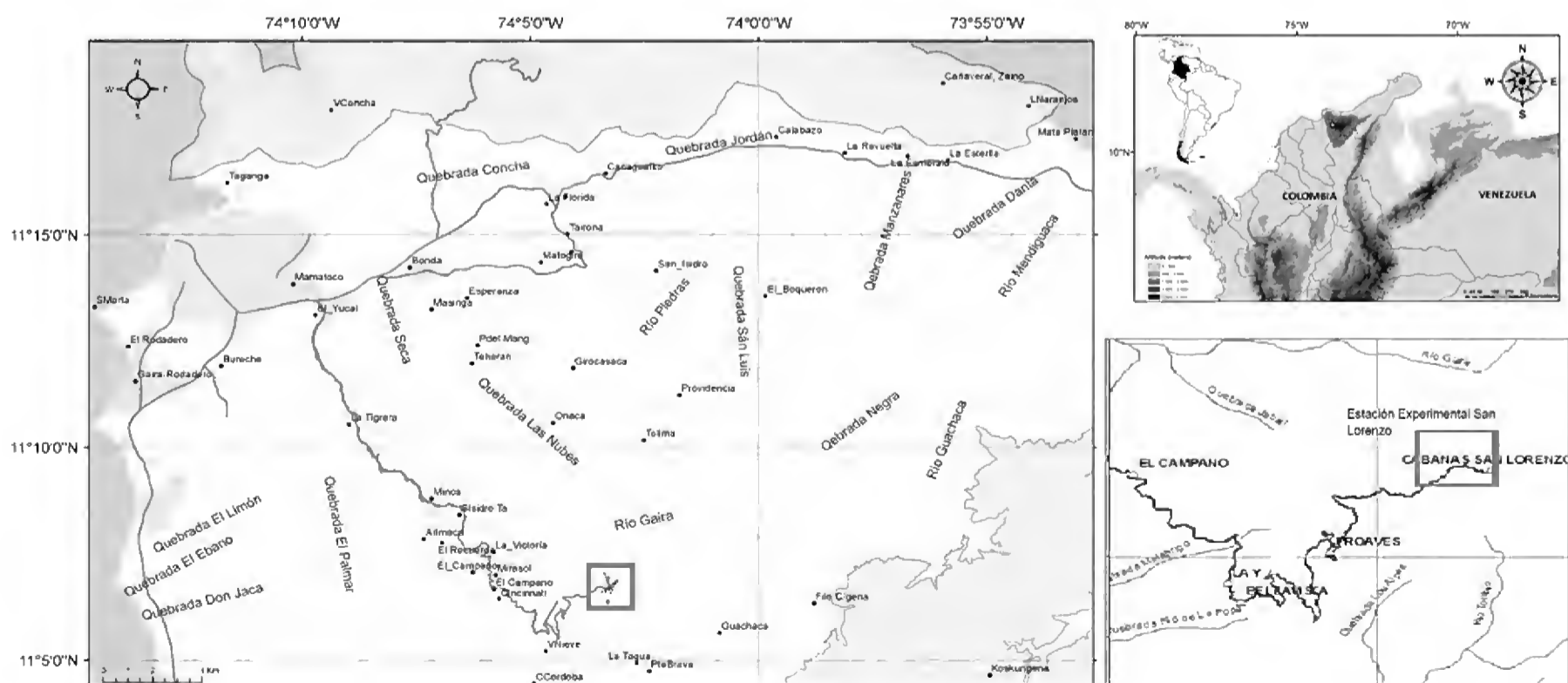
are still vulnerable to the threat imposed by *Bd* and other emerging diseases. For example, it is known that for the Parque Nacional Natural Gorgona, *Bd* has been present for at least eight years (Flechas et al. 2012), however there is no evidence of declines. Additionally, amphibians are vulnerable to diseases produced by aquatic pathogens, due to their dependency on aquatic environments. (Bosch 2003). For this reason authorities and administrators of protected areas in Colombia consider emerging diseases (especially chytridiomycosis) a challenge to the protection of threatened amphibians.

The implementation of recurrent epidemiological surveillance, monitoring of key amphibians species, and biosecurity protocols, become fundamental to the *in situ* conservation of amphibian assemblages in protected areas. This way, early alerts are generated and can be used to implement and carry out the best management practices in a timely manner, thus reducing *Bd* (or disease) outbreaks and transmission. This paper shows the implementation of a pilot program (the first program of its kind in the country) of these actions (surveillance, monitoring, and protocols for amphibian species implemented for disease control) set in a protected area, the Parque Nacional Natural Sierra Nevada de Santa Marta, considered one of the principle centers for amphibian endemism in Colombia (Lynch et al. 1997).

## Materials and Methods

### Study Area

The Sierra Nevada de Santa Marta (SNSM) was declared a reserve of the Biosphere in 1979 by United Nations Educational Scientific and Cultural Organization (UNESCO). Situated within three departments of the Colombian Caribbean (Fig. 1, 2A.); 383,000 hectares belong to the protected area (PNNC 2015). This area, comprises mul-



**Fig. 1.** Map of the Serranía de San Lorenzo, Sierra Nevada de Santa Marta, Colombia. Red square area highlights the Estación Experimental de San Lorenzo to 2,200 meters.





**Fig. 2.** Serranía de San Lorenzo (B) Querbrada San Lorenzo (A) Sierra Nevada de Santa Marta, Colombia. Photographs by Luis Alberto Rueda Solano.

tiple ecosystems, including dry and wet tropical forests, sub-Andean and Andean forests, moors, and zones with perpetual snow cover (PNNC 2015; ProSierra 2015). With approximately 17 species of amphibians, 12 of reptiles, 14 of birds and one mammal, all of them endemic to the area (PNNC 2015; ProSierra 2015), it is considered one of the greatest centers of endemism in the country and one of the irreplaceable protected areas of the world (Le Saout et al. 2013; Lynch et al. 1997).

The study site is the Estación Experimental de San Lorenzo (11° 6' 41.61" N 74° 3' 17.13" W), located in the Serranía de San Lorenzo, on the northwestern slope of the SNSM, department of Magdalena, Colombia (Fig. 2A) at 2,200 m and comprising an area of 400 ha. The surrounding vegetation is comprised of well conserved partially mountainous primary and secondary Andean forest, which include tropical and subtropical rainforests of the isomesothermic jungles (14 °C to 24 °C) (Hernández-Camacho and Sánchez-Páez 1992). The site's native flora includes woody species of *Gustavia speciosa*, *Sloanea* sp. and some palm species of the genus *Geonoma* sp. and *Chamaedorea* sp. (Cleef and Rangel 1984; Rangel and Garzon 1995). Nevertheless, some hectares of non-native vegetation are present. Coniferous forests with species of *Pinus patula* and *Cupressus lusitanica* introduced in the early 90s, may influence the amphibian assemblage that inhabit this area (Camero and Chamorro 1999). This sector presents one rainy season, between April through

November, with a dry period between December through March (Tamaris-Turizo et al. 2007). The mean annual temperature is 12.8 °C, the mean annual precipitation is 2,446 mm and the relative humidity oscillates between 73–98% (Tamaris-Turizo and López-Salgado 2006).

### Epidemiological Surveillance

Since 2008, population census have been performed employing the visual encounter survey (VES) method (Rodda et al. 2001; Rueda et al. 2006; Heyer et al. 1994) in determining numbers of animals with clinical signs of diseases, present deformities and/or individuals found dead in the surrounding areas of the Estación Experimental San Lorenzo. Epidemiological surveillance has been carried out through programmed visits every six months by researchers and biology students from the Universidad de Magdalena and through scheduled or nonscheduled visits by staff of the Parque Nacional Natural SNSM. We looked for anurans presenting lethargy, macroscopic lesions, abnormal postures, hyperemia, ulcers or the presence of fungi or other corporal anomaly, such as deformities. Data was collected for all individuals presenting clinical signs of a potential *Bd* infection. Swab samples from individuals were obtained following the protocol described by Hyatt et al. (2007). Frogs were captured using fresh disposable nitrile gloves and held individually in bags until the sample was taken. Each animal was swabbed by running a cotton swab ten times over the ventral surface, the inner thigh area, and the plantar surface for a total of 50 strokes. Cotton swabs were kept dry and then stored at -20 °C until processing of future laboratory analysis.

One single specimen of *Pristimantis megalops* with clinical disease signs was analyzed using histological methods. The samples of the skin areas affected with lesions were fixed in FAA (Formalin alcohol-acetic) for 24–48 hr at 6 °C. Subsequently, dehydrated in graded series of alcohols (30, 40, 50, 60, 70, 80, 90, 95, and 100%) and two cleared steps in xylene for two hours, then embedded in Paraplast Plus (Mc Cormick®) for 12 hr at 55 °C (Luna 1968; Suvarna et al. 2012). Transverse and longitudinal sections were obtained with a rotary microtome Leica® model (RM2125) set to 4–5µm thick. These were stained with hematoxylin-eosin for general descriptions and were previously stained using the Van Gieson technique with alcian blue and Gill III hematoxylin (changes made by the authors) to demonstrate collagen in the connective tissue. The sections were examined under a light microscope Nikon Eclipse Ni-U® equipped with differential interference contrast (CDI). The photographs were obtained with DS-Fi2® Nikon digital camera using the NIS Elements of Nikon software version 3.07. The image processing was performed with Image-Pro Analyzer 6.3 program (Media Cybernetics). These analysis were carried out in the biology laboratory of the Universidad de

Antioquia (Medellin, Colombia) and photographs taken in the Laboratory of Biotechnology of the Sede de Investigación Universitaria (SIU-UdeA).

Furthermore, a single specimen of *Ikakogi tayrona* with clinical disease signs was analyzed using conventional Polymerase Chain Reaction (PCR) to determine *Bd* presence. DNA was extracted from swabs using GeneReleaser® (Bioventures Inc., Carlsbad, California, USA). We used the primers developed by Annis et al. (2004) to amplify the ITS1-ITS2 region specifically in *B. dendrobatidis*: *Bd1a* (5'-CAGTGTGCCATATGT-CACG-3') and *Bd2a* (5'-CATGGTTCATATCTGTC-CAG-3'). Amplifications were performed in an MJ Research Peltier Thermal Cycler (PTC-200), as follows: an initial two minute denaturation at 95 °C followed by 35 cycles of DNA amplification (i.e., 45 sec at 95 °C, 45 sec at 55 °C, and one min at 72 °C). A final extension at 72 °C for 10 min completed the amplifications. Each reaction consisted of 0.5 µL of each primer (1 M), 3.0 µL of doubly distilled DNA-free water, 6 µL of GoTaq® Green Master Mix (1X; Promega), and 2 µL of the DNA extract. The amplified fragments were separated by electrophoresis through 1% agarose gels. These analysis were carried out in the genetic laboratory of the Universidad de los Andes (Bogotá, Colombia).

### Biosecurity Protocols

To date the presence of *Bd* has not been reported at Serranía de San Lorenzo. To prevent and reduce the risk of

transmission of *Bd*, since 2008 park administrators from SNSM have been implementing a biosecurity protocol for all foreign and national personnel that enter the Estación Experimental de San Lorenzo. The protocol consists of disinfection of field equipment (boots, nets, measuring devices) used as much by researchers as by tourists who visit the protected area (Phillott et al. 2010).

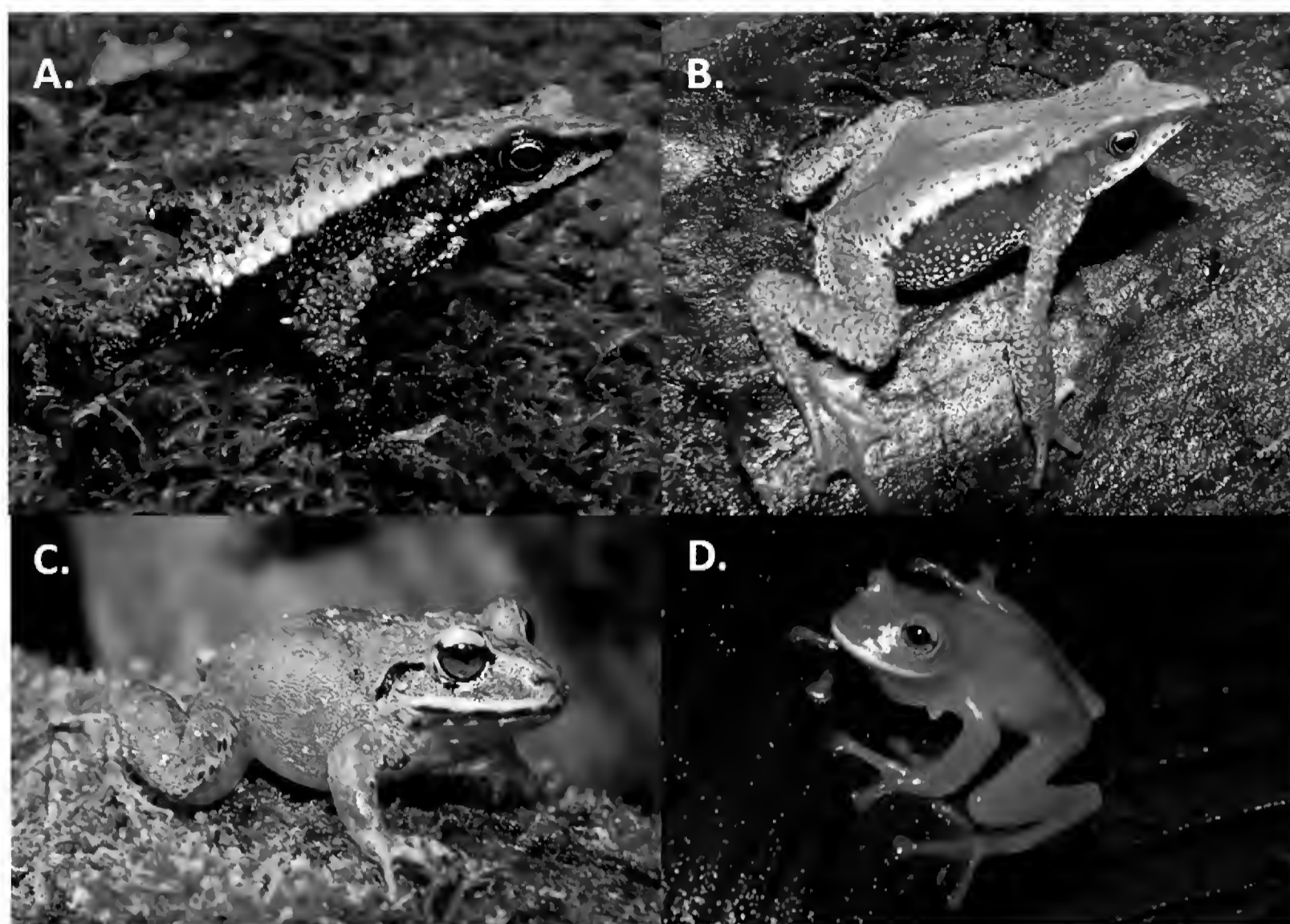
Nevertheless, as a preventive measure *in situ*, field boots are washed with approximately 200 mL of a commercial sodium hypochlorite solution diluted in three liters of water.

### Amphibian Assemblage

To describe the amphibian assemblage from the San Lorenzo sector, an inventory was carried out during seven surveys throughout the months of October and November of 2008, March of 2009, April and October of 2013, and April and November of 2014. No surveys were made in the years 2010–2012. Each field trip had a duration of four days, and the study site was surveyed for eight hours daily, employing the VES method in diurnal periods (9:00–12:00 and 15:00–17:00 hrs) and nocturnally (18:00–21:00 hrs). During the surveys, for each individual data associated with the habitat and time of day of the observation were registered. The number of researchers varied among surveys (from two to seven), and thus each survey had different effort levels (between 16 and 80 hours × person). To determine the relative abundance (RA) of different species in the sector, a classification of

**Table 1.** Epidemiological surveillance from the year 2008 to 2014 for each of the reported amphibians within the sector of the experimental station of San Lorenzo, 2,200 m altitude, Sierra Nevada of Santa Marta, Northern Colombia. Very rare (VR); Rare (R); Common (C); Abundant (A); Very Abundant (VA).

Species	<i>n</i>	RA	Microhabitat	Habit	N° diseased individuals	Disease type	N° deformed individuals	N° dead individuals	Year of reported sick individual
<i>Atelopus laetissimus</i>	128	VA	Terrestrial/Shrubs	Nocturnal	1	Cutaneous ulcers	1	0	2013
<i>Atelopus nahumae</i>	10	R	Terrestrial/Shrubs	Diurnal/Nocturnal	0	—	1	0	2013
<i>Ikakogi tayrona</i>	26	C	Shrubs	Nocturnal	1	Undetermined	0	0	2008
<i>Pristimantis delicatus</i>	13	R	Shrubs	Nocturnal	0	—	0	0	—
<i>Pristimantis carmelitae</i>	23	C	Terrestrial	Nocturnal	0	-	0	0	—
<i>Pristimantis cristinae</i>	5	VR	Shrubs	Nocturnal	0	-	0	0	—
<i>Pristimantis insignitus</i>	4	VR	Terrestrial	Nocturnal	0	-	0	0	—
<i>Pristimantis megalops</i>	477	VA	Terrestrial	Diurnal/Nocturnal	9	Fibropapilloma (tumors)	0	0	2008; 2013; 2014
<i>Pristimantis ruthveni</i>	15	Rare	Shrubs	Nocturnal	0	—	0	0	—
<i>Pristimantis sanctaemartae</i>	211	VA	Shrubs	Nocturnal	0	—	0	0	—
<i>Pristimantis tayrona</i>	48	A	Phytotelmata	Nocturnal	0	—	0	0	—
<i>Pristimantis</i> sp. nov. 1	29	C	Shrubs	Nocturnal	0	—	0	0	—
<i>Pristimantis</i> sp. nov. 2	21	C	Terrestrial/Shrubs	Nocturnal	0	—	0	0	—
<i>Bolitoglossa savagei</i>	44	A	Shrubs/Phytotelmata	Nocturnal	0	—	0	0	—



**Fig. 3.** Healthy individuals of *Atelopus laetissimus* (A) (Bufonidae); *Atelopus nahumae* (B) (Bufonidae); *Pristimantis megalops* (C) (Craugastoridae); and *Ikakogi tayrona* (D) (Centrolenidae). Photographs by Luis Alberto Rueda Solano.

very rare, rare, common, abundant and very abundant, according to the of individuals (ind.) recorded during all the surveys was established. Species were very rare, if it was observed equal to or less than nine ind.; rare, if it was observed between 10–20 ind.; common, if it was observed between 21–30 ind.; abundant if it was observed between 31–50 ind.; and very abundant, if it was observed over 50 ind. in the total of all surveys. For identification of the species belonging to the genus *Pristimantis* we employed the synopsis of Lynch and Carranza (1985) and for the remaining species we used the information provided by the American Museum of Natural History from its online reference Amphibian Species of the World (Frost 2014).

#### Monitoring of *Atelopus laetissimus* and *Atelopus nahumae*

Due to the importance represented by the species of *Atelopus*, for being one of the most affected genus for *Bd* (La Marca et al. 2005; Lotters 2007), we monitored both species reported at this locality, *A. laetissimus* and *A. nahumae* (Ruiz-Carranza et al. 1994). These species were monitored during the same months of the amphibian inventories of the sector. Nevertheless, these surveys were done during two additional days. Employing a low lying transect of 50 m in length and five m wide over the stream known as “La Quebrada San Lorenzo” located about 500 m north of the Estación Experimental de San Lorenzo at 2,100 m (Fig. 2B). Surveys were done twice daily for individuals of *Atelopus*, in the morning (9:00–12:00 hrs)

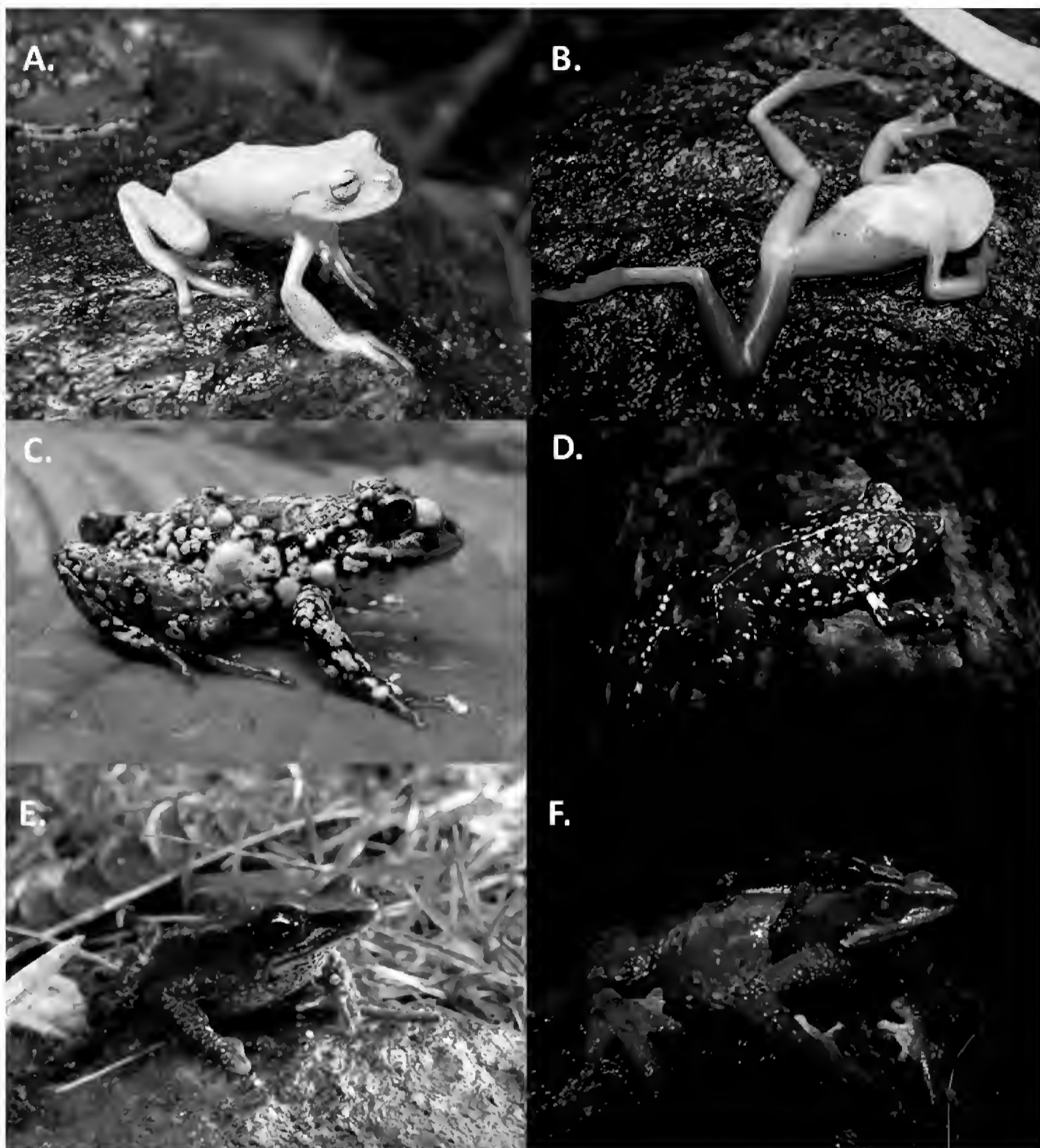
and at night (18:00–00:00 hrs). For individuals of *Atelopus*, SVL, weight, and sex have been systematically recorded since 2013. Each individual was handled with a new pair of gloves as part of the biosecurity protocols to prevent the transmission of *Bd*.

## RESULTS

### Epidemiological Surveillance

To date no mass mortality events have been reported, or individuals with field evidence of *Bd* infections in the assemblage of the 15 endemic species of amphibians at the San Lorenzo sector, corresponding to 1,375 ind. mostly healthy individuals of Bufonidae, Craugastoridae, Centrolenidae, and Plethodontidae (Table 1, Fig. 3). However, 13 sick individuals of the species *Ikakogi tayrona*, *Atelopus laetissimus*, *A. nahumae*, and *Pristimantis megalops* were recorded (Table 1, Fig. 4).

The sick individual of *Ikakogi tayrona* presented symptoms similar to those of chytridiomycosis, such as lethargy, pale skin, and hyperemia of the ventral skin (Fig. 4A, B). Nevertheless, the conventional PCR laboratory analysis yielded negative results for *Bd*. *Pristimantis megalops*, presented the greatest number of sick individuals (Table 1), they were affected by white colored tumors, bulgy and with a hard consistency (Fig. 4C, D). Healthy skin of *P. megalops* is smooth and dark-brown in color (Fig. 4, 5), while the area associated with the tumor was hyperplastic with white coloration (Fig. 5A). The



**Fig. 4.** Sick individuals of *Ikakogi tayrona* (A, B same individual); *Pristimantis megalops* (C, D), and malformation in *Atelopus nahumae* (E) and *Atelopus laetissimus* (F) found in epidemiological surveillance 2008–2014 in La Estación Experimental de San Lorenzo (2,200 meters), Sierra Nevada de Santa Marta, North of Colombia. Photographs by Luis Alberto Rueda Solano (A, B, D), Cesar Molina (C); Andres Rocha Usuga (E, F).

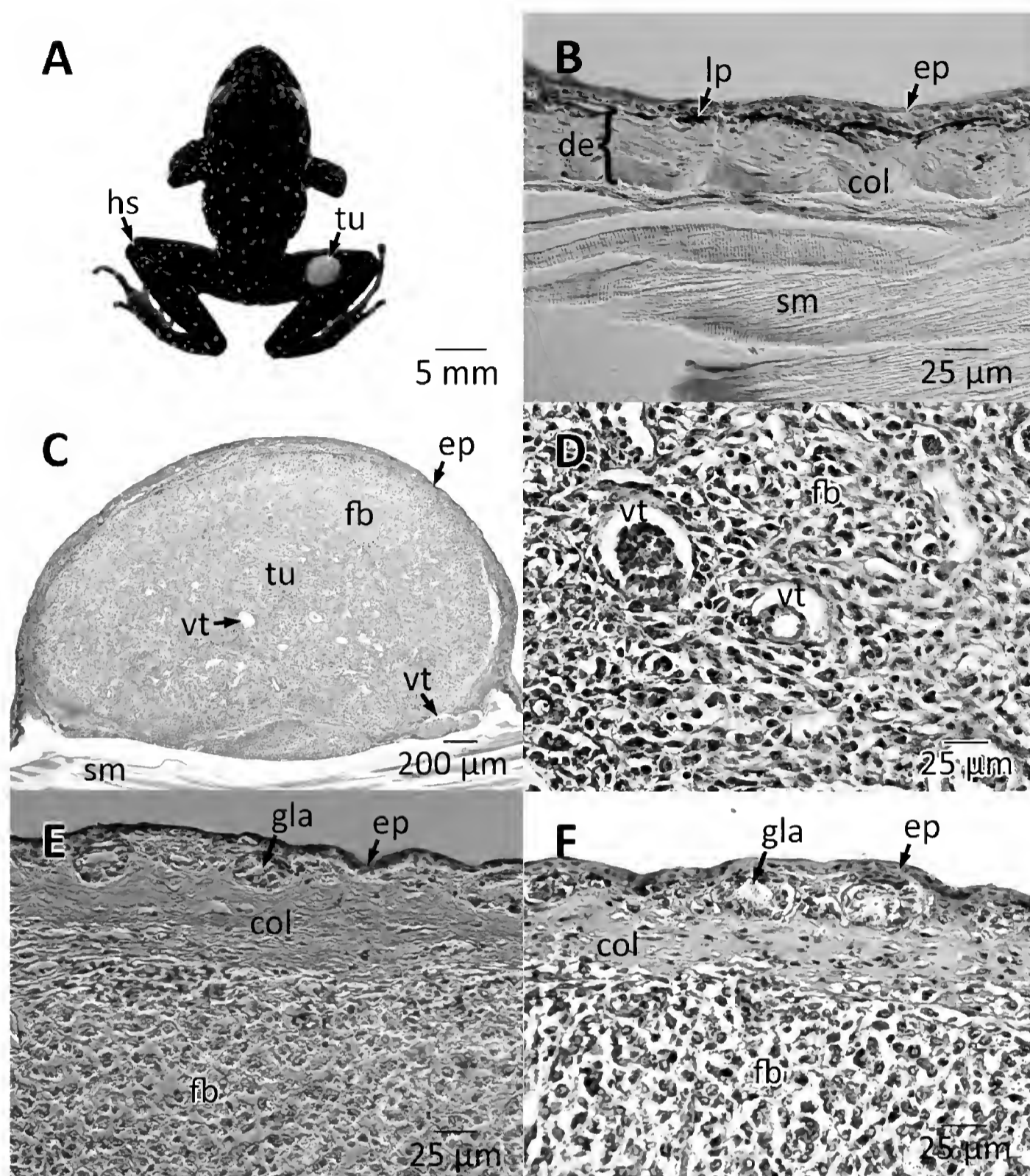
epidermis of the areas that are not associated with tumors is thin with few cell layers and is delimited by a layer of strongly pigmented skin cells (Fig. 5B). The dermis is thick and has large collagen layers that are joined closely to the underlying striated muscle through connective tissue (Fig. 5B). Neoplasia histologically corresponds to a skin fibropapilloma or fibroma, formed mainly of fibroblasts and marked anisocariosis characterized to be heavy vascularization (Fig. 5C, D). The fibropapilloma is white in color because the pigmented layer of the dermis present in healthy skin disappears completely (Fig. 5E, F). Also, dermal collagen layers are less compact, unorganized, and not associated with the muscle due to the profuse growth of fibroblasts in the tumor (Fig. 5F). The epidermis associated with neoplasia can present areas of few cell layers similar to healthy skin or otherwise be hyperplastic (Fig. 5F). Despite these individuals presenting cutaneous disease, they did not present symptoms of lethargy or malnutrition. No other similar characteristics

pertaining to these tumors were observed in other species of the assemblage.

Regarding *Atelopus laetissimus* only one individual presented a cutaneous ulcer on the abdomen, and two individual of *A. laetissimus* and *A. nahumae* were found with malformations of the extremities and face (Fig 4 E, F). Skin swab samples were collected for future *Bd* analysis for *Atelopus* species. In 2013 we collected 11 samples for *A. laetissimus* and one sample for *A. nahumae* and in 2014, 13 samples for *A. laetissimus* and four for *A. nahumae* were collected. These samples were deposited dry and cool (-20 °C) at the biology laboratory of the Universidad de Magdalena.

### Amphibian Assemblage

All species for which epidemiologic surveillance was carried out at the sector of San Lorenzo are endemic to the SNSM. The majority of individuals (61.5%) belong



**Fig. 5.** Healthy skin and fibropapilloma in *Pristimantis megalops* (A). Cross section of the healthy skin of *Pristimantis megalops* (B): The epidermis is thin, can be seen pigmented layer and layers of collagen in the dermis. Cross section of Fibropapilloma (C). Detail of fibroblasts forming fibropapilloma (D): the tissue is highly vascularized. Detail of the epidermis and collagen layers covering fibropapilloma (E–F). Histochemical staining in Fibropapilloma (F): The layers of collagen in the dermis can be seen in magenta color. **fb**: fibroblasts; **col**: layers of collagen; **de**: dermis; **ep**: epidermis; **gla**: dermal glands; **hs**: healthy skin; **lp**: pigmented layer; **sm**: striated muscle tissue; **tu**: tumor or fibropapilloma; **vt**: vascular tissue. Photographs by Edgar Javier Rincón Barón.

to the genus *Pristimantis*, with ten species (of 15 in total); dominating in abundance, two of these are in the process of being described (Table 1). *Pristimantis megalops* and *P. sanctaemartae* were the most abundant. In this sector both genera of endemic and monotypic species *Ikakogi tayrona* and *Geobatrachus walkeri* were recorded. Only one species of salamander was observed, *Bolitoglossa savagei* with a moderate abundance (Table 1). Similarly, both described species of *Atelopus* for this sector were reported, *A. laetissimus* are more abundant; conversely individuals of *A. nahumae* were rarely observed (Table 1).

### Monitoring of *Atelopus laetissimus* and *Atelopus nahumae*

In relation to the monitoring of the *Atelopus* species at the San Lorenzo stream, 128 records of *A. laetissimus*

were obtained over the course of all the surveys, with an annual mean of 18 ind. ( $n = 7$  surveys  $SD \pm 5.46$ ). In 2008 a mean of 19.5 ind. ( $n = 2$  surveys;  $SD \pm 2.12$ ) (Fig. 6), in 2009 only one survey was done in which nine ind. were recorded (Fig. 6). From the year 2010 through 2012 no monitoring was done at the San Lorenzo stream. In 2013 a mean of 16 ind. ( $n = 2$  surveys  $SD \pm 1.41$ ) of *A. laetissimus*, making this the year with the fewest number of records in comparison to other years (Fig. 6). Finally, in the year 2014 the greatest mean was recorded with 24 ind. ( $n = 2$  surveys;  $SD \pm 2.82$ ) (Fig. 6). Although the sequence of years is incomplete, the tendency in the past two years has been a slight increase in the number of observations of *A. laetissimus* individuals (Fig. 6).

Around 90% of the records of *A. laetissimus* observed have been males, principally in the night hours perched on leaves. Males had a snout-vent-length (SVL) and average weight of 4.05 cm ( $n = 80$   $SD \pm 0.46$ ) and 4.87 g

( $n = 80$   $SD \pm 1.65$ ) respectively. Only 11 females were observed, with SVL and weight averages of 5.0 cm ( $n = 11$   $SD \pm 0.46$ ) and 6.5 g ( $n = 11$   $SD \pm 1.23$ ) respectively. For *A. nahumae*, 10 individuals were recorded, two male individuals in the year 2008, one female in the year 2013 and three males and four females in 2014. Due to the scarce observations it was not possible to discern a population trend.

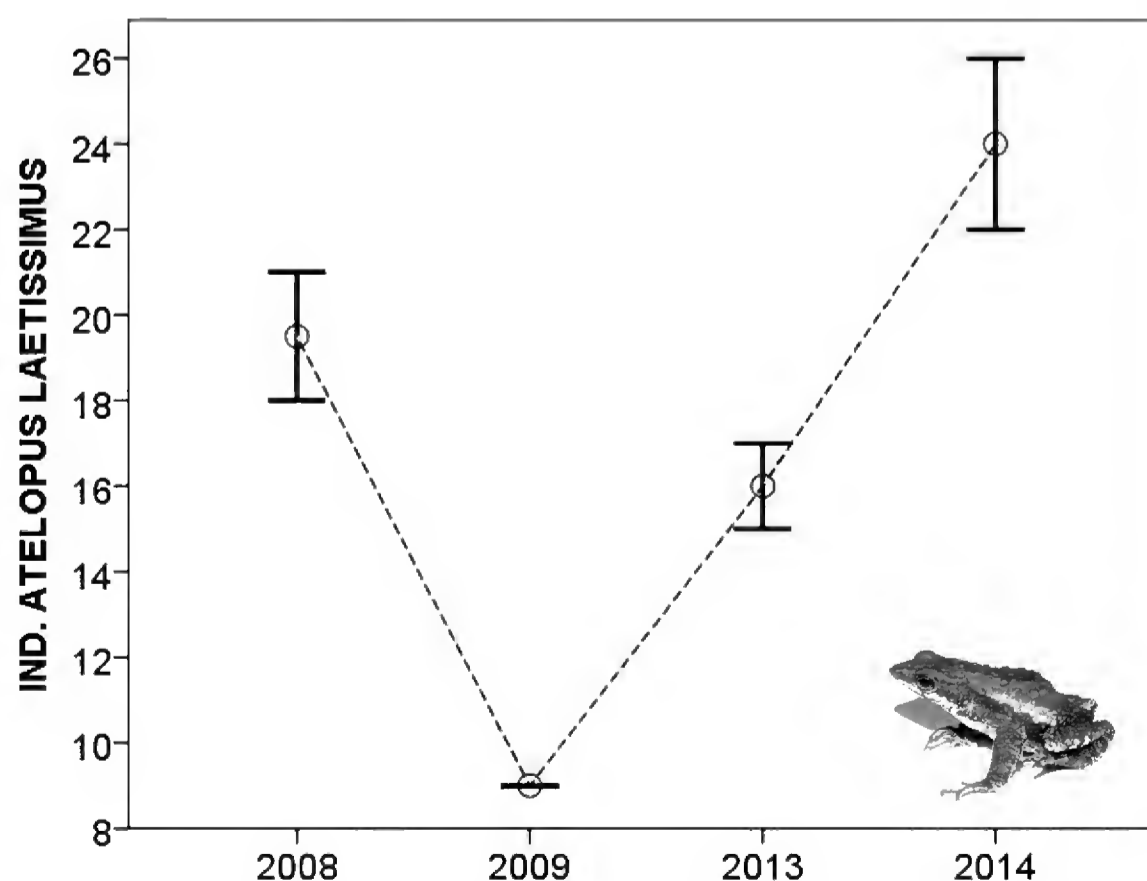
## Discussion

Epidemiological surveillance in the amphibian assemblage of the San Lorenzo sector showed a low number of individuals with symptoms of disease and no dead individuals were found over the years 2008, 2009, 2013, and 2014. This contrasts with other localities in protected areas and nonprotected areas of the American continent in countries such as Canada (Greer et al. 2005), United States (Green et al. 2002), Costa Rica (Crump et al. 1992; Lips and Papendick 2003), Panama (Crawford et al. 2010; Lips et al. 2006; Lips 2003), Venezuela (Bonaccorso, Guayasamin, Méndez, and Speare 2003), and Ecuador (Bustamante et al. 2005; Merino-Viteri et al. 2005), where mass mortalities and population and amphibian assemblage collapses have been reported due to emergent diseases.

Only one mass mortality event has been reported in Colombia in the year 1997 in Serranía de los Paraguas between the departments of Choco and Valle del Cauca (Lynch and Grant 1998), nevertheless anecdotal information shows the absence of some amphibian species in other localities which were previously more diverse and abundant, for example the Parque Nacional Natural Chingaza (A. Amézquita and C. Navas, pers. comm.).

This would not be the case for the amphibian assemblage of the protected area of the San Lorenzo sector, given that all the species reported have historical type localities from this mountain range (Lynch and Carranza 1985) including the species of *Atelopus* (Ruiz-Carranza et al. 1994) which would be vulnerable to declines due to chytridiomycosis (Lips et al. 2003). Additionally, we did not find evidence that any of these species are currently experiencing population declines. On the contrary, in this study new species were reported increasing the diversity of endemic amphibians for this locality and for the biogeographic region. Although, some anuran species still inhabit this mountain range, we could not survey them occurring in remote locations with restricted access; this is the case of *Atelopus arsyecue*, *A. walkeri*, and *A. carrikeri* (Rueda-Solano 2008). Therefore, our conclusions only focus on the populations of the protected area of the Serranía de San Lorenzo, for other localities we do not know the conservation status of amphibian assemblages. Although biosecurity protocols are an important aspect in the conservation of endemic amphibians, this study does not formally test or validate their decontamination protocols in this protected area.

In the special case of sick individuals found throughout the epidemiologic surveillance, the signs of illness of the individual of *Ikakogi tayrona* were very similar to those presented by individuals affected by chytridiomycosis (Daszak et al. 1999). Nevertheless, with negative *Bd* results the possibility exists that the signs detected in the field may be misinterpreted as typical of chytridiomycosis, when in reality are part of the symptomatology of other diseases which have not been previously diagnosed. However, our results are limited mainly to the detection of signs of chytridiomycosis in the field, it is al-



**Fig. 6.** Monitoring of *Atelopus laetissimus* through the years in the Quebrada San Lorenzo Serranía de San Lorenzo, SNSM. Circle = average number obtained from individuals in each year ( $n = 2$  samples for the years 2008, 2013 and 2014) ( $n = 1$  sample for 2009); Error Bars = maximum and minimum individual in each year. Dotted line = trend in the number of individuals over time.

ways recommended to corroborate the field observations with laboratory analysis for accurate detection of *Bd*.

In respect to the sick specimens of *Pristimantis megalops*, we are unaware of any reference regarding the symptoms presented by these individuals in Colombia, however similar neoplastic diseases are documented in others amphibian species (Green and Harshbarger 2001; Khudoley and Mizgireuv 1980). The origin of these spontaneous neoplasms are still unclear and often limited to specific species or populations (Stacy and Parker 2004), as a matter of fact; *P. megalops* was the only species in the assemblage that presented this cutaneous anomaly. Similarly, we are unaware if these tumors may be lethal for these individuals, above all because the individuals reported to have these tumors did not appear to have affected locomotion or nutritional state. It is necessary to conduct more studies about the tumor effects on these individuals, at the physiological, behavioral, and ecological level. Additionally, it should be corroborated whether this disease is unique to *P. megalops* or it can be found among populations of amphibians at different localities.

Historically the populations of *Atelopus laetissimus* and *A. nahumae* found at the Serranía de San Lorenzo have been abundant (Carvajalino-Fernandez et al. 2008; Granda-Rodríguez et al. 2008, 2012; Ruiz-Carranza et al. 1994). Nevertheless, at our study site, *A. laetissimus* is predominantly abundant, while *A. nahumae* is ecologically rare, it may be due to our surveys coincide with the upper extreme of its altitudinal distribution. This is corroborated at lower localities (approx. 1,500 m) where this species is predominantly abundant and *A. laetissimus* is ecologically rare (Carvajalino-Fernandez et al. 2013; Rueda-Solano, pers. observ.). In relation to the abundance of *A. laetissimus* at the San Lorenzo stream, we do not have sufficient data to estimate a population trend, nevertheless, in the last two years of monitoring (2013 and 2014) we have found a similar abundance, which may suggest a stable population for this species in the sector of the Sierra Nevada de Santa Marta. This conclusion coincides with previous studies which show relative abundances of *A. laetissimus* and *A. nahumae* (Carvajalino-Fernandez et al. 2008; Granda-Rodríguez et al. 2008, 2012). At present *A. laetissimus* and probably *A. nahumae* have stable and abundant populations, this strongly contrast with the problematic declines and extinctions of the entire *Atelopus* clade in the neotropics principally those which inhabit high elevations (La Marca et al. 2005; Lotters 2007). Nevertheless, we are unaware of the historical population trend for these species, especially during the years where reported declines were in Colombia (Lynch and Grant 1998), where *Bd* may have caused declines in these species, however it was not documented and may currently coexist with *Bd*, as occurs for other species of *Atelopus* (Flechas et al. 2012; Tarin et al. 2014), or in other amphibian communities where there is a higher prevalence of *Bd* and no evidence it affects the natural populations (Guayasamin et al. 2014). We suggest ret-

rospective studies including historical demography and *Bd* diagnosis using museum specimens (Rodríguez et al. 2014; Cheng et al. 2011).

Similarly, the majority of records for *Atelopus laetissimus* correspond to male individuals found active during nocturnal surveys, perched on leaves of bushes adjacent to streams. These observations coincide with historical data, in similar proportions (Granda-Rodríguez et al. 2008). In respect to females of these species, we can only deduce they possess different home ranges than males and encounters occur during reproductive seasons. Our results provide strong evidence about the nocturnal habits of the males of this species, which would employ leaves to perch and forage from (Rueda-Solano, pers. observ.). These observations are inconsistent with almost all the species of the genus *Atelopus*, which are diurnal (Lotters 1996), with one exception previously described for *Atelopus nocturnus* (Bravo-Valencia and Rivera-Correa 2011).

## Conclusion

The implementation of epidemiological surveillance, monitoring of key amphibian species, and biosecurity protocols at the San Lorenzo area have been constituted as important strategies for the conservation management of the endemic amphibians within the protected area of the Sierra Nevada of Santa Marta. It is expected that these actions be sustained and replicated at other protected areas in Colombia and the world, with amphibian assemblages susceptible to *Bd*. At a minimum they should serve as a baseline in establishing amphibian conservation methods and best management practices for *in-situ* programs. In like manner these actions should be complemented with the utilization of laboratory methods for the detection of *Bd* and other diseases.

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demiological surveillance and the timely understanding of environmental factors and anthropogenic ones which may influence wildlife conservation.

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## Epidemiological surveillance and amphibian assemblage status



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## PERSPECTIVES

# Tadpoles, froglets, and conservation: A discussion of basic principles of rearing and release procedures

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**Abstract.**—We outline component features of the captive environment and the natural world that should be considered when designing a program for head-starting and releasing amphibians, presumably as part of a conservation project. The main points indicate the importance of accounting for features of the basic biology of amphibian larvae, the biology of the focal species, and highlight the types of error risks based on generalities, human convenience, and logistical limitations. Similarly, we urge consideration and evaluation of the quality of the metamorphs that are produced over the sheer quantity produced and released. While most of the examples are taken from pond-breeding species, the general principles are relevant, and details may be modified to fit amphibian species with larvae in other habitats.

**Key words.** Amphibians, conservation, larvae, reintroductions, translocations, head-start, captive breeding

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Captive rearing of endangered frogs is a large, expensive (Mattioli et al. 2006), and time-consuming component of conservation efforts. We discuss a number of factors intended to improve the chances of successful rearing and release of captive individuals (McGregor and Zippel 2008). Central themes are 1) acceptance that captive-reared animals differ in a number of ways from those from natural populations (e.g., Griffiths and Pavajeau 2008; Gawor et al. 2012); 2) rearing protocols must address the specific biology of each species (e.g., Morrison and Hero 2003); 3) avoidance or reduction of activities that cater only to our conveniences; and 4) evaluation of criteria to judge success based on metrics other than just numbers released. We emphasize the need for long-term monitoring of the success of the releases. Most suggestions center around testing, improving, and standardizing

species-specific procedures once those that produce individuals of the highest probable fitness are verified, not just the most individuals. Tadpole mortality varies across experimental venues (Melvin and Houlahan 2012), so survival and fitness likely vary according to husbandry regime, release protocols, and even captive breeding itself.

We argue that each taxon-specific system should directly address several types of questions. Do artificial environments and particularly the food sources used in captive programs alter reared froglets relative to what wild individuals experience (i.e., rapid acclimation to captivity of Griffiths and Pavajeau 2008)? Do these alterations adversely manifest themselves in the survival and fitness of 1) the released animals; 2) the population in which the animals are released; or 3) the meta-

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populations with which they interact? The real question is: do the introductions contribute in a beneficial way to conservation, or is there the appearance of conservation (through numbers) when actions actually degrade the fitness of the population (e.g., via artificial selection)? How do the morphological, immunological (Venesky et al. 2012), and hematological (Davis and Maerz 2011; Davis 2012) conditions of reared froglets compare to those of wild individuals? For example, Burns et al. (2009) found that the first-generation of captive-bred and reared guppies had smaller brains than wild individuals, and Fraser (2008) noted that re-introduced leopard frogs showed abnormal behaviors in the wild. It must be recognized that captive breeding, often with limited choices of mates and usually with small numbers of individuals, constrains or eliminates the possible beneficial effects of sexual selection (Williams and Hoffman 2009). Captive rearing of larvae from the wild may avoid problems related to sexual selection, but nonetheless impose selective pressures on the offspring. Because fisheries researchers have faced these problems for years, they have a good perspective based on the pitfalls of releasing captive-reared individuals into the wild. Studies from fisheries science have indicated that the quality or fitness of captive-reared individuals are not necessarily equivalent to those of wild individuals (e.g., Araki et al. 2007; Christie et al. 2012). Amphibian conservationists would be advised to review the references cited herein and create means of measuring and assessing the biological quality of wild and captive tadpoles.

Within these contexts, we recognize the limitations of funding, the expectations of administrators, and the expertise of personnel sometimes can work against the success of a given program. Stakeholders and participants in conservation programs may be pressured to, or evaluated on, the release of as many individuals as possible, as soon as possible, with minimal cost. These realities can lead to the implementation of inordinate or biologically inappropriate actions that may not increase the chance of the timely release of more viable individuals. For example, we have heard comments such as “*I do not understand. Some of them took off swimming across the pond and some of them got balled up in sand like they did not know what was going on.*” In fact, reared individuals may not perform successfully at basic tasks like prey recognition or seeking refuge, but they can be given a chance to learn (i.e., pre-release training of Griffiths and Pava-jeau 2008, or the “soft-release” concept as it is termed in some conservation programs). Other statements that we have overheard include “*That skinny tadpole likes to lie on its side,*” “*I do not know why that tadpole swims in a spiral,*” “*These tadpoles constantly swim up and down the wall of the aquarium.*” All of these cases describe tadpoles that either are sick, stressed, or otherwise unsuitable for release, and examples of at least the first two cases should certainly be culled to improve the collective quality of the cohort (e.g., Nye and Cameron 2005).

We looked at two kinds of relevant information from the natural world to get a relative idea of what is faced in terms of the odds of survival in amphibian breeding. This perspective is crucial for evaluating captive programs, but seems to be poorly considered by practitioners. Some stakeholders we have observed seem to evaluate programmatic success by trying to maximize the number of metamorphic individuals that are released. Survival rates from egg to metamorphosis range from 0–20 % and are commonly 1–5 % (Wells 2007: table 14.5, fig 14.9). Survival from metamorphosis to first reproduction ranges from 6–26% (Herreid and Kinney 1966; Licht 1974). Greenberg and Tanner (2005) tracked the success of 23 breeding events of *Scaphiopus holbrookii* at eight sites in Florida over nine years; five of these events were considered successful by producing a minimum of 100 metamorphs likely derived from > 107 eggs deposited on site. Semlitsch et al. (1996) reported only one reproductive event at one site that produced significant numbers of metamorphs of *S. holbrookii* over 16 years. High levels of mortality are typical of many amphibian reproduction efforts, and efforts of husbandry to avoid such mortality may not be desirable.

Survival and fitness are correlated with environmental conditions and diet. Dietary requirements of metamorphs may be easily underestimated. For example, a grand cohort of 7,000 (1,000 each of seven species) metamorphs can consume at least  $2.3 \times 10^6$  insects the size of *Drosophila* in the first post-metamorphic month (JRM and RA, unpubl. data). This number, about 2,250 g, will produce about 930 g of frog tissue (RA, unpubl. data). A frog needs about 20 cal/day/g body weight for maintenance at 20 °C (Mazur 1968). At 5796.6 cal/g dry weight of fly tissue (Cummins and Wuycheck 1971), one can calculate that a frog could consume about 2.2–4.2 times the calories needed for maintenance during the first post-metamorphic month. Also, it must be recognized that specific conditions at one point in the rearing process can influence the quality of an individual much later in ontogeny (e.g., Scott et al. 2007; Gervasi and Foufopoulos 2008; Gagliano and McCormick 2009; Uller et al. 2009; Van Allen et al. 2010) or perhaps more importantly, in subsequent generations (Frost et al. 2010). Because the behavioral, immunological, morphological, and physiological qualities of reared individuals seldom are measured or evaluated in amphibian programs, there is little idea if techniques (e.g., diet) are producing individuals of good quality. There are many factors that modify various qualities of metamorphs and postmetamorphs (e.g., Alford 1986; Blouin 1991, 1992; Gramapurohit et al. 2004; Relyea and Auld 2005, and many others). In light of these data, the percentage of individuals produced are likely quite a poor estimator of success after release, and success should not be equated with the introduction of the largest possible number of tadpoles or metamorphs into the wild.

## The Natural World

We emphasize that not all aquatic larvae are adapted to similar aquatic environments. For example, phytotelmata are very different environments than are streams or ponds. Yet, the default rearing vessel for amphibians in captive programs is a stock, straight-walled aquarium. Focusing on the natural pond, for this example, let us consider this is an environment with a large surface area per volume for gas exchange and waste dispersion, wind and temperature induce water movements, and populations of plants and animals dispense with metabolites. The sloping banks allow tadpoles to escape some predators and seek water of the desired temperatures during development, and those approaching metamorphosis can safely switch from gas exchange across the gills to the lungs. During their daily movements about the pond, tadpoles learn directions to proper areas involving temperature, food, and refuge. The default enclosure with vertical walls and uniform depth violates all of these natural conditions, and the larvae have no exposure to differing microclimates, a natural light cycle, or the myriad ecological conditions that wild individuals encounter. Careful consideration of the egg deposition sites chosen by breeding frogs, and post-hatching behaviors of tadpoles should inform all aspects of the rearing enclosure, its placement, and environment. We should consider whether it is even possible to raise high-quality frogs indoors, or tropical frogs in a typical Nearctic zoo?

With respect to the natural world, and returning to the idea of pre-release training (Griffiths and Pavajeau 2008), we suggest that survival after release would increase appreciably if the simple tenets of Y-axis orientation (Ferguson et al. 1965; Taylor and Ferguson 1969) were implemented. This type of orientation allows individuals to move about their habitat productively as they receive input visually or via the pineal complex (i.e., nonvisual, including the patterns of light polarization; Taylor and Adler 1970). An accurate sense of time is involved, and the biological clock must be reset each day by witnessing sunrise in order to stay synchronized with changes in day lengths. A fixed light-dark cycle in the laboratory does not entrain the animals in any way because there is no appropriate movement of the “sun” and no changes in day length. Naive tadpoles released without training may have a higher probability of being predated, and a released metamorph (i.e., small size with poor locomotor skills, large surface-volume ratio and thus rapid water loss, likely with small energy reserves, and no idea of the locations of proper refuges) that makes one wrong directional choice has a high probability of dying.

We advocate that the adoption of the research protocol of Taylor and Ferguson (1969) into the release procedures would surely improve the success of the project. All that is needed to follow our pond-breeding example is: construct a meshed, wire cage with a top and bottom (about 100 × 50 × 30 cm placed with its long axis about

a third on land and two-thirds in the water, place tadpoles or metamorphs in the water and include moist cover for froglets, and wait for at least two days). Tadpoles and froglets of stream-breeding species (Preininger et al. 2012) obviously would also benefit from this procedure with some innovation of different meshed enclosures for stream species. The point is that, by experiencing even two sunrises, the animals will know the Y-axis, and when they are released, the animals can be expected to have a much better chance at survival because they are more likely to make the appropriate decisions.

## The Culture World

We comment on several related topics that we feel are important; in all cases, stage refers to Gosner’s (1960) table. The various forms of egg clutches (Altig and McDiarmid 2007) may be quite fragile, but individual eggs are much more robust than one would assume. Clutches can be pulled or cut apart without damaging the ova to improve the surface area/volume of the groups. In the case of pond breeding species, eggs should be placed in water not much deeper than the groups of eggs. Different protocols are advisable, of course, for species that breed in substantially different environments (e.g, streams or phytotelmata). The natural history of the species and the specific conditions under which its larvae develop must be considered and incorporated into the rearing protocols.

With respect to tadpoles, consider those of the Costa Rican Leaf Frog *Agalychnis lemur* that occur in very shallow, virtually non-moving swamps in nature; this must be considered when rearing this species in the lab. Many zoos and labs rely on stock aquaria, or similarly shaped tanks, that often are poor choices for rearing containers. They have a small surface area/volume, and this is a problem exacerbated by the tendency to put too many individuals in a given tank. If a caretaker laments the management of air stones and water changes, then the system is incorrect by definition. Information on management of water quality are reviewed in Poole and Grow (2012) and Pessier and Mendelson (2010). Tadpoles that swim up and down the glass are signaling that they are stressed by inappropriate temperatures, oxygen concentrations, or lack of naturalistic gradients of these crucial variables. Patterns of temperature variation can unpredictably influence developmental rate and morphology (Arrighi et al. 2013). Similarly, inappropriate quantities of food or refugia, waste buildup, or population density will also cause stress in tadpoles. As an example of our concept, consider a hypothetical pond-breeding species. Shallow pans are not recommended. They have a reasonable surface area/volume, but their total volume is small and thus water chemistry is quickly overwhelmed by food and feces, and catastrophic water loss to evaporation is easy to miss. A plastic wading pool or some similarly shaped, shallow enclosure is the best

because of the large surface area/volume. Aeration is not needed and water changes will only be necessary 1–2 times during the rearing of a batch of 300 tadpoles in a 143-cm diameter pool. Water depth in these pools does not need to exceed 6–8 cm and usually 4 cm is sufficient for most pond-breeding species; it is the surface area that is important because deeper water does not increase the usable space for more tadpoles. Flow-through systems decrease the manual work involved but are probably the worst at providing the animals the chance to acquire the proper intestinal bacteria.

We contend that the fear of the chytrid fungus, other pathogens, and caretakers' zeal for cleanliness has stimulated keepers to create overly clean environments, and this action deprives the tadpoles of acquiring intestinal symbiosis (Pryor and Bjørndal 2005a,b). Coprophagy, especially after the material has developed large populations of microbes and protozoan, is a viable feeding tactic of tadpoles (Steinwascher 1978). Careful management of diseases in captivity is based more on common-sense husbandry protocols, rather than absolute sterilization (Pessier and Mendelson 2010). Proper work-flow regimes, dedicated equipment, and vigilant monitoring of water quality are recommended over routine water-changes (in the absence of measurements to validate such) and bleaching of tanks and substrates.

Tadpoles and metamorphs have been fed many different foods (e.g., Modzelewski and Culley 1974; Claussen and Layne 1983; Jörgensen 1989; Pryor 2003; Hailey et al. 2007; Pramuk and Gagliardo 2008; and many others), but decisions and choices of foods do not appear to be based on data derived specifically from developmental performances, size, or energetic reserves present at metamorphosis. Amphibians sequester most of their body fat in the fat bodies attached to the anterior ends of the kidneys. Assessing the size of the fat body (i.e., dissection of a few specimens, or perhaps development of techniques using sonography or electrical conductivity; Walsberg 1988) of individuals raised on different diets would be a valuable endeavor. Part of the problem is that caretakers are just starting to distinguish between the consummatory and digestive diets of tadpole, with the real distinction being between what tadpoles ingest versus what they digest (Altig et al. 2007; Schiesari et al. 2009; Whiles et al. 2009). How oral structures influence feeding performances (e.g., Venesky et al. 2010a,b) also remains to be determined. The main point is that tadpoles swallow large quantities of the products of primary productivity (e.g., plants or algae), but when one considers periphyton, it is likely that these plants or algae may not represent the primary energetic intake (Altig et al. 2007). For example, one might consider adding a bit of clay soil to the rearing containers (Hailey et al. 2007; Akers et al. 2008). Adding supplements of natural foods, such as wild-collected aquatic plants or algae, is a viable idea, but one can inadvertently add vicious predators as well as potential pathogens and parasites. Algae can be allowed

to develop in tanks, and there are protocols for cleaning plant materials before introduction to tanks (Pessier and Mendelson 2010); in either case, the important periphyton will develop. Tetramin<sup>®</sup> fish foods (TetraWerke, Melle, Germany), which contain considerable amounts of animal-based material, and powdered rabbit pellets have been used successfully by the authors, but there are no data on actual developmental performances. There are now many recipes for tadpole diets applied to a number of different programs for ranids (Abrahamse and Hayes 2009) and other taxa. These kinds of feed promote rapid production of microorganisms which likely serve as the primary food source for tadpoles. The point to keep in mind is that the knowledge-base for tadpole diets in the wild and nutritional needs is poor and far from taxonomically complete. Basic research in the form of controlled studies is necessary for virtually all species.

If the program is designed to release post-metamorphic individuals and rearing conditions have been suitable, then the majority of tadpoles will reach metamorphic stages 41–42 (i.e., eruption of front legs) at nearly the same time (Wells 2007). If a large proportion (e.g., 75–90 %) of the tadpoles do not metamorphose over a short period of time, then one may assume an excessive population density or some other factor has impeded normal growth. Metamorphs should be held until tail resorption is complete because tailed individuals have reduced locomotor abilities. If post-metamorphic frogs are to be maintained in captivity, then abundant and diverse small prey must be available. A single-species diet of fruit flies alone does not match the diversity of nutrients available to free-ranging froglets.

## Release of Reared Individuals

After considering the release options outlined above, the release of tadpoles and froglets should be coordinated with when metamorphosis of the target species occurs at the release locality. This detail will increase the chances of there being proper weather conditions and sufficient food available, and one might consider verifying the local prey base (Goldstein 2007). Iterative assessments, via monitoring, and appropriate modifications of the release environment may be required. In the real context of the interactions of biotic and abiotic conditions, local populations increase and decrease through time. Populations in “good localities” (sources) persist for long periods, and populations in sites of some unknown lesser conditions (sinks) appear and disappear abruptly on short time scales. If a population did not succeed at the target site under the natural conditions, one should question the logic of a restoration attempt at that site; at least we should understand the reasons for its original failure. Also, the concept of source-versus-sink populations presents a difficult decision if one wishes to establish a population at an entirely new site (e.g., Pellitteri-Rosa et al. 2008; McMurry et al. 2009; Ruiz et al. 2010). In any case, popu-

lations at sink-sites may sometimes represent important, if ephemeral, connectivity across the meta-population landscape. It is quite unlikely that any two adjacent pools present the same conditions, and in such a case, one must set aside human notions and conveniences, know the biology of the target species very well, evaluate the new site in detail, and diversify as much as is feasible with the number of individuals available. Various factors that change as a site undergoes succession can also change the likelihood of a given site being a viable site for reintroduction. Also, released individuals likely perform differently relative to other local taxa (Tingley et al. 2011).

Although there can be repercussions in doing so, one might consider reducing the chance of predation. Snakes can decimate tadpoles and froglets, but predatory fishes usually are not a problem in ephemeral sites, streams, or other types of sites where many frogs breed. Examples of the types of things to be considered for pond sites include comparisons of sites with no aquatic vegetation or short, sparse vegetation; stands of tall vegetation (e.g., especially cattails), zones covered by water lilies, and dense stands of emergent vegetation are not acceptable. These vegetation structures provide excessive organic debris that can reduce oxygen concentrations and excessive shade that inhibits proper periphyton growth. Dense stands of filamentous algae and algal mats are not acceptable because these populations reduce the oxygen concentration and some of these organisms are toxic. All manners of emergent, submergent, and floating vegetation must be considered with direct respect to the anticipated micro-habitat use of the released species.

One should also consider the qualities of adjacent terrestrial areas. High densities of froglets can occur at release sites, so additional refuges ought to be provided if one suspects that refugia could possibly be a limited resource. Artificial refugia made from PVC pipe (narrow gauge; cap on the bottom and a T-cap at the top; small drain hole about two cm from bottom to avoid filling with water; painted black) placed upright in the local environment will be used by post-metamorphic treefrogs (RA, unpubl. data). Pushing a rod into soft soil at a low angle and removing it leaves preliminary burrows for toads and ranid frogs, and pieces of PVC pipe laid on the ground and covered with soil to avoid overheating provides similar burrows. At the same time, this technique is subject to invasion by introduced fire ants (*Solenopsis invicta*) in southeastern North America; the ants use the tubes to help establish a mound and consume any froglets that may enter. Untreated wooden cover boards also can be quite useful as retreats if there is enough local moisture.

As an alternative to rearing tadpoles in pools in the laboratory, and the various concerns raised here above, it may be preferable to simply transfer eggs to the new site. Breaking a clutch into smaller pieces to enhance aeration would be prudent because the eggs likely are not placed in the same manner (e.g., attached to a twig off

the bottom) as was done by the ovipositing frogs. Protection from egg predators (e.g., mesh enclosures) would be advisable. Egg transfers between already inhabited sites can facilitate genetic connectivity between sites, if that is what the management plan recommends. The program should not reduce genetic diversity. Reintroduction to a new or extirpated site may need multiple releases, not only for establishment, but for genetic management (e.g., if only  $F_1$ 's from captive adults were released, and there is no connectivity to other populations, there would be inbreeding depression, genetic drift, etc.). Long-term genetic maintenance should be considered when deciding where and how often animals are released.

## Conclusions

The study of amphibian declines is difficult, and the search for solutions is frustrating (e.g., Beilby et al. 2009). When release programs either succeed or fail, we often are never sure of the reasons why in either case, and volumes of anecdotal information are produced. Knowledge of the genetic diversity of the populations that are released (e.g., Charmantier and Garant 2005) is crucial, and throughout our rearing attempts, we must be certain that caretakers are not perpetuating any initial problems (e.g., Walker et al. 2008). We understand that some of the points we have raised may violate restrictions of funds, personnel, facilities, and time. But, we suggest that the bar should be raised at every available chance. If implemented at the design-phase of a conservation project, our recommendations require fewer resources than traditional programs so long as the crucial component of long-term post-release monitoring is equivalent. More field data on the biology of the species involved are needed, and many of the practical or financial limitations can be overcome by rather minor changes in techniques based on better knowledge of species biology. No protocol will ever approach total success, especially when details of why the targets met their demise in the first place. Some researchers who have made multiyear releases of head-started frogs at a site, but have not yet started routine monitoring seem uninformed. Perhaps the biggest idea in this discussion is that it must be remembered that imposing non-natural conditions (Gawor et al. 2012) on tadpoles and froglets by the seemingly simple act of culturing these organisms (Denver and Middlemis-Maher 2010) should underscore all aspects of the design and evaluation of a conservation program. The quality of the released individuals, the release protocol, and post-release monitoring are the most important factors to reconsider in any amphibian reintroduction or relocation program.

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# A new case of facultative paedomorphosis in Smooth Newts, *Lissotriton vulgaris* (Caudata: Salamandridae), in Turkey

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**Abstract.**—A report of the occurrence of a dimorphic population of Smooth Newts in Lake Sazlı (Izmir, Western Anatolia, Turkey). During fieldwork on March 2015, 29 aquatic individuals (seven males, 22 females) were captured and of these individuals, five were paedomorphic (four males, one female). The metamorphic (83%) and paedomorphic (17%) ratio of the population is skewed to metamorphic. The mean snout-vent length (SVL) was 28.30 mm (range = 26.00–30.41), and total length (TL) was 57.66 mm (53.35–61.40) in paedomorphs. The average exterior gill length was 4.12 mm (2.64–4.71). The SVL was 39.99 mm (range = 33.44–39.93), and TL was 69.06 mm (66.19–79.17) in metamorphs. The possible reasons for the presence of facultative paedomorphosis in the population are discussed, with the dimorphic paedomorph hypothesis supported.

**Key words.** Salamander, Izmir, Lake Sazlı, Gediz Delta

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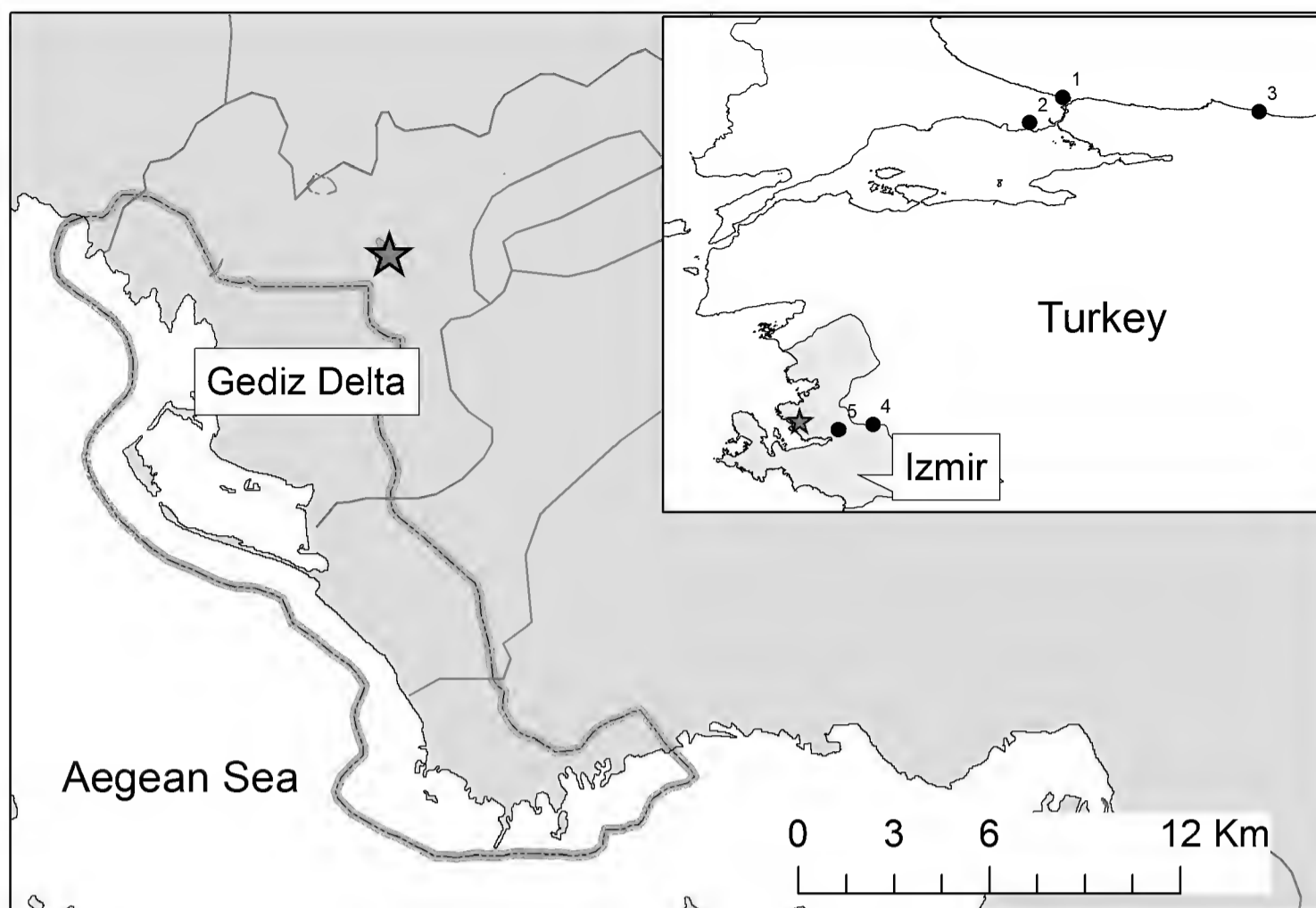
Facultative paedomorphosis is an environmentally-induced polymorphism that results in the coexistence of sexually mature, gilled, and fully aquatic paedomorphic individuals and transformed, metamorphic individuals in the same population (Denoël et al. 2005a). Paedomorphosis has been known for more than one century and can be either obligate or facultative in 57 urodeles species (Denoël et al. 2005a). Facultative paedomorphosis has been particularly reported in numerous European newts (e.g., Kalezić et al. 1994; Denoël 2005; Denoël et al. 2009a; Skorinov et al. 2009).

*Lissotriton vulgaris* has a high tendency to be paedomorphic (Beebee and Griffiths 2000) and there are many reports of the phenomenon throughout its distribution range (e.g., Skorinov et al. 2009; Litvinchuk et al. 1996; Litvinchuk 2001; Denoël et al. 2009b; Covaciu-Marcov et al. 2011; Stănescu et al. 2014). In Turkey, there are four records of facultative paedomorphosis in *L. vulgaris* from Thracian and Marmara and Aegean regions

of Turkey (Yılmaz 1983; Çevik et al. 1997; Çiçek and Ayaz 2011; Bozkurt et al. 2015, Fig. 1).

On March 13, 2015, during an amphibian and reptile monitoring survey in the Gediz Delta, paedomorphic Smooth Newts were observed in a population sampled at Lake Sazlı, which is located in the northeastern part of the Gediz Delta, approximately 15 km west of Menemen (38.600149°N, 26.911006°E, at about sea level, Fig. 1). The subspecies *Lissotriton vulgaris schmidtlerorum* (Raxworthy 1988) inhabits the Izmir region. The lake surface area is nearly 30 ha, and is surrounded by agricultural (corn, cotton, and wheat) areas and *Quercus* sp. dominated shrubs (Fig. 2a). Emergent aquatic vegetation of the lake includes Common Reed (*Phragmites australis*), Reed Mace (*Typha* sp.), Common Spike Rush (*Eleocharis* sp.), Tufted Sedge (*Carex* sp.), and Rushes (*Juncus* sp.) (Gediz Delta Management Plan 2007). Climatic conditions in the study area (Izmir) are mainly Mediterranean, with a mean annual temperature

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**Fig. 1.** The map of Gediz Delta (Izmir, Turkey). 1: Kumköy, Istanbul, 2: Küçükçekmece, Istanbul, 3: Ihsaniye, Karasu, Sakarya, 4: Lake Sülüklü, Manisa, 5: Lake İkiz, Izmir, The star shows the record (Lake Sazlı, Gediz Delta, Izmir), The dotted line shows Ramsar protected area borders.

of 18 °C (8.9–28 °C) and annual rainfall of 690 mm (20–1,430) (1950–2015, Turkish Meteorological Service 2016).

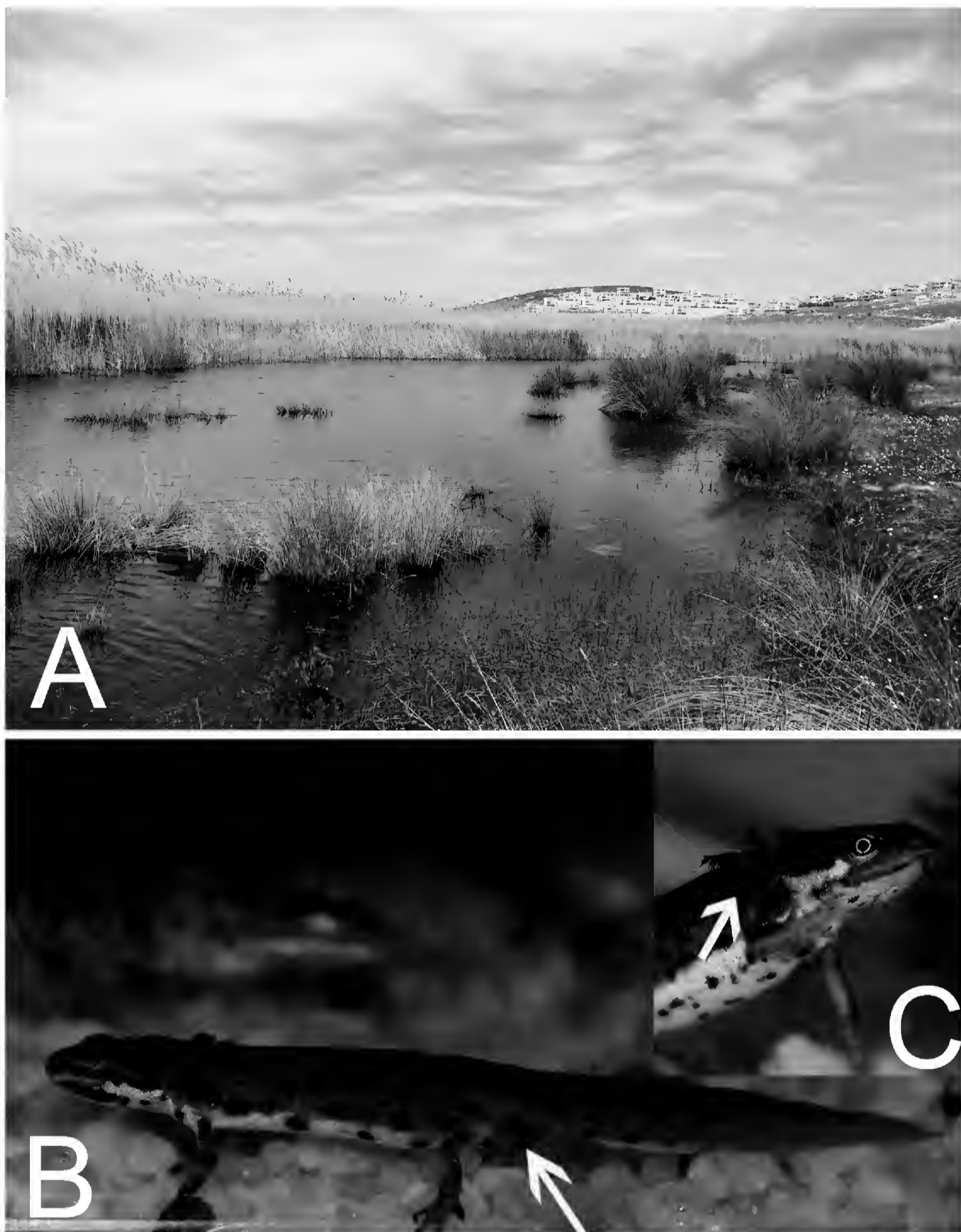
Sampling was conducted from the shore to one m depth by two persons using 45 cm radius dip nets. 29 aquatic individuals (seven males, 22 females) were captured, and of these individuals, five were found to be paedomorphic (four males, one female, Fig. 2b, c). The paedomorphs were captured 30–50 cm depth on shore of lake. The males presented sexual characteristics, including the distended cloacae, spots on the side, and a well-developed dorsal crest. The paedomorphs were easily discerned from the metamorphs by their gills. The sex of the captured individuals was recorded; snout-vent length [from the tip of the snout to the posterior of the cloacal opening], total length, and exterior gill length (mm) were measured with digital callipers to 0.01 mm precision. Three paedomorphic individuals were brought to the laboratory and kept in an aquarium (40 × 30 × 30 cm, in 25 cm natural spring water) for a brief period to be photographed. The paedomorphs were then released back to where they had been captured.

The mean snout-vent length (SVL) was 28.30 mm (range = 26.00–30.41, SE = 0.76), and total length (TL) was 57.66 mm (53.35–61.40, SE = 1.09) in paedomorphs. The average exterior gill length (GL) was 4.12 mm (2.64–4.71, SE = 0.38). The SVL was 39.99 mm (range = 33.44–39.93, SE = 0.81), and TL was 69.06 mm (66.19–79.17, SE = 1.33) in metamorphs (Table 1).

According to these data, the metamorphic (83%) and paedomorphic (17%) ratio of the population is skewed to metamorphic. This species shares its habitat with four

other amphibians: The Balkan-Anatolian Crested Newt (*Triturus ivanbureschi*), Levant Water Frog (*Pelophylax bedriagae*), Oriental Tree Frog (*Hyla orientalis*), and the Variable Toad (*Bufo variabilis*); five reptiles, the European Pond Turtle (*Emys orbicularis*), Western Caspian Turtle (*Mauremys rivulata*), Grass Snake (*Natrix natrix*), Dice Snake (*Natrix tessellata*), and East-Four-Lined Ratsnake (*Elaphe sauromates*). Fifteen fish species belonging to eight families are cited in the Gediz Delta Management Plan (2007), including the introduced exotic Western Mosquitofish (*Gambusia affinis*).

Baçoğlu et al. (1994) stated that when *L. vulgaris* larvae complete their metamorphosis, their TL can be 35 to 40 mm, adults can be 70 to 80 mm in TL in Western Anatolia. In the western Anatolian population, the adult snout-vent length ranged from 28.8 to 35.4 mm in males, and 30.5 to 36.4 mm in females, while TL ranged from 54.6 to 65.9 mm in males, and 56.1 to 66.8 mm in females (Olgun et al. 1999). The average SVL of *L. vulgaris* was 40.15 mm (34.7–43.7), 40.77 mm (35.3–44.6) in females (Colleoni et al. 2014). Stănescu et al. (2014) reported that the SVL of paedomorphs was 32.7 mm for females, 34.5 mm for males from the Danube Delta Biosphere Reserve (Romania). They also indicated metamorphs were larger than paedomorphs. Colleoni et al. (2014) reviewed sexual dimorphism in newts and found female-biased sex size dimorphism in the species. Bozkurt et al. (2015) found paedomorphic *L. v. koswigi* from (Sakarya) northwestern Turkey and they measured 31.60 mm for males and 30.06 mm for females. The authors claimed that the size of paedomorphs is larger than metamorphs. In the Montenegrin Smooth Newts paedomorphs may or may



**Fig. 2.** The general view of habitat (A) and a male paedomorphic *Lissotriton vulgaris* (B, C) from Lake Sazlı (Izmir, Turkey). The arrows show the cloaca (B) and the gills (C).

not be bigger than metamorphs in their developmental pathway (i.e., metamorphosis versus paedomorphosis), as well as their species and population (Denoël et al. 2009a).

The gill length (GL) of paedomorphs measured 2.58 to 7 mm in northwestern Turkey (Bozkurt et al., 2015), 5.88 mm (3.30–7.90) in Lake Sülüklü (western Turkey, Çiçek, and Ayaz, 2011), 6.7 mm in Romania (Covaciu-Marcov and Cicort-Lucaciu 2007), and 1.5 mm in Ukraine (Litvinchuk 2001). Our data was within the range of previous reports. GL varies among populations and might be connected to the oxygen level or other water quality parameters, but this remains to be demonstrated.

According to Semlitsch (1987), facultative paedomorphosis is controlled by environmental and

genetic factors that allow individuals to cope with habitat variation, take advantage of environmental heterogeneity in the presence of open niches, and increase their fitness. It can occur in a variety of habitats from deep oligotrophic alpine lakes to small eutrophic temporary ponds, arid areas and humid forests (Whiteman 1994; Denoël et al. 2001). In addition, paedomorphs are encountered in different regions and latitudes, independently of environmental and habitat conditions (Whiteman 1994; Denoël et al. 2001; Denoël et al. 2005a).

Three main hypotheses explain the appearance and maintenance of facultative paedomorphosis: i) the best of a bad lot, ii) the paedomorphic advantage, and iii) the dimorphic paedomorph hypothesis (Whiteman 1994). The paedomorph advantage hypothesis corresponds to the

**Table 1.** Summary statistics of paedomorphic and metamorphic *Lissotriton vulgaris* from Lake Sazlı (Izmir, Turkey).

	Paedomorphic Males			Paedomorphic Females		
	SVL (mm)	TL (mm)	GL (mm)	SVL (mm)	TL (mm)	GL (mm)
<b>Mean</b>	28.30	57.52	4.02	26.00	53.35	4.52
<b>Range</b>	26.91–30.41	55.49–61.40	2.64–4.71	—	—	—
<b>SE</b>	0.75	1.09	0.38	—	—	—
	Metamorphic Males			Metamorphic Females		
	SVL (mm)	TL (mm)	GL (mm)	SVL (mm)	TL (mm)	GL (mm)
<b>Mean</b>	37.46	68.80	—	36.95	68.48	—
<b>Range</b>	35.53–39.93	66.19–73.48	—	33.44–38.37	66.62–79.17	—
<b>SE</b>	1.08	1.54	—	0.84	0.89	—

basic model of Wilbur and Collins (1973). It predicts large, fast-growing individuals in good growing habitats to be paedomorphic, while individuals smaller than minimum size for the paedomorphs completely metamorphose to escape competition with larger paedomorphs. More unlikely, the best of a bad lot model predicts the reverse solution in poor habitats with low growth conditions. The larger larvae metamorphose, while the smallest ones keep a larval form and become reproductively mature. The latter hypothesis, the dimorphic paedomorph, suggests that the phenomenon results from the two other hypotheses according to the local conditions experienced by each individual (Whiteman 1994). The metamorphic and paedomorphic ratio of populations can exhibit variations across populations and species (Denoël et al. 2001). The fluctuations in natural populations of paedomorphic urodeles may be related to both natural and anthropogenic factors (Denoël et al. 2005b). The absence of predators and the abundance of food resources are known to favor the delay of metamorphosis and the appearance of paedomorphs (Denoël et al. 2001). At Lake Sülüklü (Çiçek and Ayaz 2011), the metamorph/paedomorph rate changes from year to year with no paedomorphs found in five samples in 2015 (K.Ç., pers. obs). The fluctuation in the population size of fish could affect the metamorph/paedomorph rate (Denoël et al. 2015). The Lake Sazlı population could favor the third hypothesis due to the observation that the size of paedomorphs is lower than metamorphs and presence of potential predators in the habitat.

Habitat is an essential key in the persistence of facultative paedomorphosis in natural populations of newts (Denoël 2005). Denoël and Ficetola (2014) compared the likelihood of multiple potential environmental determinants impacting facultative paedomorphosis. They observe that paedomorphs prefer deep ponds, with conditions favorable to aquatic breathing (high oxygen content), with no fish and surrounded by a suitable terrestrial habitat. Despite the presence of predators, Lake Sazlı has dense aquatic vegetation, abundant food sources and is surrounded by unsuitable terrestrial habitat. There is limited available shelter to hide and save terrestrial forms. Particularly, the presence of aquatic shelters has been shown to favor the coexistence between newts and fish (Winandy et

al. 2015). This might have allowed the co-occurrence of newts with fish in the studied population but more surveys are needed to explore these patterns.

Although several studies have documented the presence and the cause of facultative paedomorphosis in Europe (Denoël et al. 2005a and reference herein), data remain limited on Asian species and subspecies. Better monitoring of facultative paedomorphosis in this part of the world would help to explore hypotheses that may provide a more comprehensive understanding of this phenomenon.

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# First population assessment of two cryptic Tiger Geckos (*Goniurosaurus*) from northern Vietnam: Implications for conservation

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**Abstract.**—The Cat Ba Tiger Gecko *Goniurosaurus catbaensis* Ziegler, Nguyen, Schmitz, Stenke and Rösler, 2008 is a recently discovered species endemic to Cat Ba Island, Hai Phong, Vietnam. Morphologically, *G. catbaensis* resembles *G. luii* Grismer, Brian, Viets and Boyle, 1999, which was originally described in 1999 from southern China and was recorded in 2006 also in northeastern Vietnam. Both species inhabit remote limestone habitats, which suffer ongoing degradation and fragmentation due to agricultural development and the expansion of touristic sites. Tiger Geckos experience increasing interest in the international pet trade, which already resulted in local population extirpation of *G. luii* due to unsustainable overexploitation for commercial use. However, impacts of anthropogenic pressures on wild populations, distribution ranges, and population sizes of *Goniurosaurus* species remain imperfectly studied. Herein we used a capture-recapture method to provide preliminary population size estimation of the endemic island dwelling species, *G. catbaensis*, in comparison to its cryptic continental relative, *G. luii*, in order to evaluate their conservation status and assess the level of threats. Our study revealed relatively small population sizes and provided evidence for the negative impact of humans on the two *Goniurosaurus* species. Our research emphasizes the necessity to support the conservation of the species and their natural habitats, especially on the Cat Ba Archipelago. We further provide a new provincial record of *G. luii* in Vietnam from Lang Son Province and record for the first time evidence for the occurrence of *G. catbaensis* on further offshore island in the Ha Long Archipelago.

**Key words.** Eublepharidae, distribution, population size, new record, endemism, conservation measures

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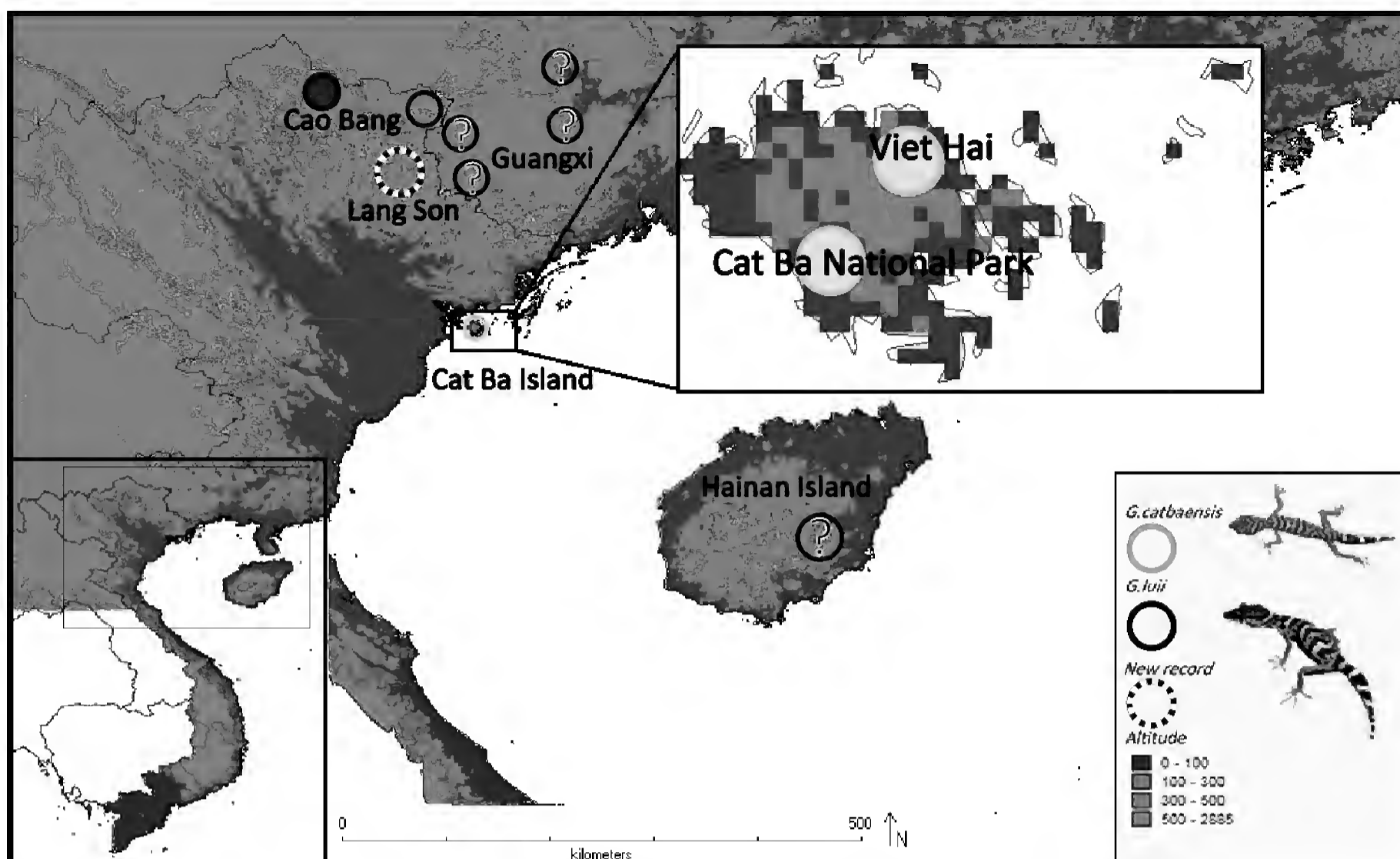
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## Introduction

Tiger Geckos of the genus *Goniurosaurus* have a restricted distribution range in Asia, from northern Vietnam over southern China eastwards to the Ruykuyu Archipelago of Japan. Currently, 17 species are recognized; most of them are endemic to small areas (Grismer et al. 1994,

1999; Seufer et al. 2005; Yang and Chan 2015; Ziegler et al. 2008). Tiger Geckos are popular in the international pet trade and the species *G. luii* was reported being extirpated at its type locality in southern China shortly after its description (Stuart et al. 2006). Although Tiger Geckos are considered to be threatened by extinction due to overexploitation for the illegal trade and habitat de-

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**Fig. 1.** Distribution of the cryptic species *Goniurosaurus catbaensis* (gray circles) and *G. luyi* (black circles): Filled circles represent study areas; dashed circle represents new record of *G. luyi*; questions marks indicate former localities or sites where the current presence is unclear. Occurrence records were represented as big circles to prevent showing exact locality data.

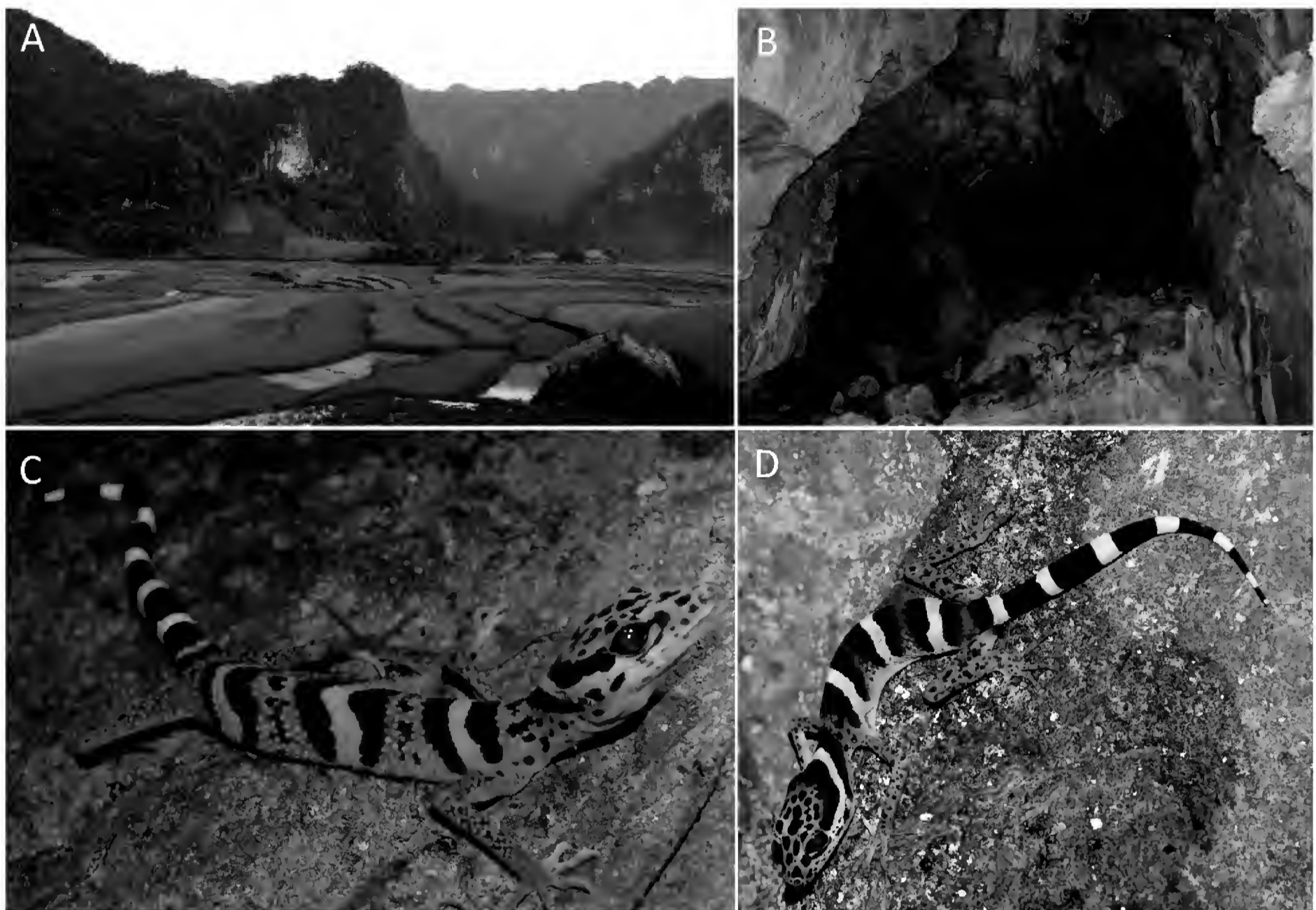
struction, only the members of the *G. kuroiwae* group are listed on the IUCN Red List of Threatened Species (Ota 2010). Knowledge about the status of the remaining *Goniurosaurus* populations is poor and for most species are not yet available. This study aims to contribute towards a better understanding about the population status of the two species, *Goniurosaurus catbaensis* and *G. luyi* in Vietnam. Both species are members of the *G. luyi* species group, which contains eight known species, *G. araneus* Grismer, Viets and Boyle 1999 from the northern portion of Vietnam, *G. bawanglingensis* Grismer, Haitao, Orlov, and Anajeva 2002 from Hainan Island, China, *G. luyi* Grismer, Viets and Boyle 1999 from northern Vietnam and southeastern mainland of China, *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke, and Rösler 2008 from Cat Ba Island of Vietnam, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen, and Ho 2008 from northern Vietnam, *G. liboensis* Wang, Yang, and Grismer 2013 from the border region between Guangxi and Guizhou provinces, *G. kadoorieorum* Yang and Chan 2015, and *G. kwangsiensis* Yang and Chan 2015 from Guangxi Province, China. The members of this species group are morphologically very similar and their phylogenetic relationships are only partly resolved (Grismer et al. 1994, 1999; Seuffer et al. 2005; Vu et al. 2006; Yang and Chan 2015; Ziegler et al. 2008). Hence, we investigated one of the most poorly known species, *G. catbaensis*, which is an endemic flagship species for Cat Ba Island in the Gulf of Tonkin, northern Vietnam. This island belongs to one of the most attractive tourist sites in Vietnam, but the impact of the

tourism on this ecosystem and its biodiversity is not yet fully understood.

Population size estimations provide essential information for the classification of the threat level of a species and are crucial for wildlife management and management of the long-term survival of populations and species (Reed et al. 2003; Traill et al. 2007). We therefore conducted the first population assessment of *G. catbaensis*, including population density, size, and structure, and evaluation of human impacts on the population. In comparison, we likewise studied its cryptic sibling species *G. luyi* on the mainland of northern Vietnam, which is also karst adapted, and occupies a similar ecological niche (Grismer 1999; Ziegler et al. 2008). *Goniurosaurus luyi* indeed shows a wider distribution range, but its natural history is still poorly known and data on its abundance in Vietnam is completely lacking to date (Grismer 1999; Yang and Chan 2015). By comparing populations of two closely related species, one from an island with the other in mainland ecosystems, we expected lower population densities in the mainland, stronger human impacts at tourist sites, and finally aimed to gain insights for improved conservation strategies for the Tiger Geckos in the future.

## Materials and Methods

**Study areas:** Study sites were selected based on previous surveys of the authors' on Cat Ba Island, Hai Phong City and in Ha Lang District, Cao Bang Province, north-

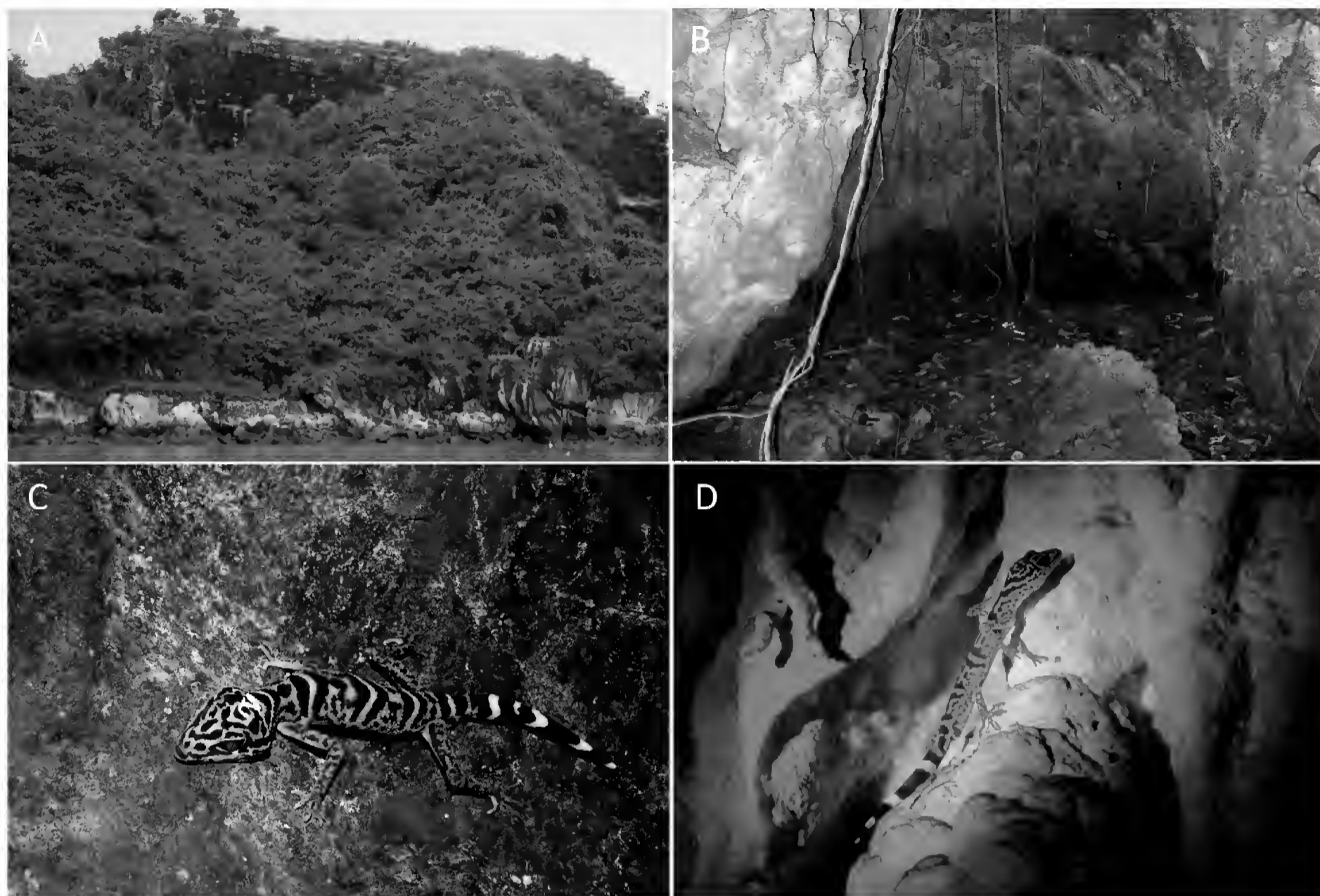


**Fig. 2.** **A:** Macrohabitat and **B:** Microhabitat of *Goniurosaurus luyi* in Ha Lang District, Cao Bang Province, North Vietnam; specimens of *G. luyi* from **C:** Cao Bang Province and **D:** Lang Son Province (new record). Photos Mona van Schingen, Marta Bernardes, and Tao Thien Nguyen.

ern Vietnam (e.g., Lehmann, 2013; Ziegler et al. 2008, see Fig. 1). Both areas comprise large limestone karst formations with secondary evergreen forest and share zoogeographic affinities (Fig. 2A, 3A). The limestone karst forest provides diverse habitats for a unique flora and fauna, and is recognized as arcs of biodiversity (Clements et al. 2006). While habitats in Cao Bang Province—situated at the border to China—lie outside protected areas, Cat Ba Island was recognized as “Cat Ba Archipelago Biosphere Reserve” (CBBR) by the UNESCO in 2004, due to its significant ecosystem and biodiversity values (CBBR Authority 2013). Besides the recent discovery of *G. catbaensis* by Ziegler et al. (2008), Cat Ba Island harbors 282 further species of terrestrial vertebrates, of which 22 are listed in the Red Data Book of Vietnam. Cat Ba is renowned for the endemic, Critically Endangered Golden-headed Langur (*Trachypithecus poliocephalus*). Cat Ba Archipelago as well as the adjacent Ha Long World Heritage Area account as the most popular tourist destination in Vietnam, annually attracting more than one million tourists (CBBR Authority 2013), and thus is facing several challenges from rapid tourism as well as aquaculture development, and climate change. During the present study, two sites on Cat Ba Island, which distinctly differed in the number of tourists, were selected in

order to evaluate if the presence of tourists might impact wild populations of *Goniurosaurus* (Fig. 1).

**Field surveys:** During a field survey in 2012 several *Goniurosaurus* cf. *luyi* specimens were observed in Lang Son Province. One voucher specimen was collected and deposited in the collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi (TD-LS2012.1). The specimen was determined by comparison with further specimens of *G. luyi* from Cao Bang Province (ML-19, TAO-182, IEBR 3252, CB-2012.2, IEBR 3254, and IEBR 3253) and based on data from the literature (Grismer et al. 1999; Yang and Chan 2015). Exact locality data is not presented herein to prevent poaching (see also Yan and Chan 2015). For assessment of the population status of *G. catbaensis* and *G. luyi*, field surveys were conducted between June and August 2014, May 2015 and during a short time in June 2015, which is the non-hibernation season of *Goniurosaurus* (Grismer et al. 1999). Seven transects (1,100 to 4,200 m in length) along limestone cliffs or caves were repeatedly surveyed in periods of several days on Cat Ba Island and in Cao Bang Province. Surveys took place after sunset between 7:30 and 11:30 pm, when lizards were found active or foraging. Captured animals were individually marked with a



**Fig. 3.** **A:** Macrohabitat of *Goniurosaurus catbaensis* at the coast of Cat Ba Island; **B:** Limestone cliffs, the typical microhabitat of *G. catbaensis*; **C:** Adult male of *G. catbaensis* marked for population assessment; **D:** First evidence for the occurrence of *G. catbaensis* within limestone cave of small offshore Island in Ha Long Bay archipelago. Photos Hai Ngo, Tao Thien Nguyen, and Minh Le Pham.

permanent pen (Edding Eraser) and released on the same spot after taking measurements (see Fig. 3C). This marking technique has the advantage of being non invasive, inexpensive, and enables the short-term identification of animals, while markings last until the next shedding of the animals.

**Population analysis:** To estimate population sizes, we applied a “Capture-recapture Method” after Huang et al. (2008) by using an “Invisibility Rate Index,” which compensates for animals present but not detected during surveys. The method is described in more details in Huang et al. (2008) and van Schingen et al. (2014). Estimated population sizes were only applied for the specific surveyed sites, and did not encompass the entire populations of the species. Since it is impossible to survey all suitable habitats, density estimations in reference to the transect line were used as relative abundances of respective species. To assess the population structure, lizards were categorized into three age classes based on snout-vent length (SVL > 105 mm = adult, SVL > 85 mm = subadult, and SVL < 85 mm = juvenile), sexes, and in case of females, into gravid and non-gravid specimens. To test for differences in population structure between the island species *G. catbaensis* and the continental *G. luyi*, a Chi<sup>2</sup> test with  $\alpha = 0.05$  was applied with GraphPad

Prism version 5.0 for Windows, GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com). We further compared densities of *Goniurosaurus* in areas, which are frequently visited by tourists with areas where access is limited in order to evaluate if tourism affects wild populations.

**Molecular analysis:** In order to confirm the taxonomic assignment of the newly collected specimens of *Goniurosaurus* cf. *luyi* from Lang Son and Cao Bang, a fragment of the mitochondrial ribosomal gene, 16S, was amplified using the primer pair 16Sar and 16Sbr (Palumbi et al. 1991) for four samples (TD-LS2012.1, TAO-182, ML-19, IEBR-3254). Tissue samples were extracted using DNeasy blood and tissue kit, Qiagen (California, USA). Extracted DNA from the fresh tissue was amplified by PCR mastermix (Fermentas, Canada). The PCR volume consisted of 21  $\mu$ l (10  $\mu$ l of mastermix, five  $\mu$ l of water, two  $\mu$ l of each primer at 10 pmol/ $\mu$ l, and two  $\mu$ l of DNA or higher depending on the quantity of DNA in the final extraction solution). PCR condition was: 95 °C for five minutes to activate the taq; with 40 cycles at 95 °C for 30 s, 50 °C for 45 s, 72 °C for 60 s; and the final extension at 72 °C for six minutes.

PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen). Gels

## First population assessment of two cryptic Tiger Geckos

**Table 1.** Totally observed specimens, densities and estimated population size of *Goniurosaurus catbaensis* and *G. luii* in 2014 and 2015.

		2014		2015
		June	July	August
		May		
<b>Total Cat Ba Island</b>				
Species	<i>G. catbaensis</i>			
Total observed	—	17	12	14
D[ind/km of transect]	—	1.3	1.0	1.3
Population size	—	24	16	16
<b>Cat Ba National Park</b>				
Species	<i>G. catbaensis</i>			
Total observed	—	5	6	3
D[ind/km of transect]	—	0.9	1.1	0.9
Population size	—	5	8	3
<b>Viet Hai Commune</b>				
Species	<i>G. catbaensis</i>			
Total observed	—	12	6	11
D[ind/km of transect]	—	1.7	0.8	1.5
Population size	—	19	8	13
<b>Ha Lang District, Cao Bang Province</b>				
Species	<i>G. luii</i>			
Total observed	15	—	—	—
D[ind/km of transect]	0.8	—	—	—
Population size	21	—	—	—

were stained for 10 minutes in 1X TBE buffer at two pg/ml of ethidium-bromide, and visualized under UV light. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification Kit (Fermentas, Canada). Purified PCR products were sent to Macrogen Inc. (Seoul, South Korea) for sequencing.

Sequences generated in this study were aligned with one another using De Novo Assemble function in the program Geneious v.7.1.8 (Kearse et al. 2012). They were then compared with other sequences using the Basic Local Alignment Search Tool (BLAST) in GenBank.

## Results

**New population record of *G. luii*:** Four sequences of 574 bps were obtained from the *Goniurosaurus* specimen collected in Lang Son Province. The sequences are almost identical to each other, except in two positions, and 99% to 100% similar to those of *Goniurosaurus luii* from GenBank, specifically the sequences with accession numbers EU499390, EU499391, KC765083, KM455054. The results confirmed that *Goniurosaurus* samples collected in Lang Son Province and in Cao Bang Province are conspecific with *G. luii*. Thus, our finding represents the first record of *G. luii* and the second recorded *Goniurosaurus* species from Lang Son Province, northern Vietnam. Previously, only *G. huuliensis* was known from Huu Lien Nature Reserve, Huu Lung district

in the South of Lang Son Province occurring at elevations of about 370 m (Orlov et al. 2008). *Goniurosaurus luii* was recorded from the north at similar elevations of about 364 m above sea level. Based on our current knowledge no sympatric occurrence of the two species has been recorded so far, but exact distribution boundaries remain unknown. The microhabitats of *G. luii* in Lang Son Province were densely vegetated limestone caves, which are similar to those observed in Cao Bang Province (Fig. 2B). The Geckos had been found active during night on cliffs or cave walls about 0.5–2.5 m above the ground. Most interestingly, our morphological examination of the newly recorded *G. luii* specimens from Lang Son Province showed that no significant differences in diagnostic characters compared with the recently described *G. kadoorieorum* (see Table 1, Fig. 3D). But the newly recorded specimens of *G. luii* from Lang Son Province slightly differed from both *G. luii* and *G. kadoorieorum* in having more nasal scales surrounding the naris (9 vs. 6–7 in *G. kadoorieorum* and 6–8 in *G. luii*) and more cilia (59–60 vs. 47–54 in *G. kadoorieorum* and 50–56 in *G. luii*) (see Yang and Chan 2015).

**Extended distribution range of *G. catbaensis*:** In this study *G. catbaensis* was recorded—besides already known sites in Cat Ba National Park (NP) and Viet Hai commune—on karst formations at the coastline of Cat Ba Island (Fig. 3B). An adult female was found on rocks

**Table 2.** Morphological characters of *Goniurosaurus luyi* from Guangxi (type series; Grismer et al. 1999), Lang Son and Cao Bang Province compared with *G. kadoorieorum* (Yang and Chan 2015). Length given in mm.

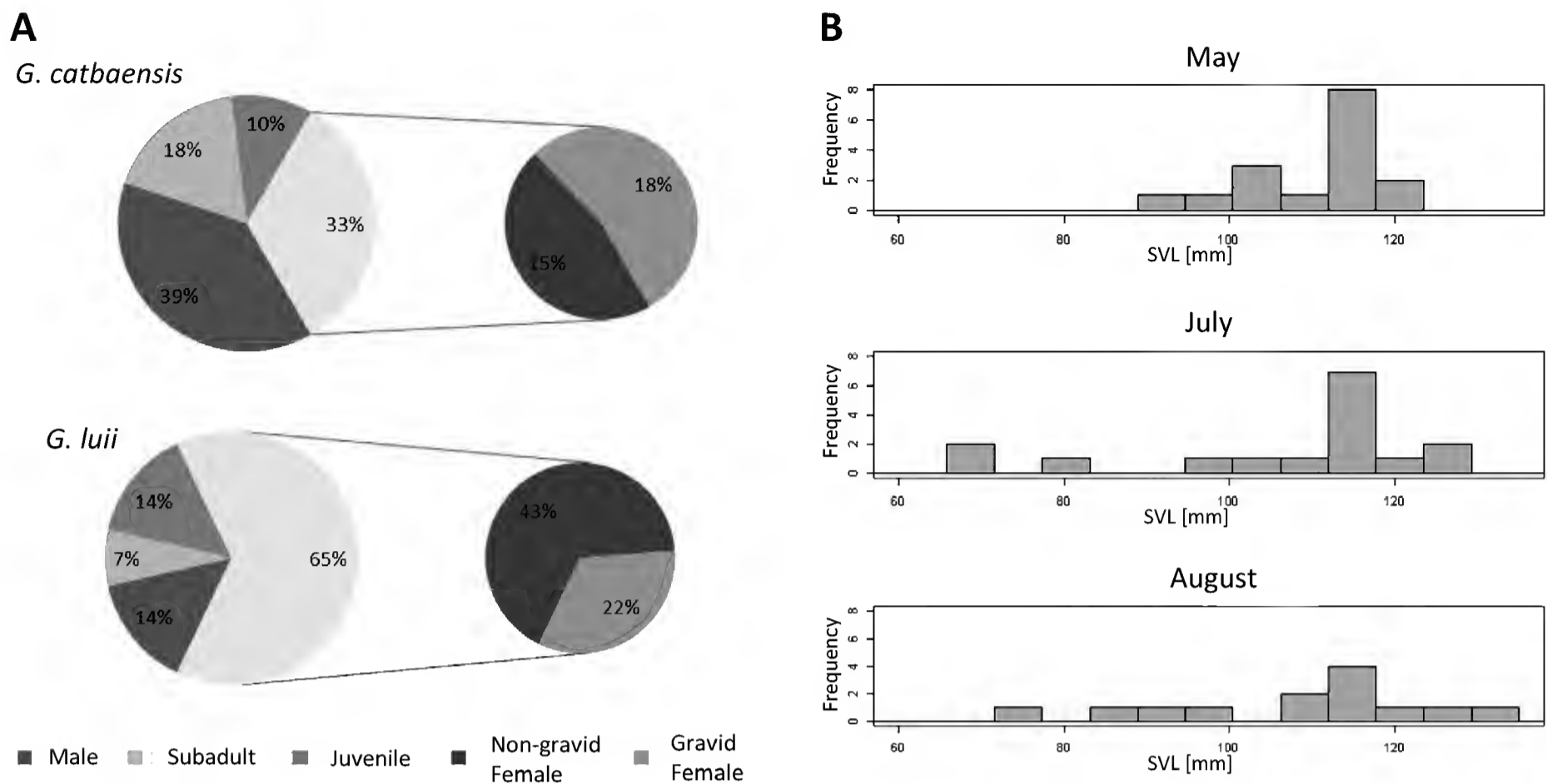
	<i>G. luyi</i> (type series, Guangxi, China, n=6)	<i>G. luyi</i> (Lang Son) (n=1)	<i>G. luyi</i> (Cao Bang) (n=6)	<i>G. kadoorieorum</i> (n=5)
SVL	—	112.13	78.93-121.21 (104.0±18.4)	68.9-118 (104.28±20.3)
TaL	—	62.42	54.95-65.67 (58.37±6.25)	59.8-69.5 (60.59±5.38)
AG	—	53.41	36.58-60.1 (49.41±9.24)	30.5-55.6 (49.3±10.61)
HL	—	28.92	21.74-30.68 (27.9±4.12)	18.3-30.5 (26.82±4.98)
HW	—	20.46	14.99-21.8 (18.72±3.09)	12.5-20.4 (18.28±3.46)
HH	—	12.85	7.89-14.95 (11.38±2.82)	8.3-13.3 (11.64±1.98)
SE	—	12.24	9.13-12.87 (11.35±1.71)	7.6-12.6 (11.28±2.11)
EE	—	11.58	8.35-12.36 (10.23±1.78)	6.5-10.9 (9.5±1.77)
SVL:TaL	—	1.79	1.35-2.34 (1.86±0.42)	1.15-1.83 (1.56±0.36)
SVL:HL	—	3.88	3.51-3.96 (3.71±0.17)	3.77-3.98 (3.88±0.08)
SVL:AG	—	2.09	2.07-2.17 (2.11±0.06)	2.05-2.26 (2.13±0.08)
HL:HW	—	1.41	1.39-1.66 (1.49±0.1)	1.39-1.5 (1.47±0.05)
SE:EE	—	1.06	1.03-1.23 (1.11±0.08)	1.14-1.25 (1.19±0.04)
SPL	9-12 (9.5±0.55)	10/9	10-12 (10.92±0.67)	10-11 (10.3±0.48)
IFL	9-11 (10±0.63)	10/10	8-11 (9.9±0.9)	9
N	—	9/9	6-8 (7.25±0.75)	6-7 (6.2±0.42)
IN	—	2	3-1 (1.5±0.84)	2
PostIN	—	6	3-5 (4.0±0.89)	3-9 (5.2±2.49)
PM	2-4 (3±0.89)	5	2-5 (3.83±0.98)	4-5 (4.8±0.45)
GP	—	8	7-11 (8.67±1.37)	8-11 (9.6±1.52)
PO	14-17 (15.8±1.17)	—	—	15-19 (16.7±1.16)
CIL	57-61 (59.5±1.87)	59/60	50-56 (53.83±1.75)	47-54 (51.7±2.58)
MB	119-144 (134.5±12)	122	105-132 (118.5±11.47)	124-132 (129.2±3.11)
GST	9-14 (12.2±1.34)	11/12	9-12 (11.2±0.94)	11-13 (12±1.05)
TL	33-34 (33.8±0.75)	31	32-35 (33.3±1.21)	30-34 (32.6±1.67)
DTR	—	22	19-23 (21.5±1.52)	22-24 (23.2±0.84)
LF1	—	9/10	9-10 (9.83±0.39)	10-11 (10.2±0.42)
LF4	—	19/20	19-20 (19.54±0.69)	17-19 (17.8±0.79)
LT1	—	11/9	9-10 (9.9±0.32)	10-11 (10.6±0.52)
LT4	21-24 (23.5±1.38)	24/24	22-25 (23.5±1.08)	21-24 (22.3±0.95)
PP (male)	23-29 (26±2.58)	—	17-24 (20.5±4.95) (n=2)	26-28 (26.75±0.96)
PP (female)	—	22 (pitted)	18-24 (20.0±3.5) (pitted, n=3)	Absent
PAT	—	2/2	1-2 (1.75±0.45)	1-2 (1.4±0.52)

on the ground at an elevation of eight m above sea level. This sighting is the first observation of *G. catbaensis* in immediate proximity to the sea and provides an extended distribution range from forested areas to completely open areas close to the sea. In addition, first evidence for the occurrence of *G. catbaensis* on a small island within the Ha Long Bay is recorded based on a photo documentation by a tourist (Fig. 3D). The specimen was observed on the wall of a limestone cave on a very small offshore island. Based on color pattern and discernible scalation, the photographed specimen revealed to be *G. catbaensis*, although not all diagnostic characters for that species

could be confirmed due to the lack of a voucher specimen.

**Population status:** During the present study, *G. catbaensis* and *G. luyi* were found along five and seven transects, respectively. A total of 43 individuals of *G. catbaensis* and 15 individuals of *G. luyi* were captured. Based on an estimated invisibility rate index of 0.6, the *G. catbaensis* population on Cat Ba Island was estimated to comprise 16, 24, and 16 individuals in May, July, and August, respectively (Table 1). Furthermore, the encounter rates of *G. catbaensis* were always higher in Viet Hai

## First population assessment of two cryptic Tiger Geckos



**Fig. 4. A:** Average population structure of *Goniurosaurus catbaensis* and a continental *G. luyi* population from Cao Bang Province (May–August vs. June, respectively); **B:** Frequency histogram of Snout-vent length of *G. catbaensis* for the months May, July, and August.

Commune than in other sites near the headquarters of Cat Ba NP, where more tourists frequented. In comparison, the continental subpopulation of *G. luyi* was estimated to comprise about 21 individuals within the investigated site (Table 1). Monthly mean densities of *G. catbaensis* ranged between 1 and 1.3 individuals per km of surveyed transect, while densities were generally higher in Viet Hai Commune than in other sites within Cat Ba NP (Table 1; Fig. 4). With regard to temporal variations, the highest density of *G. catbaensis* was observed during the month of August compared to May and July. In comparison, the continental subpopulation of *G. luyi* was estimated at the density of 0.8 individuals per km/transect, slightly lower than the density of *G. catbaensis*.

The investigated population of *G. catbaensis* on average consisted mainly of adult males, followed by adult females, subadults, and juveniles (39%, 33%, 18%, 10%, respectively; see Fig. 4). In comparison, the population structure of continental *G. luyi* slightly differed, with adult females constituting the major proportion of the local subpopulation, followed by adult males, juveniles, and subadults (65%, 14%, 14%, 7%, respectively, see Fig. 4). Females were more abundant in populations of *G. luyi* than in those of *G. catbaensis* (Fig. 4). However, population structures did not differ significantly between the two cryptic species ( $\text{Chi}^2 = 5.2$ ;  $\text{df} = 3$ ;  $p = 0.158$ ). Most of the observed adult females were gravid (33% in Cao Bang vs. 54% on Cat Ba) between May and July. In July, all five encountered females of *G. catbaensis* were gravid, while no gravid females were observed in August. Frequency histograms of SVL showed a tendency of a monthly shift in presence of age classes in *G. catbaensis* ( $\text{Chi}^2 = 1.227$ ,  $\text{df} = 6$ ,  $p = 0.9755$ ; Fig. 4). Individuals with SVL less than 90 mm were only found

from July onwards. Similarly, animals with SVL larger than 120 mm were observed from July onward, while the largest individuals (SVL up to 110 mm) were recorded in August (Fig. 4).

## Discussion

**Distribution:** While most of the *Goniurosaurus* species are endemic and restricted to a small distribution range, our new record of *G. luyi* from Lang Son Province extended the distribution of this species in northern Vietnam. However, the distribution range of *G. luyi* probably overlaps with its cryptic relatives, i.e., *G. araneus*, *G. kadoorieorum*, and *G. kwangsiensis* (Chen et al. 2014; Yang and Chan 2015). Chen et al. (2014) indicated that *G. luyi* and *G. araneus* occur sympatrically in Guangxi Province, southern China, being only divided by a river system as a geographic boundary, which also is the barrier for *G. yingdeensis* and *G. indet.* The recently described *G. kadoorieorum* and *G. kwangsiensis* were also discovered from Guangxi Province, although information about exact locality was not provided (Yang and Chan 2015). Our morphological investigation of *G. luyi* from Vietnam revealed no distinct morphological differences between *G. luyi* and *G. kadoorieorum*. Thus, the validity of the newly described *G. kadoorieorum* should be verified by genetic analyses in the future. A similar case of cryptic diversity within a small geographic range is found in the *G. kuroiwae* complex, consisting of five species, in the Ryukyu Archipelago, Japan (Chen et al. 2014). Definite overlaps in distribution ranges of different *Goniurosaurus* species have only been reported for less closely related congeners, *G. lichtenfelderi* and *G.*



*luii* thus far. Although *G. luii* is the second known species of *Goniurosaurus* (after *G. huuliensis*) from Lang Son Province, they are not sympatric inhabitants. While *G. luii* was recorded from northern Lang Son, *G. huuliensis* had been only recorded from Huu Lien Nature Reserve in the southern part of the province. It can be assumed that the two species are geographically separated, however exact distribution boundaries have not yet been identified.

At present, *G. catbaensis* is endemic to Cat Ba Archipelago and it is expected to be found on other offshore islands in Ha Long Bay. Besides, other reptile species such as *Pseudocalotes brevipes* and *Rhynchophis boulengeri* have been observed at the coastline of Cat Ba Island, while *R. boulengeri* was even observed swimming in the ocean (Nguyen et al. 2011). In addition, two specimens of the *Gekko palmatus* complex were sighted on a small offshore island in Cat Ba Archipelago in 2015. These findings highlight the potential of Cat Ba Archipelago and Ha Long Bay as a laboratory for future studies to understand island biogeography of tropic lizards. These studies are recommended to investigate species communities, species relationships, and explore if similar speciation processes are underway on these small offshore islands comparable to those reported in *Anolis* lizards on Caribbean islands (e.g., Losos and Schluter 2000; Losos and Thorpe 2004).

**Population status:** Species with restricted distribution ranges are especially vulnerable to anthropogenic threats, such as habitat loss or degradation, overexploitation, and climatic changes (Hanski 1991; Reed et al. 2003; Traill et al. 2007). The population size plays a crucial role in long term survival of species, and a minimum viable size of at least 3,000–7,000 individuals is required to maintain a stable population over a longer time period (Reed et al. 2003; Traill et al. 2007). Preliminary estimates of *G. catbaensis* revealed a small population size that varied monthly between 16 and 24 individuals. These values only reflect the situation at the two known sites of the species on the island and might not capture the population over the entire range of the taxon. We assume that future surveys will probably uncover further occurrences, which is supported by the recent sighting of potential *G. catbaensis* on a small offshore island. However, *G. catbaensis* is still relatively restricted in its distribution and exclusively relies on the presence of limestone habitats in remote areas. Thus, the total population size of the species is assumed to be relatively small, and not exceeding the size of a minimum viable population.

Accordingly, *G. catbaensis* had been found in a fairly low density of 1.2 individuals per km, which only occurs in the sites containing suitable habitats such as limestone cliffs and caves. The habitats only cover a portion of the Cat Ba Archipelago, since karst formations alone represent only one of several ecosystems present on Cat Ba Island with an area of about 170 km<sup>2</sup> (CBBR Authority,

2013). Our findings suggest that higher abundances of *G. catbaensis* were generally found in remote sites, which were less frequently visited by tourists. The results might indicate a negative impact of tourism on the population of *G. catbaensis*. As tourism is developing rapidly on the island, wild populations and suitable habitats are likely to steadily decrease in the future.

A comparative investigation of the continental *G. luii* population in Cao Bang Province revealed similar size estimations of about 21 individuals (vs. 16–24 individuals of *G. catbaensis* on Cat Ba), based on the same number of surveyed transects. The sites, where *G. luii* was observed, were remote and far away from human settlements. This observation affirms our assumption that the presence of humans negatively impacts the occurrence of *Goniurosaurus* species.

**Population structure:** In both investigated species, adult females represented the dominant group, which might be an indication for a territorial or aggressive behavior between males, which probably disperse more than females (Vitt and Pianka 1994). The proportion of males tends to be higher in the island population of *G. catbaensis* compared with the continental population of *G. luii*. This finding might be explained by the limited dispersal ability on the island due to limited availability of suitable habitats. Accordingly, the fact that relatively higher numbers of gravid females were found on the island, compared with the continental population, might have resulted from the respective higher density of males in the population. Furthermore, gravid females in both populations of *G. catbaensis* and *G. luii* were encountered between May and July, confirming the observation of Grismer et al. (1999) that July is the reproduction season of *G. luii*.

## Implications for Conservation

Tiger Geckos, in particular *Goniurosaurus luii* and *G. araneus*, have been used for traditional medicine by local people and became very popular in the trade since the 1990s (Grismer et al. 1999; Chen et al. 2014; Yang and Chan 2015; Ziegler et al. 2015). Grismer et al. (1999) reported an exemplary case of one dealer exporting over 10,000 individuals of *G. luii* and *G. araneus* to the USA for the pet trade. Already before its description in 1999, *G. luii* had been overexploited for commercial use in China, which presumably led to the extirpation from its type locality in Pingxiang (Grismer et al. 1999; Stuart et al. 2006). According to Yang and Chan (2015), local villagers mentioned to have been paid by dealers for collecting large quantities of live *Goniurosaurus*, which is a common scenario within the non-sustainable reptile trade (e.g., Huang et al. 2008). A similar scenario might have taken place simultaneously in Vietnam. As a result, even extensive field surveys, e.g., Nguyen et al. (2009), Orlov et al. (2008), Ziegler et al. (2008), and by our team in

2010 and 2014 in Cao Bang Province, failed to record any specimen of *G. araneus*.

These findings emphasize how fast local populations of range-restricted species can be extirpated due to over-exploitation (e.g., Huang et al. 2008; van Schingen et al. 2015). The international demand for *Goniurosaurus* species among hobbyists still remains high. The long term monitoring of local pet markets and internet sources by Yang and Chan (2015) showed that almost all *Goniurosaurus* species are subject to extensive pet trade. Sometimes, the species fetch alarmingly high prices. Observations by our team confirmed the regular trade in respective species in international reptile fairs, e.g., in Hamm and Dortmund, Germany, or on internet platforms such as www.terrarium.com. The species are available for sale from as low as 15 EUR up to several hundred Euros per individual.

In many cases, the origin of the species and their legal export permits remain questionable. Among them, *G. catbaensis* has been observed in European pet markets, even though it was only described relatively recently. Anthropogenic threats, such as poaching, habitat degradation, and introduced predators together with a small distribution range of 1,600 km<sup>2</sup> imperiled the insular *G. kuroiwae* species group, endemic to the Ryukyu Archipelago of Japan, leading to its inclusion in the IUCN Red List as Endangered (Ota 2010). Yang and Chan (2015) argued that most *Goniurosaurus* species from China and Vietnam are similarly or even more threatened than the Japanese species, since Japan is more advanced in species conservation management.

Our study suggests that the insular *G. catbaensis* is very sensitive to the impacts of humans, and subject to overexploitation to supply the international pet trade. Besides illegal collection, habitat destruction for touristic purposes has dramatically increased the pressure on the wild population of *G. catbaensis*. According to interviews with local villagers, several karst areas of Cat Ba Island, comprising unique and important habitats for the species, have recently been converted to a huge tourist resort and further tourism development has been planned. Such development would seriously threaten *G. catbaensis* and the unique fauna and flora of the Cat Ba Archipelago, which requires urgent conservation measures to protect the species from imminent extinction.

## Recommendations

Due to the restricted distribution range of *G. catbaensis* and the rising anthropogenic threats to its natural populations, we recommend to include this species in the IUCN Red List. Since this study provided evidence for negative impact of tourism on the presence of *G. catbaensis*, the public access to core habitats of the species needs to be restricted by local authorities. Based on our results, *G. catbaensis* was found more frequently at some spots in the vicinity of Viet Hai Village. The sites should there-

fore be considered a priority zone for the species conservation. Future surveys will evaluate the relevance of further sites as key habitats for conservation of *G. catbaensis*. Furthermore, the Vietnamese authorities should strictly control illegal collection of *G. catbaensis* as well as other *Goniurosaurus* species. Currently, all *Goniurosaurus* species are considered to be threatened by commercial use (Chen et al. 2014; Grismer et al. 1999; Yang and Chan 2015; Ziegler et al. 2015) and the international demand for Tiger Geckos still remains high. Because of their restricted distribution ranges and low densities, all *Goniurosaurus* species are especially vulnerable to unsustainable harvest, which already caused the local extinction of at least one species. As a first step to reduce poaching and to control the international trade in *Goniurosaurus* species, we further recommend assessment of trade status for all species of the genus *Goniurosaurus* with a view to including them in the appendices of the Convention of International Trade in Endangered species (CITES).

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**Hai Ngoc Ngo** is a young scientist who has been involved in several projects at the Institute of Ecology and Biological Resources (IEBR) since 2013, and has been a researcher of the Vietnam National Museum of Nature since 2014. He recently graduated with his M.S. in 2015 at Ha Noi University of Science. He has participated in numerous field surveys to study herpetology in Vietnam and has much experience in field research and conservation work. He is now focusing on ecology, phylogeny, and conservation of endemic endangered lizards in Vietnam.



**Thomas Ziegler** has been the Curator of the Aquarium/Terrarium Department of the Cologne Zoo since 2003. He is also the coordinator of the Cologne Zoo's Biodiversity and Nature Conservation Projects in Vietnam and Laos. Thomas studied biology at the University Bonn (Germany), and conducted his diploma and doctoral thesis at the Zoological Research Museum Alexander Koenig in Bonn, focusing on zoological systematics and amphibian and reptile diversity. As a zoo curator and project coordinator he tries to combine *in situ* and *ex situ* approaches, viz., to link zoo biological aspects with diversity research and conservation in the Cologne Zoo, in rescue stations and breeding facilities in Vietnam, and in Indochina's last remaining forests. Beginning in February 2009 he has been an Associate Professor at the Zoological Institute of Cologne University. Since 1994, Thomas has published 355 papers and books, mainly dealing with herpetodiversity. He was involved in the first record of *Goniurosaurus luii* from Vietnam (Vu et al. 2006) and in the discovery of *Goniurosaurus catbaensis* (Ziegler et al. 2008).



**Truong Quang Nguyen** is a researcher at the Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST), and is a member of the Biodiversity and Nature Conservation project of the Cologne Zoo. He finished his Ph.D. in 2011 at the Zoological Research Museum Alexander Koenig (ZFMK) and the University of Bonn, Germany (DAAD Fellow). From 2011 to 2014 he worked as a postdoctoral student in the Zoological Institute at the University of Cologne. He conducted numerous field surveys and is the co-author of seven books and more than 150 papers relevant to biodiversity research and conservation in Southeast Asia. His research interests are systematics, ecology, and phylogeny of reptiles and amphibians of Southeast Asia.



**Cuong The Pham** is a Ph.D. candidate and researcher at the Institute of Ecology and Biological Resources (IEBR) Vietnam Academy of Science and Technology (VAST). He is member of the Cologne Zoo's Biodiversity and Nature conservation projects in Vietnam. He has published several papers mainly dealing with Vietnam's herpetodiversity. He is experienced in biodiversity and field research and has conducted numerous field surveys in Vietnam.



**Tao Thien Nguyen** is the curator of herpetology and the current head of the Department of Nature Conservation at the Vietnam National Museum of Nature (VNMN) of the Vietnam Academy of Science and Technology. His research interests are in the taxonomy, evolutionary origin, and diversification of amphibians and reptiles, as well the practical elucidation of the phylogeny of various amphibian and reptile groups. Tao obtained his Ph.D. at the Kyoto University, Japan with a focus on the molecular and morphological systematics and distribution pattern of various rhacophorid species. He has extensive experience in taxonomy and ecology of amphibians and reptiles throughout Vietnam. Since 2007, he has published more than 70 papers on herpetology.



**Minh Duc Le** has been working on conservation-related issues in Southeast Asia for more than 15 years. His work focuses on biotic survey, wildlife trade, and conservation genetics of various wildlife groups in Indochina. He is currently working on projects which characterize genetic diversity of highly threatened reptiles and mammals in the region. He also pioneers the application of molecular tools in surveying critically endangered species in Vietnam. Minh has long been involved in studying the impact of the wildlife trade on biodiversity conservation in Vietnam, and is developing a multidisciplinary framework to address the issue in the country.



**Mona van Schingen** is Ph.D. candidate at the Zoological Institute of the University of Cologne and the Cologne Zoo, Germany, where she also graduated with her B.S. in Biology in 2011 and her M.S. in 2014, respectively. Since 2011 she has been investigating the lizard fauna of Southeast Asia integrated in the working group of Thomas Ziegler and is highly experienced in tropical field research, conservation work, and project management. Her current research is focused on ecology, population dynamics, and conservation of endangered, specialist and range-restricted lizard species in Vietnam.