AMPHIBIAN & REPTILE CONSERVATION

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Rediscovery of *Andinophryne olallai* Hoogmoed, 1985 (Anura, Bufonidae), an enigmatic and endangered Andean toad

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Abstract.—We report the rediscovery of *Andinophryne olallai*, an endangered species only known from a single specimen, collected in 1970. At the type locality, Tandayapa, Pichincha Province, numerous follow-up surveys after 1970 failed to record the species suggesting that the population is extinct. The rediscovery of *A. olallai* took place in 2012 at Río Manduriacu, Imbabura Province, Ecuador. Two surveys suggest that a healthy population of *A. olallai* survives at the site, with observations of froglets, juveniles, and adults across numerous stream systems. However, the extent of known occupancy of the population is small (<1 km²). Further data are presented to update knowledge of the distribution, ontogeny, morphology, and conservation status of the species. The population at Río Manduriacu is surrounded by logging, mining, and hydroelectric developments that could compromise its future survival. There is an urgent need to establish a monitoring program and to protect its remaining population and habitat in the region.

Key words. *Andinophryne olallai*, rediscovery, Tandayapa Andean toad, Andinosapo de Olalla, Bufonidae, Endangered species, Ecuador

Introduction

The small and understudied toad genus *Andinophryne* (Bufonidae) is restricted to the western slopes of the Andes in Colombia and Ecuador. Three species of *Andinophryne* have been described: *Andinophryne atelopoides* (Lynch and Ruiz-Carranza 1981), *Andinophryne colomai* (Hoogmoed 1985), and *Andinophryne olallai* (Hoogmoed 1985). Until recently, all three species were only known from five or fewer adult individuals at the type localities: *A. atelopoides* (Cauca Department, Colombia, 1980), *A. colomai* (Carchi Province, Ecuador, 1984), and *A. olallai* (Pichincha Province, Ecuador, 1970).

The paucity of information available on *Andinophryne* has led to many questions about the taxonomic and conservation status of all three species. *Andinophryne atelopoides*, the only species endemic to Colombia and only known from two specimens, was originally placed in the genus *Bufo* by Lynch and Ruiz-Carranza (1981). Four years later, following the discovery of two similar bufonid species (*A. colomai* and *A. olallai*) in northern Ecuador, and the reexamination of information presented on *B. atelopoides* by Lynch and Ruiz-Carranza (1981), Hoogmoed (1985) created the genus *Andinophryne* (Bu-

fonidae), and placed all three species within the new genus.

Despite numerous attempts by trained scientists and over 150 search hours, subsequent visits to the type localities of *A. colomai* and *A. olallai* in Ecuador have failed to record either species (Coloma et al. 2004; Ron and Frenkel 2013). Then, in 2005, Murillo et al. (2005) reported a 160 km range extension for *A. olallai* in Río Ñambi, Department of Nariño, Colombia. This observation marked the first record of any *Andinophryne* species in more than two decades. However, as part of our recent work with *Andinophryne*, a member of our team recently examined a specimen from Río Ñambi and determined that it was not *Andinophryne olallai* but a different species (Santiago Ron, unpubl. data). This identification has been confirmed by additional fieldwork and specimens collected at Río Ñambi by Paul David Gutiérrez-Cárdenas (pers. comm.). Therefore, *A. olallai* is the rarest of all *Andinophryne* species, with the only known record being the original type specimen from Tandayapa, Pichincha Province, Ecuador in 1970.

Forty-three years after the original description of *Andinophryne olallai*, we report the rediscovery of a population of *A. olallai* from Río Manduriacu (herein Manduriacu), Imbabura Province, Ecuador. We also pro-

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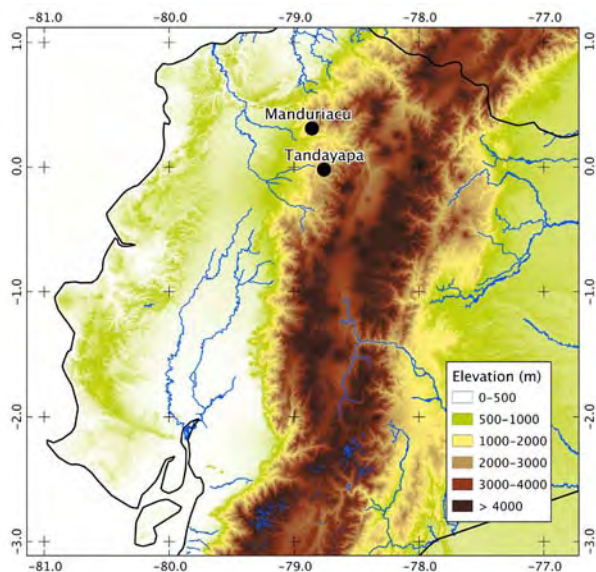
Fig. 1. Known records of *Andinophryne olallai* in northwest Ecuador; Tandayapa: Type Locality, Manduriacu: New Locality.

vide the first information on the species' natural history, geographic range, ontogeny, and conservation status, and present the first published color photos of live individuals across different age classes.

Materials and Methods

Our surveys took place in the premontane tropical forest and cloud forests of Manduriacu in NW Ecuador (1,100–1,400 m), 40 km N of the type locality of *A. olallai* and near the south border of the Cotacahi-Cayapas Ecological Reserve (Fig. 1). Surveys were conducted on 18 November 2012 (original rediscovery) and 13–15 May 2013 using Visual Encounter Surveys (VES) along stream transects between 19:00 and 01:00 h.

The objectives of the surveys were: (1) determine the population status of *A. olallai*; (2) determine the extent of its occupancy in Manduriacu; and (3) obtain information about the behavior and natural history of the species. Surveys were carried out along small rocky streams with overhanging herbaceous vegetation (Fig. 2). A total of three nights were spent surveying four stream systems neighboring the site of initial discovery (approximately 100 m between streams; < 1 km² area total).

Information collected in the field included: air temperature (°C), relative humidity (%), time of encounter (24 hr), perch height (cm), snout-vent length (SVL, mm), sex (when possible), and age class (froglet, juvenile, adult). Froglets (i.e., recently metamorphosed individuals) were defined as individuals with heavily patterned dorsum, lack of pronounced parotoid glands, and SVL between 10–20 mm. Juveniles were defined as individuals with faint dorsal patterning, more pronounced parotoid glands, and SVL between 20–30 mm. Adults were defined as

individuals with no dorsal patterning, very pronounced parotoid glands, presence of large cream-tan colored tubercles on the flanks, and SVL above 30 mm.

Perch height for each individual was measured using a marked meter stick and SVL measurements were taken using dial calipers. Climate information was recorded using a handheld Kestrel 3500 Weather Meter. Individual toads were only handled when necessary, and always with use of latex gloves to prevent transferring pathogens such as amphibian chytrid fungus (*Batrachochytrium dendrobatidis*).

Results and Discussion

During the first survey of Manduriacu on 18 November 2012, two adult *A. olallai* were encountered perched on leaves overhanging a small running stream. Elevation of the observation site was 1,253 m, and perch heights of the individuals were 1.5 m and 2.0 m above ground. Both individuals appeared to be females, based on size, with SVL of 57 and 58 mm, however sex could not be determined with complete certainty in the field because no secondary sexual characteristics are evident in live

Table 1. Reptiles and amphibians associated with *Andinophyne olallai* at Manduriacu, Imbabura Province, Ecuador and their current (August 2013) IUCN and FaunaWebEcuador Red List status (NE – Not Evaluated, DD – Data Deficient, LC – Least Concern, NT – Near Threatened, V – Vulnerable, EN – Endangered). IUCN Red List available at: http://www.iucnredlist.org/; FaunaWebEcuador Red List available at: http://zoologia.puce.edu.ec/vertebrados/anfibios/EspeciesEstadoConservacion.aspx.

| Species	Fauna Web Ecuador Red List	IUCN Red List
Caecilia guntheri	DD	DD
Centrolene peristictum	NT	V
Epipedobates darwinwallacei	EN	NE
Espadarana prosoblepon	LC	LC
Hyloscirtus alytolylax	NT	NT
Pristimantis achatinus	LC	LC
Pristimantis calcarulatus	LC	V
Pristimantis labiosus	NT	LC
Pristimantis luteolateralis	NT	NT
Pristimantis muricatus	V	V
Pristimantis scolodiscus	DD	EN
Rulyrana orejuela	DD	DD
Alopoglossus festae	NT	NE
Anolis aequatorialis	NT	NE
Anolis gemmosus	LC	LC
Basiliscus galeritus	NE	NE
Bothriechis schlegelii	NT	NE
Cercosaura vertebralis	DD	NE
Diaphorolepis wagneri	NT	NE
Lepidoblepharis conolepis	EN	NE

animals. This initial observation yielded two significant findings: the first evidence of an *A. olallai* population in 43 years and the second known locality for the species extending its known range 40 km N from its type locality.

During the course of the survey in May 2013 a total of 18 *A. olallai* were observed across four stream systems. Average nightly environmental conditions during the three nights of surveys in May were: air temperature 18.3 °C and relative humidity 92.8%. We recorded the presence of adults, juveniles, and froglets, indicating ongoing population recruitment (Fig. 3). Eleven of the nineteen individuals encountered were adults, and although their sex could not be determined, eggs were visible in the abdomen of two gravid females. The sex of one preserved adult male (QCAZ-A 55561) was confirmed by internal gonad examination. The confirmed adult females had SVL of 57 mm and 60 mm, considerably larger than the SVL reported by Hoogmoed (1985) for the holotype (♀, 39.6 mm). The single confirmed male had a SVL of 36.5 mm. Mean SVL for adults with unknown sex was 47.1 mm (*n* = 8).

All individuals encountered were perched on branches or leaves overhanging or bordering the streams. Mean perch height was 1.4 m (*n* = 18), with adults generally perching higher than younger individuals. Maximum observed perch height was four meters. Although no official surveys were conducted during the day, no individuals were observed along streams during random daytime walks. Although further behavioral work needs to be conducted, this observation suggests that *A. olallai* may be actively foraging during the day in the forests surrounding streams. At night, they remain immobile perched on leaves overhanging the streams. Lack of movement may protect them from predators.

Ontogeny and Morphology

All information on *A. olallai* reported by Hoogmoed (1985) was based on two adult specimens. Our observations of froglets and juveniles mark the first reported information on the species' pre-adult morphology and ontogeny. Ontogenetic change in color pattern is considerable (Fig. 3), and is one of the few reported cases of such an extreme change in bufonids in Ecuador (see Hoffman and Blouin 2000). We observed a total of two froglets (mean SVL 13.1 mm) and five juveniles (mean SVL 26.6 mm). Froglets have a copper, gold, and white dorsum with a mottling pattern reminiscent of some species of *Atelopus* (Fig. 3: A, B). This contrasts with the patternless brown dorsum of the adults. The venter of froglets have a series of white undulating lines that extend the length of the body (Fig. 4). The iris in froglets and juveniles is more vibrantly red than in adults, which have a yellow copper-colored iris that is darker medially near the horizontally oval pupil. Froglets also differ from adults in lacking tubercles and parotoid
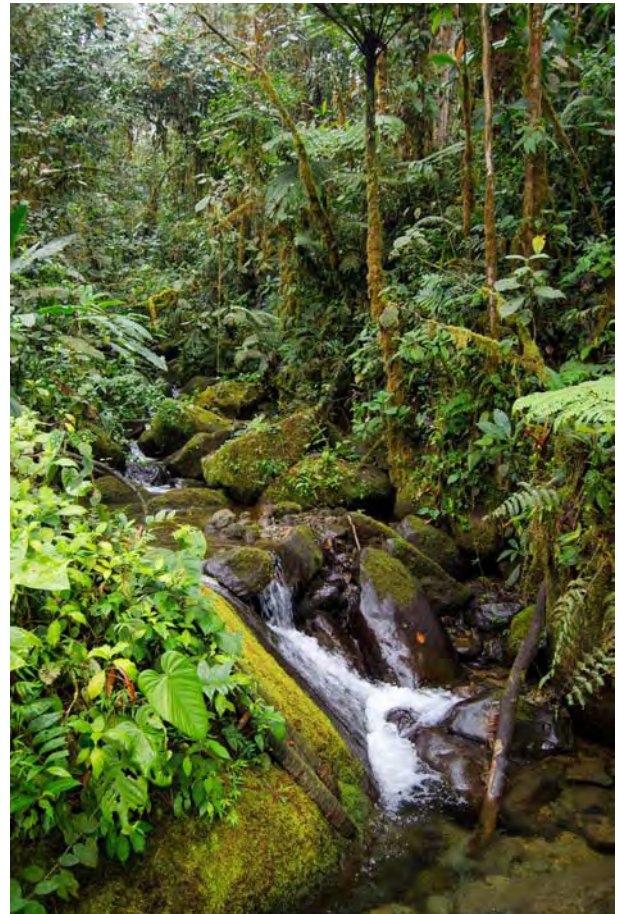
Fig. 2. *Andinophryne olallai* habitat from Río Manduriacu, Imbabura Province, Ecuador. All individuals encountered were found perched on branches or leaves along streams similar to the stream pictured here.

glands. Juveniles retained some of the mottling pattern seen in froglets (primarily posteriorly on the hind legs) and lacked the conspicuous tubercles on the flank (Fig. 3: C, D). However, they begin to show adult traits like pronounced parotoid glands, tan-brown coloration, and strongly webbed fingers.

Morphological characteristics of the adults match those of the holotype of *A. olallai* (comparisons based on photographs of the holotype, available at Link/URL: Amphibiaweb Ecuador, and Hoogmoed 1985). The holotype and the observed specimens of the population from Manduriacu differ from the other species of the genus in having more developed parotoid glands, larger body size, strongly webbed fingers, and conspicuous yellowish glands scattered on the flanks and arranged in rows or in irregular patterns (Fig. 3: E, F) (Hoogmoed 1985). The dorsal texture varies from smooth to mildly tuberculate. One individual had abundant tubercles on the anterior half of the dorsum and large scattered tubercles on the posterior half. The description of coloration given by Hoogmoed (1985) was of an animal in preservative; however, the color description falls within the variation observed in life at Manduriacu. The only notable differ-
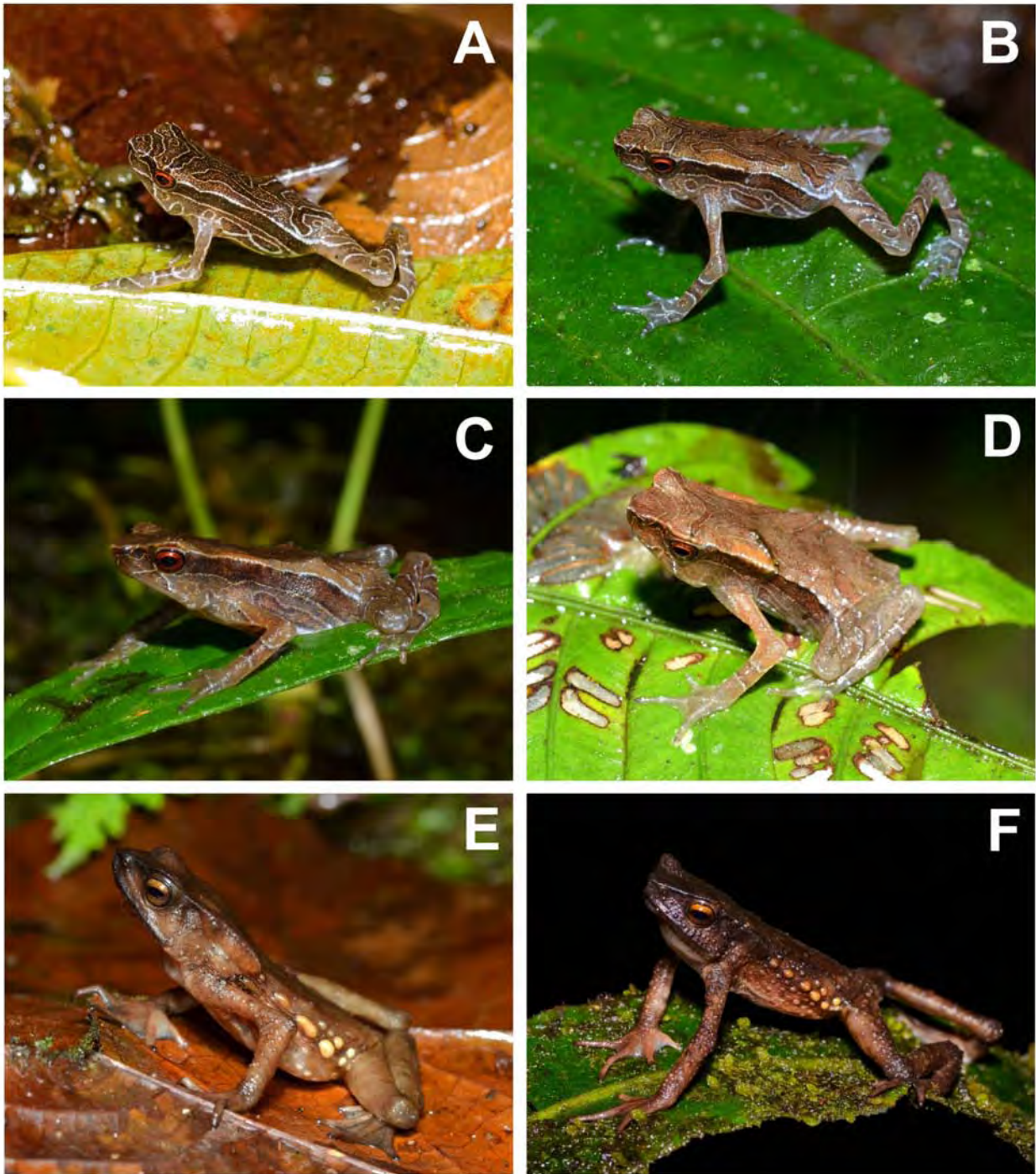
Fig. 3. Ontogenetic transformation of color and pattern in *Andinophryne olallai* from Río Manduriacu, Imbabura Province, Ecuador. (A) Froglet (11 mm SVL; *in situ*), (B) Froglet (15.1 mm SVL; *in situ*), (C) Juvenile (26.3 mm SVL; *in situ*), (D) Juvenile (28.1 mm SVL; *in situ*), (E) Adult (44.6 mm SVL; *ex situ*), (F) Adult (53.3 mm SVL; *in situ*). Note the progressive ontogenetic change in dorsal patterning from heavily mottled to no pattern; lack of parotoid glands and tubercles along the flank to presence of conspicuous parotoid glands and tubercles along the flank; a darkening of color from copper, tan, and white to dark brown; and iris color change from vibrant crimson to copper-orange.

Fig. 4. Ventral pattern of froglets of *Andinophryne olallai*. Manduriacu, Imbabura Province, Ecuador.

ence is that dorsal and flank coloration is not uniform in all individuals; the head and dorsum were darker brown than the light brown-tan flanks in most live animals observed at Manduriacu.

Sympatric Species

During our herpetofaunal surveys of Manduriacu we recorded observations of all amphibian and reptile species occurring at the site (Table 1). Most of these species are mid-elevation (1,000–2,500 m) inhabitants of premontane and cloud forests of the eastern Andes. A number of the species (i.e., *Lepidoblepharis conolepis*, *Pristimantis scolodiscus*) are either nationally or internationally listed as Endangered, and two of the species are categorized as Data Deficient or have not yet been assessed (i.e., *Diaphorolepis wagneri*, *Epipedobates darwinwallacei*) and very little is known about their biology or conservation status due to few available records or localities.

Conservation and Threats

Andinophryne olallai is currently classified as Data Deficient by the IUCN Red List (Coloma et al. 2010). However, more recent assessments considers *A. olallai* as Endangered based on its restricted range, the apparent extirpation of the species from the type locality and

Fig. 5. A recently deforested plot of land that is less than one km from the population of *Andinophryne olallai* in Manduriacu, Imbabura Province, Ecuador.

extensive habitat degradation (Coloma et al. 2011-2012; Ron and Frenkel 2013). The rarity of known distribution and a very small population size likely warrants an IUCN Red List status of Endangered.

Although we found evidence of a seemingly healthy population of *A. olallai* at Manduriacu, with the presence of all size classes across numerous stream systems, the extent of known occupancy remains extremely small (< 1 km^2). At present, pristine habitat still exists at Manduriacu, however, the surrounding forest is rapidly disappearing due to a variety of anthropogenic factors (i.e., intensive logging, mining, and hydroelectric development). These activities are expanding quickly and resulting in extensive habitat fragmentation and loss (Fig. 5). The apparent extirpation of *A. olallai* from the type locality, a site where forest has been lost and fragmented, suggests that the species is sensitive to anthropogenic habitat change. Urgent conservation measures and population monitoring are needed in order to ensure the survival of *A. olallai* in nature. It is our hope that the rediscovery of *A. olallai* will result in immediate support for greater protection of the forests in and around Manduriacu, and provide assistance in creating biological corridors between the neighboring reserves of Los Cedros and Cotacachi-Cayapas.

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Ryan L. Lynch is the lead biologist and photographer for Ecuadorian programs for The Biodiversity Group in Quito, Ecuador. He received his M.S. in wildlife ecology and conservation from the University of Florida where he used occupancy modeling to determine the status of anurans across the Florida everglades landscape. Ryan's current research interests focus on the ecology, distribution, and conservation of rare, threatened, and new species of reptiles and amphibians in Ecuador.

Sebastián Kohn is the administrator for the Antisanilla-Sunfohuaico Reserve run by the Jocotoco Foundation in Ecuador. He received his B.A. in biology and environmental studies at Whitman College in Washington State, USA. He currently directs the Río Manduriaco Cooperative in Imbabura, Ecuador, as well as the Ilitio Wildlife Rescue Center and Hacienda Ilitio in Cotopaxi, Ecuador. Sebastián is a founding member of the Andean Condor Conservation Group of Ecuador (Grupo Nacional de Trabajo del Cóndor Andino) and has been working with, and researching, both wild and captive condors for ten years.

Fernando Ayala-Varela is the director of the herpetology collection at the Pontificia Universidad Católica del Ecuador in Quito. He received his diploma at the Pontificia Universidad Católica del Ecuador, Quito in 2004. He has been interested in herpetology since childhood and has dedicated a lot of time studying the lizards of Ecuador, specifically the taxonomy and ecology of *Anolis* species. His current research interests include reproductive biology and ecology of lizards and snakes in Ecuador.

Paul S. Hamilton is the founder and executive director of The Biodiversity Group in Tucson, Arizona, USA. He holds a master's degree in biology from the University of California, Riverside, and a Ph.D. in biology from Arizona State University, and has conducted field studies in evolutionary, behavioral and conservation ecology both in the tropics and the desert southwest. In addition to his research interests in ecology and conservation of overlooked species such as amphibians, reptiles, and invertebrates, he is also a well published scientific and artistic photographer.

Santiago R. Ron is the curator of amphibians and professor at the Pontificia Universidad Católica del Ecuador in Quito. His research focuses on the evolution and diversity of neotropical amphibians with emphasis on Ecuador. Areas covered include evolution of animal communication, sexual selection, systematics and taxonomy. In the area of conservation biology Santiago is interested in the study of amphibian extinctions in the Andes. Santiago also oversees the *ex situ* amphibian conservation project Balsa de los Sapos at the Pontificia Universidad Católica del Ecuador in Quito.

A new Andean anole species of the Dactyloa clade (Squamata: Iguanidae) from western Ecuador

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Abstract.—We describe a new species of *Anolis* from the western slopes of the Andes of Ecuador, province of Bolívar. It is referred to (1) the *aequatorialis* series based on its moderate size and narrow toe lamellae, and (2) the *eulaemus* sub-group based on having a typical *Anolis* digit, in which the distal lamellae of phalanx III distinctly overlap the proximal subdigital scales of phalanx II. The new species is most similar morphologically to *A. otongae* and *A. gemmosus*, both from similar elevations on the western Andean slopes of Ecuador, but differs from these species in morphology and color patterns. We present a phylogeny based on DNA sequence data as additional evidence supporting delimitation of the new species. The new species and *A. gemmosus* are sister taxa within the "western Dactyloa clade."

Key words. Clade Dactyloa, DNA, lizard, phylogeny, South America, systematics

Introduction

With nearly 490 described species, anole lizards (*Anolis*) have proliferated impressively in the Americas (Nicholson 2002; Poe 2004), possibly prompted by ecological opportunity (Losos 2009). Although the diversity of these lizards has been extensively studied in the West Indies (Losos 2009), the same is not true for the mainland radiation, which is probably greater than previously thought. For example, all but two—*Anolis ruibali* Navarro & Garrido 2004 and *A. sierramaestrae* Holáňová et al. 2012—of the 31 new species of *Anolis* described during the last decade (2003–2013) occur in mainland Central and South America (Uetz and Hošek 2014). Improving knowledge concerning the diversity of mainland anoles is crucial to understanding the nature of this radiation.

Anole lizards represent the most species-rich clade traditionally recognized as a genus in Ecuador, with 37 species reported to date (Torres-Carvajal et al. 2014). The diversity of anole lizards in Ecuador is remarkably greater west of the Andes, with more than twice the number of species that occur east of the Andes (25 and 12 species, respectively). Of these, five species have been described during the last six years from both sides of the Andes as a result of both careful examination of existing collections and recent collecting in poorly explored areas. Here we contribute to that growing body of taxonomic knowledge with the description of a new species of *Anolis* endemic to the western slopes of the Andes in

Ecuador. We present molecular evidence supporting recognition of the new species by performing phylogenetic analyses of mitochondrial DNA sequence data.

Materials and Methods

Morphological data

All known specimens of the new species described in this paper are included in the type series, and were deposited in the Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ). Specimens of other species of *Anolis* examined in this study are listed in Appendix 1. We follow previously proposed terminology (Williams et al. 1995) for measurements and squamation. Nine morphological measurements were taken with digital calipers and recorded to the nearest 0.1 mm: head length, head width, head height, forelimb length, hindlimb length, snout-vent length, jaw length, axilla-groin length, and snout length. In addition, tail length measurements were taken with a ruler and recorded to the nearest millimeter; regenerated or broken tails were not measured. Sex was determined by noting the presence of hemipenes, which were everted in all male specimens during preparation.

Statistical analyses

Given that the new species is very similar in morphology to *Anolis gemmosus* and *A. otongae* we performed

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Fig. 1. Head of the holotype (QCAZ 3449) of *Anolis poei* sp. nov. in dorsal (**top**), ventral (**middle**), and lateral (**bottom**) views [Scale bar = 10 mm]. *Photographs by F. Ayala-Varela*.

a Principal Component Analysis (PCA) to determinate whether separation in morphological space between those species was statistically significant. Principal components (PCs) were extracted from a covariance matrix of the raw and rescaled data. The new species is most similar to *A. gemmosus*, for which we also used *t*-tests to evaluate quantitative differences between both species. One of the assumptions of the *t*-test for two samples is that the variances of both samples are equal: therefore, *F*-tests also were performed for each character to test for equality of variances. If the variances were not the same (i.e., $P < 0.05$), an unequal variance *t*-statistic was used. Statistical analyses were performed in SPSS Statistics 17 (SPSS Inc. 2008).

The distribution map was prepared in ArcMap 9.3 (ESRI, Inc.); WGS84 is the datum for all coordinates presented below.

DNA sequence data

Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Tissue samples were first mixed with Proteinase K and a lysis buffer and digested overnight prior to extraction. DNA samples were quantified using a Nanodrop® ND-1000 (NanoDrop Technologies, Inc), re-suspended and diluted to 25 ng/ul in ddH2O prior to amplification.

Using primers and amplification protocols from the literature (Folmer et al. 1994; Kumazawa and Nishida 1993; Macey et al. 1997; Schulte and Cartwright 2009) we obtained 2807 nucleotides (nt) representing the nuclear gene recombination-activating gene 1 (RAG1, 811nt), as well as the mitochondrial genes Cytochrome c oxidase I (CO1, 655nt) and a continuous fragment including the NADH dehydrogenase subunit 2 (ND2, 1038 nt), tRNATrp, tRNAAla, tRNAAsn, tRNACys (282nt), and the origin of the light-strand replication (Ol, 29nt). The new sequence data were obtained for three individuals of the new species described herein, two of *A. gemmosus*, and two of *A. otongae*. In addition we used sequence data generated by Castañeda and de Queiroz (2011) for 20 individuals of the clade Dactyloa, as well as one sequence of *A. occultus*, which was used as the outgroup in the phylogenetic analysis. Gene regions of taxa included in phylogenetic analyses along with their GenBank accession numbers are shown in Table 1.

Phylogenetic analyses

Editing, assembly, and alignment of sequences were performed with Geneious ProTM 5.3 (Biomatters Ltd. 2010). Genes were combined into a single dataset with eleven partitions, three per protein coding gene corresponding to each codon position, one with all tRNAs, and one with the Ol. The best partition strategy along with the corresponding models of evolution were obtained in PartitionFinder 1.1.1 (Lanfear et al. 2012) under the Bayesian information criterion.

Phylogenetic relationships were assessed under a Bayesian approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2003). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 20 million generations and four Markov chains with default heating values. Trees were sampled every 1,000 generations resulting in 20,000 saved trees per analysis. Stationarity was confirmed by plotting the –ln L per generation in the program Tracer 1.6 (Rambaut et al. 2013). Additionally, the standard deviation of the partition frequencies and the potential scale reduction factor (Gelman and Rubin 1992) were used as convergence diagnostics for the posterior probabilities of bipartitions and branch lengths, respectively. Adequacy of mixing was assessed by examining the acceptance rates for the parameters in MrBayes and the effective sample sizes (ESS) in Tracer. After analyzing convergence and mixing, 2,000 trees were discarded as "burn-in" from each run. We then confirmed that the four analyses reached stationarity at a similar likelihood score and that the topologies were similar, and used the resultant 72,000 trees to calculate posterior probabilities (PP) for each bipartition on a 50% majority rule consensus tree.

Systematics

The taxonomic conclusions of this study are based on the observation of morphological features and color patterns, as well as inferred phylogenetic relationships. We consider this information as species delimitation criteria following the general species concept of de Queiroz (1998, 2007).

Anolis poei sp. nov.

urn:lsid:zoobank.org:act:712687F6-CF33-4969-815D-E4600D01FB4C

Proposed standard English name: **Telimbela anoles**
Proposed standard Spanish name: **Anolis de Telimbela**

Holotype
QCAZ 3449 (Figs. 1, 2), adult male, Ecuador, Provincia Bolívar, Telimbela, 01.65789ºS, 79.15334ºW, WGS84 1,354 m, 10 June 2011, collected by Fernando Ayala-Varela, Jorge H. Valencia, Diana Troya-Rodríguez, Francy Mora, and Estefanía Boada.

Paratypes (15)
ECUADOR: Provincia Bolívar: QCAZ 3444−3448, 3451−3455, 4359, same data as holotype, except 0.1658440ºS, 79.157150ºW, 1,310 m; QCAZ 6781−6783 Telimbela, Escuela Elisa Mariño de Carvajal, 0.1665857ºS, 79.172096ºW, 27 July 2004, collected by Edwin Carrillo-Ponce and Morley Read; QCAZ 9219 Guaranda, Salinas, Recinto Tres Cruces, 01.431380ºS,
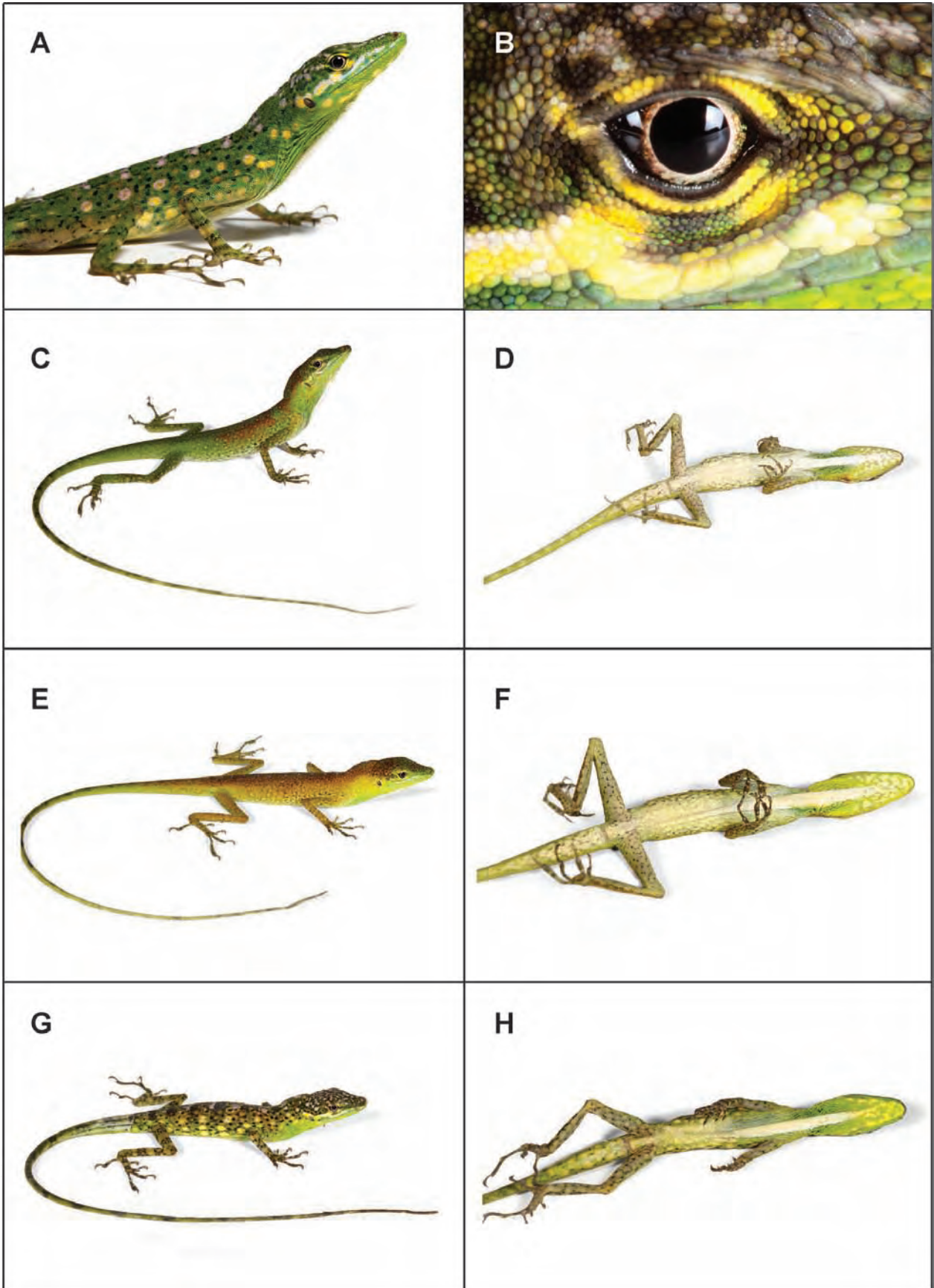
Fig. 2. *Anolis poei* sp. nov. Holotype, adult male (SVL = 59.67 mm, QCAZ 3449, **A**), eye close-up (SVL = 60.31 mm, QCAZ 3448, **B**), subadult male (SVL = 52.12 mm, QCAZ 3455, **C, D**), adult male (SVL = 59.02 mm, QCAZ 3451, **E, F**), adult male (SVL = 60.31 mm, QCAZ 3448, G, H). *Photographs by L. Bustamante (A), and O. Torres-Carvajal (B, C, D, E, F, G, H).*
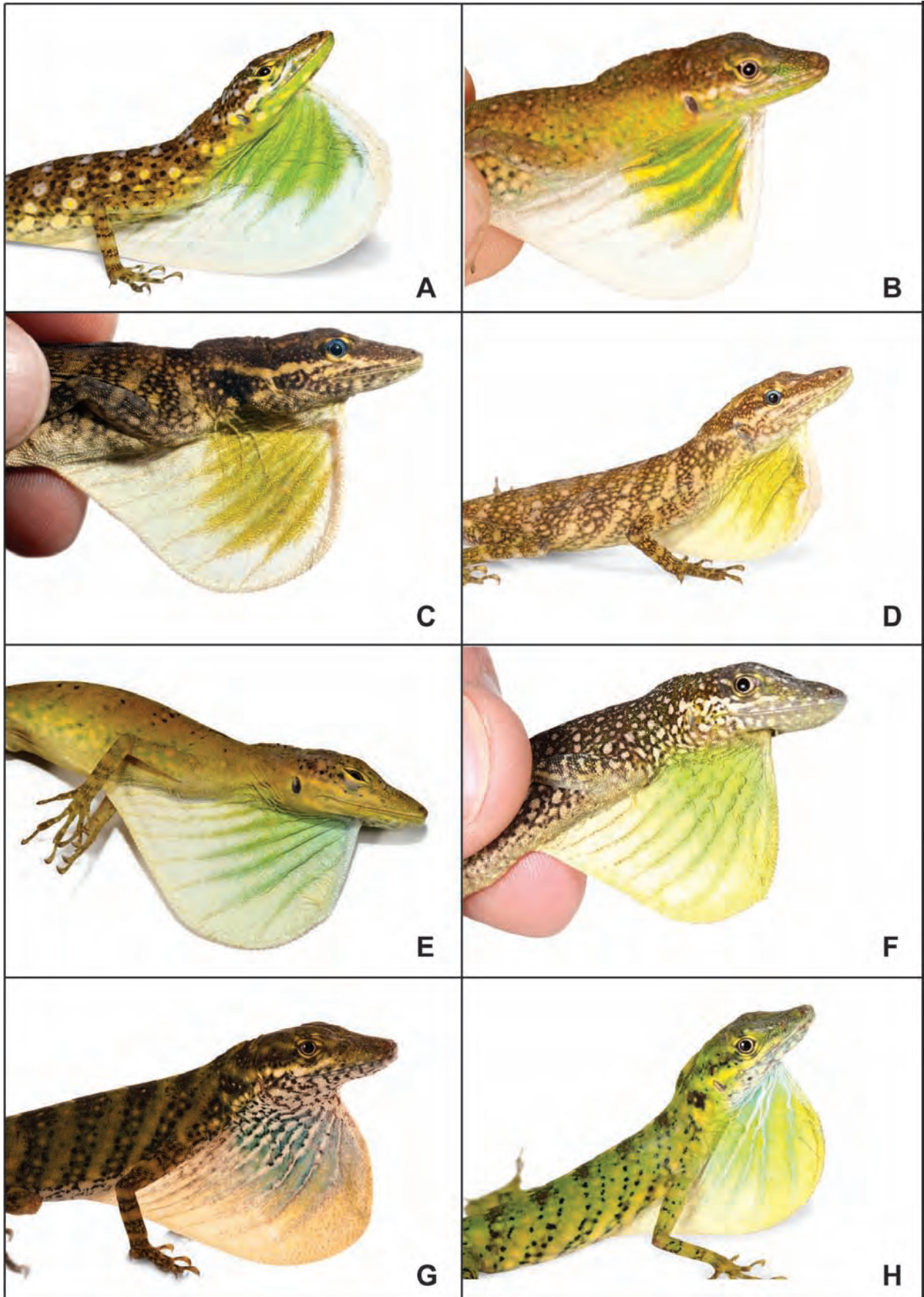
Fig. 3. Male dewlap of *Anolis poei* sp. nov. (holotype, QCAZ 3449, **A**; paratype, QCAZ 3455, **B**); *A. otongae* (QCAZ 4661, **C**; QCAZ 11791, **D**); and *A. gemmosus* (QCAZ 4385, **E**; QCAZ 4352, **F**; QCAZ 9452, **G**; QCAZ 11850, **H**). *Photographs by L. Bustamante (A), O. Torres-Carvajal (B, C, D, E, F, H), and S. R. Ron (G).*

Table 1. Species of *Anolis* sequenced in this study, voucher specimen numbers, collecting localities, and GenBank accession numbers.

Species	Voucher	Locality	GenBank Number
A. gemmosus	QCAZ 4385	Ecuador, Carchi, Río San Pablo near Chical	ND2: KJ854205 COI: KJ854219 RAG1: KJ854212
	QCAZ 4406	Ecuador, Carchi, Maldonado, Teldibi Ecological Trail	ND2: KJ854206 COI: KJ854220 RAG1: KJ854213
A. otongae	QCAZ 11790	Ecuador, Pichincha, Biological Reserve Otonga	ND2: KJ854207 RAG1: KJ854214 COI: KJ854221
	QCAZ 11791	Ecuador, Pichincha, Biological Reserve Otonga	ND2: KJ854208 COI: KJ854222 RAG1: KJ854215
A. poei	QCAZ 3444	Ecuador, Bolívar, Telimbela	ND2: KJ854209 COI: KJ854223 RAG1: KJ854216
	QCAZ 3445	Ecuador, Bolívar, Telimbela	ND2: KJ854210 COI: KJ854224
	QCAZ 3448	Ecuador, Bolívar, Telimbela	ND2: KJ854211 COI: KJ854225 RAG1: KJ854217
	QCAZ 4359	Ecuador, Bolívar, Telimbela	RAG1: KJ854218

79.097970ºW, 2,628 m, 28 May 2009, collected by Elicio E. Tapia, Silvia Aldás-Alarcón, and Eduardo Toral-Contreras.

Diagnosis

We assign *Anolis poei* both to the aequatorialis series, based on moderate to large body size, narrow toe lamellae, small head scales, smooth ventral scales, and uniform dorsal scalation; and to the eulaemus-subgroup, based on a typical *Anolis* digit, in which the distal lamellae of phalanx III distinctly overlap the first proximal subdigital scale of phalanx II (Williams 1976; Williams and Duellman 1984; Castañeda and de Queiroz 2013).

At present ten species are recognized within the eulaemus-subgroup: *Anolis anoriensis* Velasco et al. 2010, *A. antioquiae* Williams 1985, *A. eulaemus* Boulenger 1908, *A. fitchi* Williams & Duellman 1984, *A. gemmosus* O'Shaughnessy 1875, *A. maculigula* Williams 1984, *A. megalophitecus* Rueda-Almonacid 1989, *A. otongae* Ayala-Varela & Velasco 2010, *A. podocarpus* Ayala-Varela & Torres-Carvajal 2010, and *A. ventrimaculatus* Boulenger 1911. *Anolis poei* differs from them mostly in dewlap features. The dewlap in males of *A. poei* has a yellowish-green (or both yellow and green) gorgetal region, light blue border, and white sternal and marginal regions (Fig. 3). It has a blackish gorgetal region, and creamy white sternal region with light brown scales in *A. anoriensis*; brown gorgetal region, and pale brown marginal region in *A. eulaemus*; bluish-gray gorgetal region, orange stripes, pale bluish-rose anterior third, and white sternal region becoming pale blue toward the belly in *A. maculigula*; sepia background, with red narrow and irregular stripes on each side of rows in *A. megalophitecus*; white, pale yellow, or greenish-yellow gorgetal region, with white or pale-yellow marginal and sternal regions in *A. otongae* (Fig. 3); dull yellowish-green or light blue gorgetal region, shading to dull cream, greenish yellow or orange on the marginal region, with white or bluish green gorgetal rows with or without brown spots and with yellowish white, yellow or orange sternal region in *A. gemmosus* (Fig. 3). The dewlap in males of *A. poei* has wide rows of 3−7 scales separated by naked skin; the width of these rows is one scale in *A. fitchi*, 2−5 granular, minute scales in *A. podocarpus*, 1–2 scales in *A. ventrimaculatus*, 3−6 scales in *A. otongae*, and 2−3 scales in *A. gemmosus*. In addition, females of the new species lack a dewlap, which is present in females of *A. anoriensis*, *A. antioquiae*, *A. eulaemus*, *A. fitchi*, and *A. podocarpus*.

Anolis poei is most similar morphologically to *A. otongae* and *A. gemmosus* (Fig. 4). From the former species (character states in parenthesis) *A. poei* differs in having small dorsal chevrons in females (large dorsal chevrons extending onto flanks), pale yellowish-brown iris (iris dark blue), interparietal scale (if present) surrounded by small swollen scales (interparietal scale surrounded by relatively enlarged flat scales), enlarged postanal scales separated by 3−5 scales (postanal scales separated by 1−2 scales), and in lacking a dark stripe on side of head (dark coppery-brown stripe present). Additionally, PCA analyses suggested that specimens of *A. poei* have shorter jaws, as well as lower and narrower heads than *A. otongae* (Table 2, Fig. 5), with PC1 (39% of total variation) represented mainly by head height, head width, and jaw length.

The new species can be distinguished from *A. gemmosus* (Table 3) in having fewer scales between second canthals (11−14, mean = 12.08 and 12−21, mean = 15.25, respectively; t = 5.31, $P<0.005$); fewer scales between supraorbital semicircles (1−3, mean = 1.62 and 1−5, mean = 3.13, respectively; t = 4.46, P<0.005); more lamellae under phalanges III-IV of fourth toe (18−19, mean = 18.92 and 14−18, mean = 17.33, respectively; t = -7.86, P<0.005); a narrower head (head width = 7.84−8.84, mean = 8.29 and 6.97−17.41, mean = 10.82, respectively; t = -7.03, P<0.005); lower head (head height = 6.54−7.48, mean = 6.92 and 5.42−15.96, mean = 9.51, respectively; t = -6.96, P<0.005); and shorter snout (snout length = 6.75−7.30, mean = 6.92 and 5.79−14.95, mean = 10.58, respectively; t = -11.74, P<0.005).
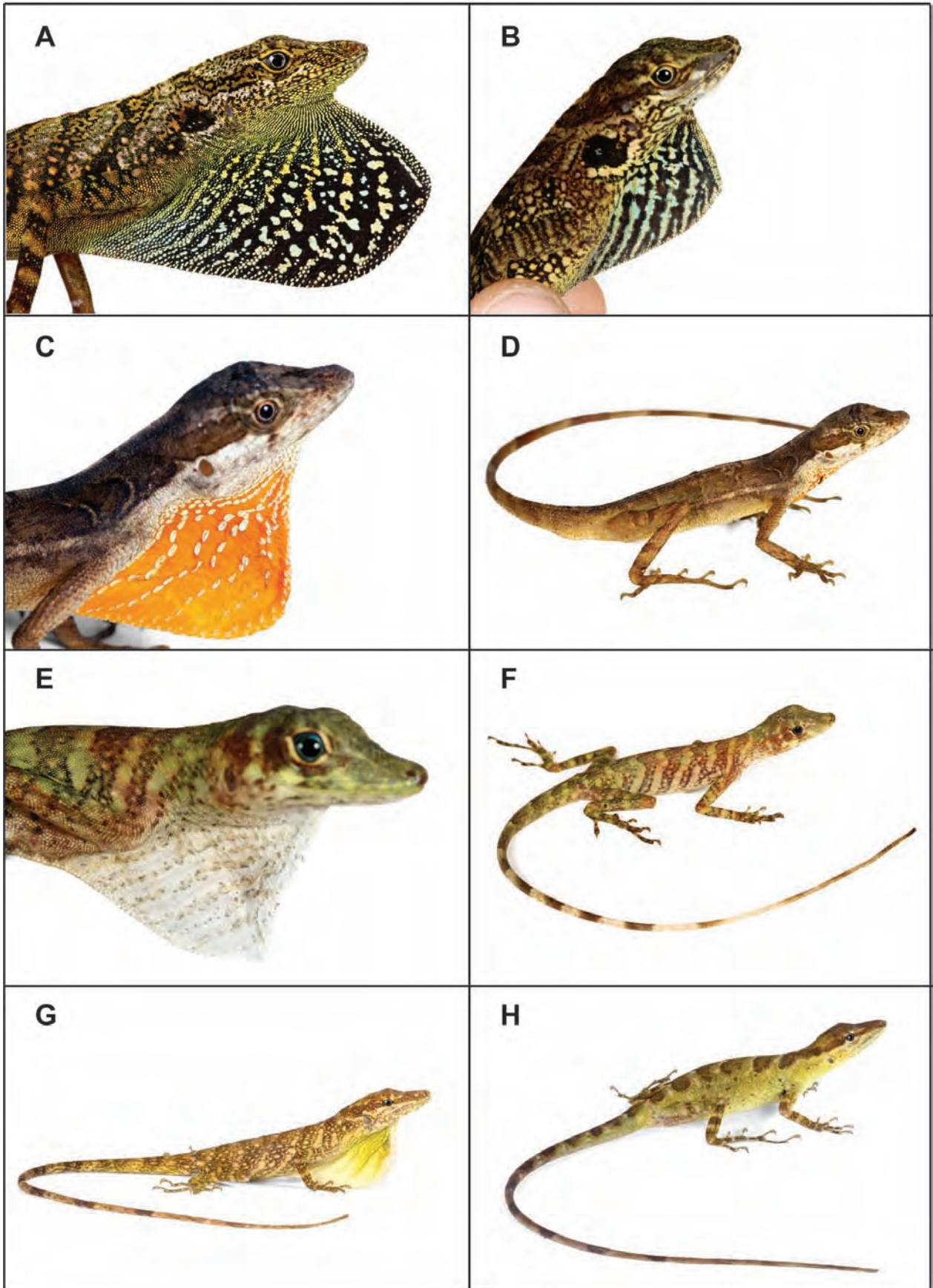
Fig. 4. Part 1. Five species of *Anolis* from western Ecuador. *A. aequatorialis*: male (QCAZ 11861, **A**) and female (QCAZ 3443, **B**); *A. binotatus*: male (QCAZ 3434, **C, D**); *A. fasciatus*: male (QCAZ 3450, **E, F**); *A. otongae*: male (QCAZ 11790, **G**) and female (QCAZ 11791, **H**).
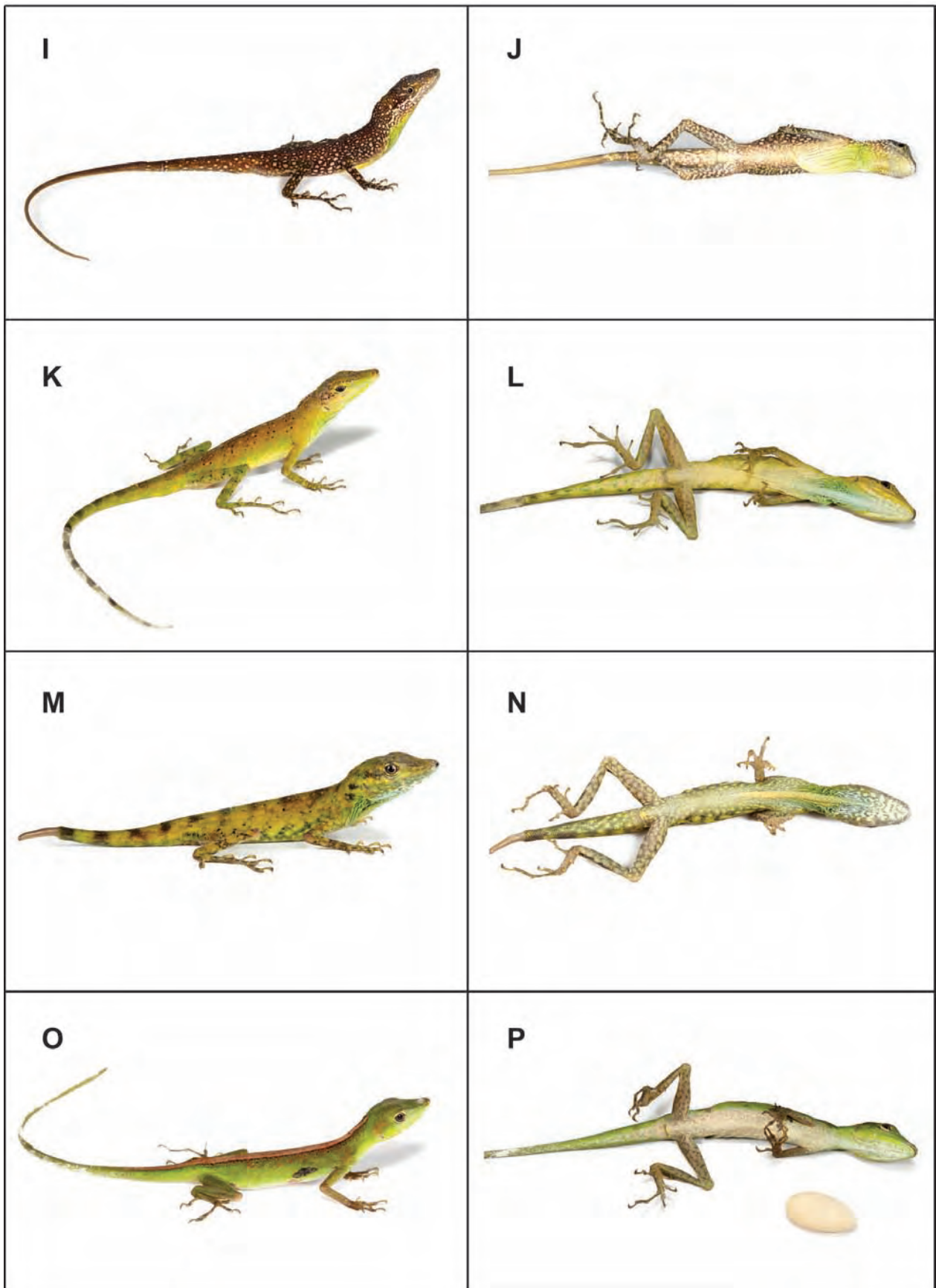
Fig. 4. Part 2. *A. gemmosus*: male (QCAZ 4352, **I, J**), male (QCAZ 4385, **K, L**), male (QCAZ 11849, **M, N**), and female (QCAZ 4393, O, **P**). *All photographs by O. Torres-Carvajal, except A, M, N (S. R. Ron).*

Description of holotype (scores for para-types in parentheses)

Male (Figs. 1, 2); SVL 59.7 mm (46.5−60.3 mm); tail length 150.2 mm (146.2−163.4 mm); head length 15.9 mm (14.8−16.5 mm); head width 8.4 mm (7.8−8.8 mm); head height 7.2 mm (6.5−7.5 mm); internasal distance 2.0 mm (1.2−2.1 mm); interorbital distance 2.4 mm (2.2−2.5 mm); interparietal absent (present, interparietal length 0.8−0.9 mm; second largest scale length near interparietal 0.3−0.4 mm); ear opening maximum length 1.6 mm (1.6−2.1 mm); snout length 6.8 mm (6.8−7.3 mm); jaw length 11.7 mm (11.7−14.4 mm); axila-groin distance 27.7 mm (27.4−30.6 mm); femur length 14.8 mm (14.4−15.6 mm); 4th toe length 12.5 mm (10.6−12.8 mm); 4th toepad width 1.2 mm (1.0−1.3 mm); forelimb length 36.2 mm (21.8−36.2 mm); hindlimb length 42.6 mm (42.6−52.7 mm).

Head scales multicarinate (same, unicarinate, or rugose) on frontal region and unicarinate (same, multicarinate or rugose) on supraocular disc; 11 (10−14) scales between second canthals; 13 (11−15) scales between first canthals; 6 (5−7) scales bordering the rostral posteriorly; anterior nasal in contact with rostral (same or inferior nasal in contact with rostral); supraorbital semicircles separated by two (0−3) scales; supraocular disk with scales heterogeneous in size; one elongate superciliary followed by a series of granules (same or one small scale instead of granules); 6 (5−8) loreal rows on left side; 49 (25−53) loreal scales; interparietal absent (same or, when present, the interparietal smaller than ear opening, with 4−7 scales between interparietal and semicircles on each side, and 8−15 scales between interparietal and nape scales); suboculars in contact with supralabials; 6 (5−7) supralabials counted up to a point below center of eye; 6 (5−7) infralabials counted up to a point below center of eye; 7 (4−7) postmentals; one enlarged sublabial on each side.

Table 2. PCA loadings conducted on nine morphological variables of *Anolis gemmosus*, *A. otongae* and *A. poei*.

	Raw			**Rotated**		
	1	2	3	1	2	3
Head height	-0.96	0.21	-0.02	-0.97	0.16	-0.07
Head length	0.24	0.34	0.05	0.22	0.35	0.05
Head width	-0.96	0.20	-0.03	-0.96	0.15	-0.07
Jaw length	0.98	0.06	-0.06	0.98	0.11	-0.03
Snout length	0.82	0.33	-0.07	0.81	0.37	-0.06
Forelimb length	-0.01	0.80	0.04	-0.05	0.80	0.00
Hindlimb length	-0.01	0.85	0.02	-0.05	0.85	-0.03
Axilla-groin length	-0.01	-0.50	-0.02	0.01	-0.50	0.01
Snout-vent length	0.06	-0.04	0.99	0.02	0.01	1.00
Eigenvalue	3.54	1.93	1.00	3.53	1.93	1.01
% var. explained	39.31	21.42	11.16	39.23	21.45	11.21
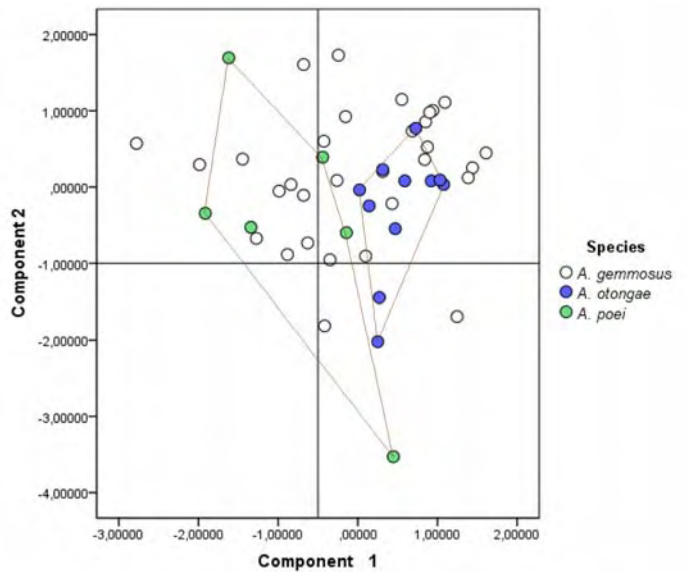
Fig. 5. Distribution of *Anolis gemmosus*, *A. otongae* and *A. poei* sp. nov. along the first and second principal components axes.

Dorsal crest or enlarged middorsal row absent; dorsal scales keeled, 11 (9−11) dorsal scales in 5% the length of SVL contained in the dorsal midline at the level of the forelimbs; flank scales more or less separated by skin; ventrals smaller than dorsals, 13 (8−13) longitudinal rows in 5% the length of SVL; ventrals smooth and granular, arranged in diagonal rows.

Toepads overlap the first phalanx in all toes; 19 (18−19) lamellae under phalanges III and IV of fourth toe (character 27 in Williams et al. 1995 and character 9 in Poe 2004); supradigitals multicarinate; tail with a double row of middorsal scales; postanals present (same or absent), with a slightly enlarged scale laterally on each side.

Nuchal fold present (absent in females and juveniles); dorsal folds absent; dewlap extending posteriorly to a point halfway between fore and hindlimbs (absent in females); dewlap with five longitudinal rows of 3−7 swollen scales, similar size to ventrals, separated by naked skin.

Sexual variation of meristic and morphometric characters in *A. poei* is presented in Table 4.

Color in life

Holotype (QCAZ 3449; Figs. 2, 3): background of head, body, limbs and tail green; head with light bluish green, dark green, and light grey irregular spots dorsally; dorsal surface of body with six light grey, small irregular blotches; dorsal surface of neck with two light grey, small irregular blotches; limbs with dark green and yellowish-cream spots; lateral surface of head with a white stripe extending posteriorly from loreal region, through subocular region, to a point anterior to the tympanum; white blotch with yellow center above tympanum; eyelids yellowish green with first row of upper and lower

palpebrals black, second and third rows both yellow and green; lateral surface of neck with dark green dots; body flanks green, with rows of yellow-centered white spots oriented ventro-posteriorly; ventral surface of head yellowish green with light yellow blotches; ventral surface of body white with bluish-green reticulations; ventral surface of limbs white with several transparent scales and dark brown reticulations; ventral surface of tail white with dark green spots anteriorly, and yellowish-green transverse bands posteriorly; dewlap skin light blue, dark yellowish green on gorgetal region, light blue on sternal region; gorgetal scales light yellowish green; marginals and sternals white; iris dark brown with a white inner ring. When stressed, the dorsal background color switched from green to yellowish brown.

Subadult male (QCAZ 3455, Figs. 2,3, differences from holotype): head with dark green and white irregular small spots dorsally; dorsal surface of body and neck with white and dark green small spots, and larger pale yellow spots; lateral surface of head yellowish green with a white stripe extending posteriorly from loreal region, through subocular region, to upper border of tympanum; lateral surface of body with rows of white and dark green small spots, and larger pale yellow spots; ventral surface of head with white blotches and light blue spots; ventral surface of body with dark green reticulations; ventral surface of limbs with brown or green reticulations; ventral surface of tail with blackish green reticulations anteriorly; dewlap skin white, yellow on gorgetal region, white on sternal region; throat, edge of mouth, and tongue pinkish white (Fig. 6). When stressed, rust-colored blotches appeared on dorsal surface of head, body, limbs and tail.

Adult female (QCAZ 3454, Fig. 7): dorsal surface of head, body and tail yellowish green; dorsal surface of body with six narrow brown chevrons, each one delimited posteriorly by a grayish white blotch; limbs yellowish green with dark green spots arranged in bands, and pale yellowish spots; tail with two brown chevrons anteriorly; lateral surface of head yellowish green; loreal region yellow; lateral surface of neck and body yellowish green with brown dots; ventral surface of head pale yellow with yellowish green reticulations, short white longitudinal stripe on throat; ventral surface of body and tail white with black reticulations laterally; ventral surface of limbs white with some transparent scales and brown reticulations on hindlimbs; ventral surface of tail with brownish green reticulations anteriorly; iris brown with a pale white ring.

Subadult female (QCAZ 3446, Fig. 7, differences with QCAZ 3454): occipital and temporal regions with brown and white small blotches; dorsal surface of neck with a distinct brown chevron delimited posteriorly by a grayish white blotch; lateral surface of body yellowish green dorsally and light blue ventrally, with white or cream spots; dorsal surface of tail with two brown chevrons, each one delimited posteriorly by a grayish white blotch.

Fig. 6. Tongue of *Anolis poei* sp. nov., subadult male (QCAZ 3455, **top**); *A. gemmosus*, adult male (QCAZ 4347, **middle**); *A. otongae*, adult male (QCAZ 4661, **bottom**). *Photographs by S. R. Ron (top), O. Torres-Carvajal (middle, bottom).*

Color in preservative

Holotype (QCAZ 3449): dorsal background of head, body, limbs and tail grayish brown; dorsal surface of head with metallic green, dark green, blue, gray and white cream irregular spots; dorsal surface of body with six black small chevrons, each delimited posteriorly by a white irregular blotch; limbs with dark brown and white spots; lateral surface of head with a white stripe extending posteriorly from loreal region, through subocular region, to a point anterior to the tympanum; upper border of tympanum with a white spot; eyelids purple with first row of upper and lower palpebrals black, second and third rows white and purple; neck flanks with black dots; body flanks grayish brown, with dark brown diagonal

Table 3. Summary of morphological characters of *Anolis poei* sp. nov. and *A. gemmosus* from Ecuador. For each quantitative character, the *F*-value, *t*-value, and corresponding *P*-values are given. Range and sample size (in parenthesis) followed by mean ± standard deviation are given.

Character	*A. gemmosus*	*A. poei* sp. nov.	*F*-value	*P*	*t*-value	*P*
Scales between second canthals	12–21 (24) 15.25 ± 1.98	11–14 (13) 12.08 ± 1.12	2.59	0.12	5.31	<0.005
Postrostrals	5–7 (24) 5.79 ± 0.72	5–7 (13) 5.92 ± 0.64	1.37	0.25	-0.55	0.59
Row of loreals	6–10 (24) 7.25 ± 1.15	5–8 (13) 6.31 ± 1.18	0.03	0.86	2.36	0.02
Scales between supraorbital semicircles	1–5 (24) 3.13 ± 1.23	1–3 (13) 1.62 ± 0.77	5.27	0.03	4.46	<0.005
Scales between interparietal (if present) and semicircles	3–8 (24) 5.67 ± 1.27	4–7 (6) 5.83 ± 1.17	0.18	0.67	-0.29	0.77
Supralabials	5–7 (24) 6.08 ± 0.50	5–7 (13) 6 ± 0.41	1.29	0.27	0.51	0.61
Postmentals	4–8 (24) 6.13 ± 1.03	4–7 (13) 5.77 ± 0.93	0.18	0.67	1.03	0.31
Lamellae under phalanges III-IV of fourth toe	14–18 (24) 17.33 ± 0.92	18–19 (13) 18.92 ± 0.28	8.71	0.01	-7.86	<0.005
Head length	13.23–18.12 (94) 15.46 ± 1.07	14.79–16.5 (7) 15.67 ± 0.51	4.67	0.03	0.93	0.37
Head width	6.97–17.41 (94) 10.82 ± 3.24	7.84–8.84 (7) 8.29 ± 0.36	32.16	<0.005	-7.03	<0.005
Head height	5.42–15.96 (94) 9.51 ± 3.32	6.54–7.48 (7) 6.92 ± 0.38	31.04	<0.005	-6.96	<0.005
Jaw length	7.31–17.43 (94) 12.32 ± 3.02	11.73–14.36 (7) 12.44 ± 0.91	19.25	<0.005	0.26	0.80
Snout length	5.79–14.95 (94) 10.58 ± 2.93	6.75–7.30 (7) 6.92 ± 0.19	41.30	<0.005	-11.74	<0.005
Forelimb length	23.41–34.34 (94) 29.43 ± 2.28	21.84–36.18 (7) 28.57 ± 4.25	0.02	0.89	-0.12	0.90
Hindlimb length	41.51–63.80 (94) 52.82 ± 4.13	42.56–52.68 (7) 49.01 ± 3.33	1.00	0.32	-2.38	0.02
Axilla-groin length	20.73–33.51 (94) 26.74 ± 2.07	27.35–30.61 (7) 28.54 ± 1.30	0.95	0.33	2.26	0.03
Snout-vent length	46.71–66.21 (94) 58.34 ± 3.65	46.47–60.31 (7) 56.87 ± 4.85	0.35	0.56	-1.00	0.32
Tail length	94.94–191 (94) 154.59 ± 18.66	146.21–163.37 (7) 154.74 ± 6.32	3.82	0.05	0.02	0.98

Table 4. Sexual variation in lepidosis and measurements (mm) of *Anolis poei* sp. nov. Range followed by mean ± standard deviation are given.

Character	Males	Females
	n = 4	*n* = 3
Scales between second canthals	11–13 11.75 ± 0.96	12–13 12.67 ± 0.58
Postrostrals	5–6 5.75 ± 0.5	6–7 6.33 ± 0.58
Row of loreals	6–8 7 ± 1.15	5–6 5.33 ± 0.58
Scales between supraorbital semicircles	1–2 1.75 ± 0.5	1–2 1.67 ± 0.577
Scales between interparietal and semicircles	Interparietal absent	6–7 6.50 ± 3.78
Supralabials to below center of eye	6	6
Postmentals	4–7 5.25 ± 1.5	6–7 6.33 ± 0.58
Lamellae under phalanges II-III of fourth toe	19	19
Head length	15.8–16.5 15.95 ± 0.38	14.8–15.62 15.29 ± 0.44
Head width	7.84–8.84 8.31 ± 0.41	8.05–8.66 8.26 ± 0.34
Head height	6.67–7.48 7.02 ± 0.39	6.54–7.27 6.8 ± 0.41
Jaw length	11.73–12.65 12.25 ± 0.38	11.86–14.36 12.70 ± 1.43
Snout length	6.75–7.04 6.87 ± 0.12	7.82–7.30 7 ± 0.26
Forelimb length	27.94–36.18 30.75 ± 3.72	21.84–28.19 25.68 ± 3.37
Hindlimb length	42.56–52.68 49.35 ± 4.59	47.50–49.56 48.57 ± 1.03
Axilla-groin length	27.35–28.17 27.76 ± 0.33	27.94–30.61 29.57 ± 1.43
Snout-vent length	58.80–60.31 59.45 ± 0.68	46.47–58.48 53.43 ± 6.22
Tail length	150.20–163.37 157.89 ± 5.89	146.21–155.38 150.53 ± 4.60
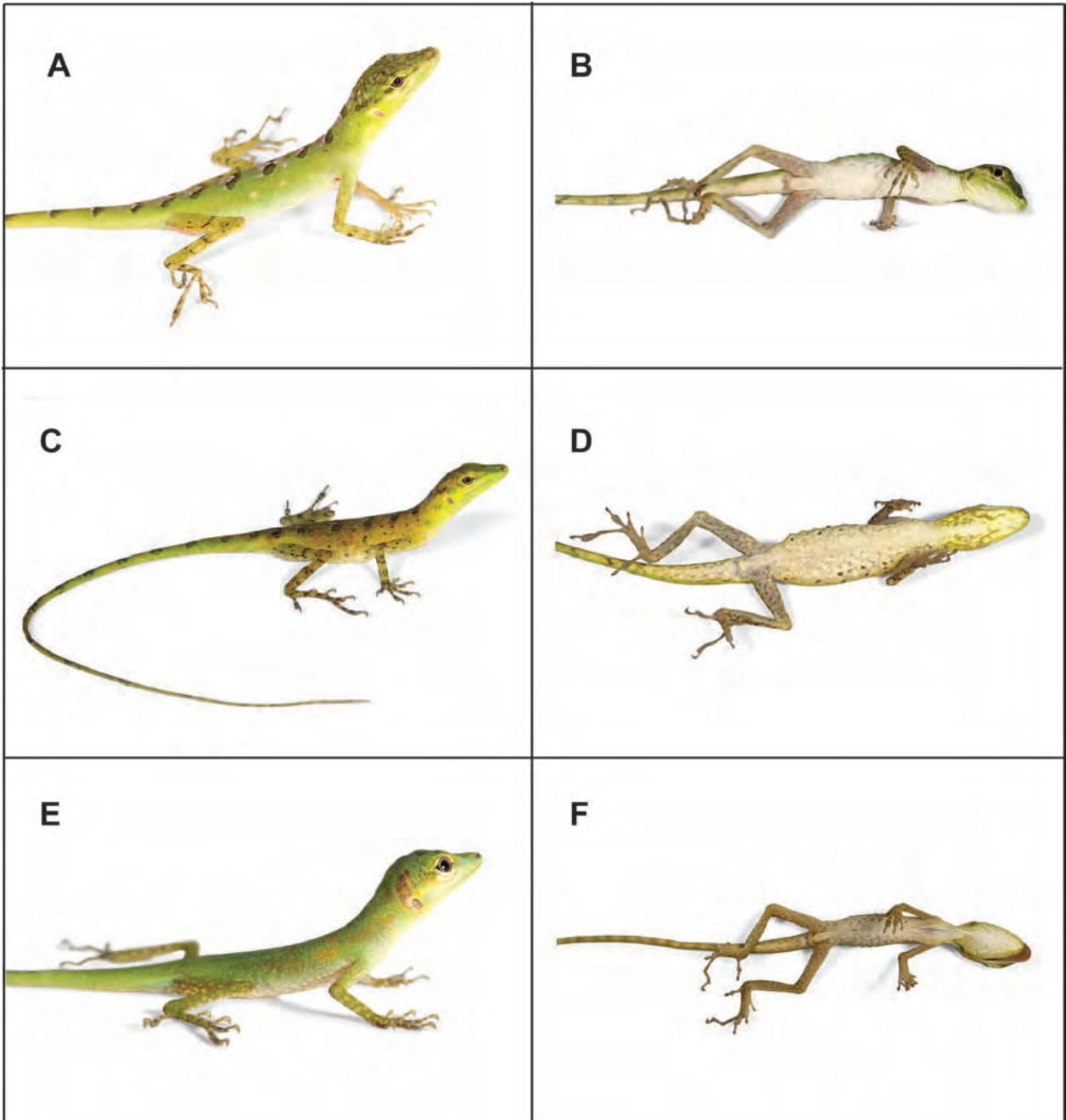
Fig. 7. *Anolis poei* sp. nov. Adult female (SVL = 46.47 mm, QCAZ 3454, **A**, **B**), subadult female (SVL = 47.99 mm, QCAZ 3446, **C**, **D**), juvenile male (SVL = 26.85 mm, QCAZ 3453, **E**, **F**). *Photographs by O. Torres-Carvajal.*

bands oriented ventro-posteriorly and intercalated with white spots; ventral surface of head white with light blue reticulations; ventral surface of body white with faint grayish purple reticulations; ventral surface of limbs grayish cream with dark brown reticulations; ventral surface of tail white anteriorly with a metallic green tint and grayish purple spots, and gray posteriorly; dewlap skin with a turquoise gorgetal region and white sternal region; gorgetal scales light brown with a gold tint internally, and dark brown externally; dewlap marginals and sternals white; throat, edge of mouth and tongue white.

Adult male (QCAZ 6783): dorsal surface of head and body dark brown with gray dots; dorsal surface of limbs dark brown, with gray dots on forelimbs; lateral surface of head dark brown with white cream dots dorsal and anterior to tympanum; body flanks dark brown with faint white dots arranged on diagonal lines that reach venter; ventral surface of head with bluish-purple infralabial and sublabial regions, and light purple gular region with white irregular spots; ventral surface of body white with faint purple reticulations; limbs creamish gray with dark brown reticulations; ventral surface of tail white with

purple mottling anteriorly, and gray posteriorly; dewlap skin with a light blue gorgetal region and white sternal region; gorgetal scales purple; sternal and marginal scales white.

Adult female (QCAZ 3454): dorsal surface of head brown with metallic blue and green frontal and supraocular regions; dorsal surface of body brown with six narrow black chevrons, each one delimited posteriorly by a white blotch; forelimbs bluish brown with white spots arranged in stripes; hindlimbs brown with dark brown bands and dots; tail with two black chevrons anteriorly; lateral surface of head brown with purple tint; labial region light purple; lateral aspect of neck and body purple with black dots; ventral surface of head white with purple brown stripes; ventral surface of body white with dark brown dots laterally; ventral surface of limbs grayish cream with dark brown reticulations on hindlimbs; ventral surface of tail white with dark brown dots.

Subadult female (QCAZ 3446, differences with QCAZ 3454): occipital and temporal regions with dark brown, small blotches; dorsal surface of neck with a distinct dark brown chevron; dorsal surface of body with six distinct, dark brown chevrons; dorsal surface of tail with two dark brown chevrons.

Phylogenetic relationships

The data matrix analyzed in this study contained 1,065 unique site patterns. Of the 2,807 nucleotide characters included in our analysis 1,703 were constant, 224 parsimony uninformative, and 880 were parsimony informative. The 50% majority rule consensus tree resulting from the Bayesian analysis (Fig. 8) is generally congruent with the phylogeny of the clade Dactyloa presented by Castañeda and de Queiroz (2011). Both the new species described here and *A. otongae* are members of the aequatorialis series of Castañeda and de Queiroz (2013), which corresponds roughly to the "western clade" of Castañeda and de Queiroz (2011). Our phylogeny supports strongly (PP = 0.99) a sister taxon relationship between *Anolis poei* and *A. gemmosus*, as well as the exclusivity (de Queiroz and Donoghue 1990; de Queiroz 1998) of both species. They form a clade sister (PP = 0.89) to *A. otongae*. The clade formed by the three species is sister (PP = 1) to a clade formed by *A. aequatorialis* and *A. anoriensis*.

Distribution and ecology

Anolis poei inhabits low montane evergreen forest (Sierra 1999) on the western slopes of the Andes in central Ecuador, Provincia Bolívar, between 1,310–1,354 m (Fig. 9).
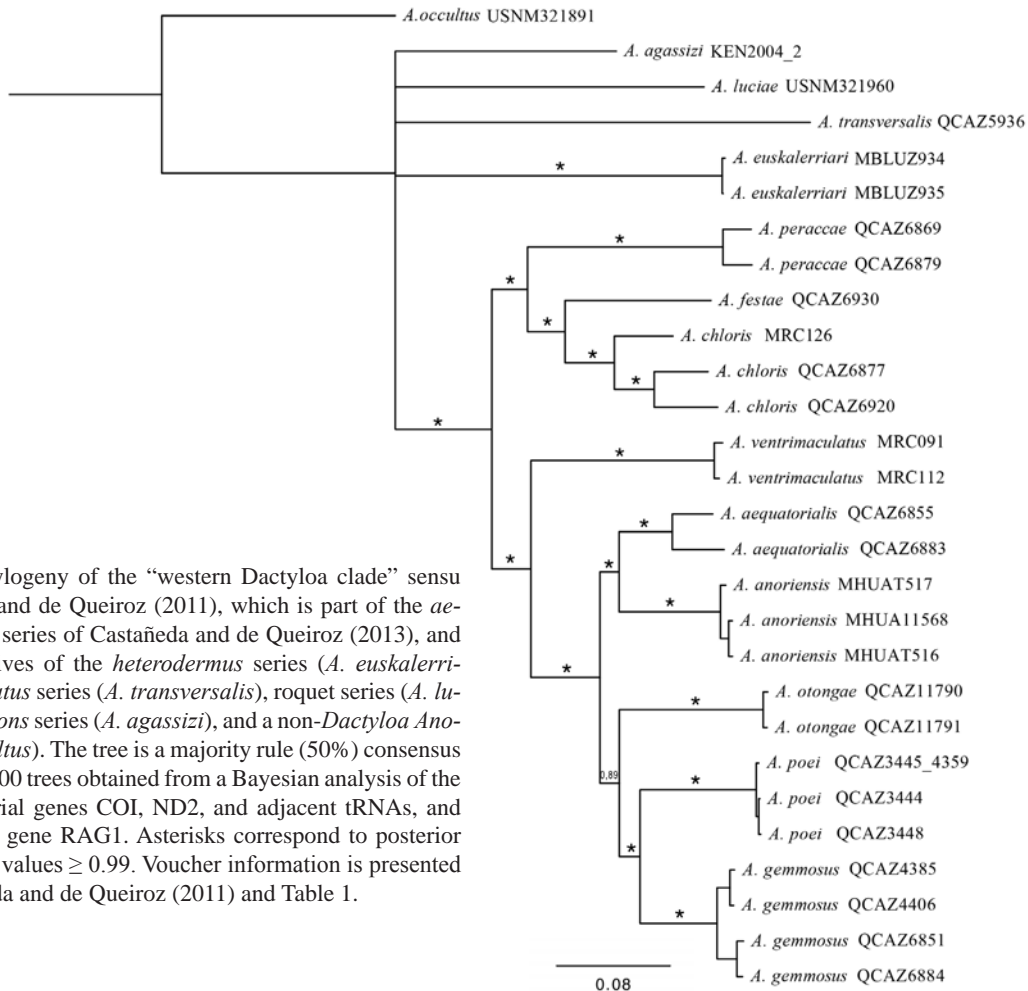
Fig. 8. Phylogeny of the "western Dactyloa clade" sensu Castañeda and de Queiroz (2011), which is part of the *aequatorialis* series of Castañeda and de Queiroz (2013), and representatives of the *heterodermus* series (*A. euskalerriari*), *punctatus* series (*A. transversalis*), roquet series (*A. luciae*), *latifrons* series (*A. agassizi*), and a non-*Dactyloa Anolis* (*A. occultus*). The tree is a majority rule (50%) consensus tree of 72,000 trees obtained from a Bayesian analysis of the mitochondrial genes COI, ND2, and adjacent tRNAs, and the nuclear gene RAG1. Asterisks correspond to posterior probability values ≥ 0.99. Voucher information is presented in Castañeda and de Queiroz (2011) and Table 1.

The new species occurs in sympatry with *A. aequatorialis*, *A. binotatus*, and *A. fasciatus* at its type locality (Fig. 4). Specimens of the new species were collected along the border of a road, close to rivers, in secondary forest, and on shrubs within pastures. All individuals were found between 20h00 and 22h00 sleeping with their heads up, or in a horizontal position on branches or vines, 0.5–4.5 m above ground or streams. The smallest individual QCAZ 3453 (SVL = 26.9 m; TL = 67.6 mm) was collected on 11 June 2011.
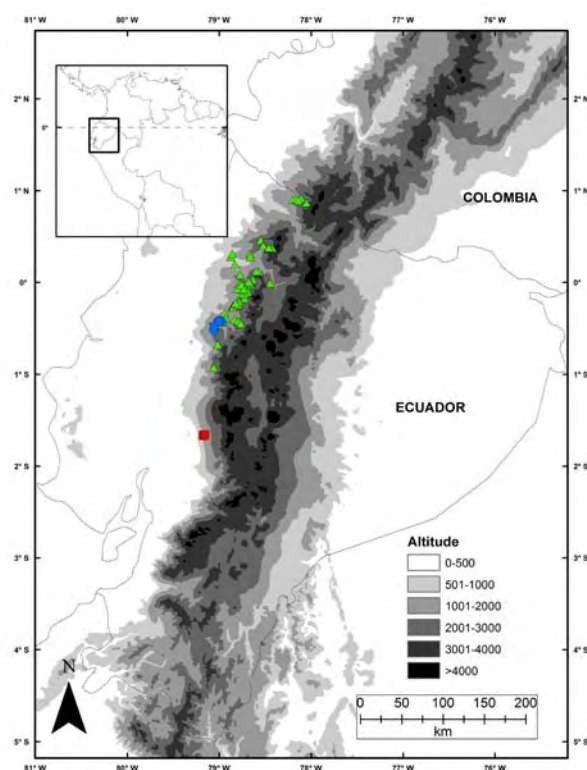
Etymology

The specific name is a noun in the genitive case and is a patronym for Steve Poe, who has published important contributions to the systematics and evolution of *Anolis* lizards (Poe 2004, 2011). During his collecting trips to Ecuador in 2009 and 2010, Poe trained several young herpetologists in field collecting techniques and inspired them to explore the diversity of anole lizards. This paper is one of the products resulting from that inspiration.

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Fig. 9. Distribution of *Anolis gemmosus* (triangles), *A. otongae* (circles) and *A. poei* sp. nov. (squares) in Ecuador.

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Appendix 1

Additional specimens examined

Anolis gemmosus – Ecuador: *Carchi*: Chilma Bajo, Finca de Aníbal Pozo, 0.86397°N, 78.04723°W, 2,022 m, QCAZ 8681-82; Chilma Bajo, Finca de Aníbal Pozo, 0.86495°N, 78.04979°W, 2,071 m, QCAZ 8683; La Centella, 0.89318°N, 78.13471°W, 1,800-2,400 m, QCAZ 11784; Maldonado, Sendero Ecológico Teldibi, 0.91301°N, 78.10782°W, 1,477-1,635 m, QCAZ 12272, 12278, QCAZ 12279-80, 4360, 4406, 4408; Río San Pablo, cerca a Chical, 0.90302°N, 78.16284°W, 1,399 m, QCAZ 4377, 4382, 4385-86,4388; Río San Pablo, cerca a Chical, 0.90327°N, 78.16201°W, 1,429 m, QCAZ 4393; Río Verde and Río Pablo, Río Estrellita, Guapil, 1,428-1,466 m, QCAZ 12289, 12294, 12302; *Cotopaxi*: 115 km Oeste de Pilaló, 0.928°S, 79.057°W, 1,500 m, QCAZ 4072; 18.2 km de Quillutuña, vía a Pucayacu, 0.67843°S, 79.01565°W, 1,420 m, QCAZ 8845-49; Alrededores de San Francisco de Las Pampas, 0.42371°S, 78.96765°W, 1,800 m, QCAZ 1440-47, 2123; Bosque Integral Otonga , 0.4194°S, 79.00345°W, 1,720-2,143 m, QCAZ 2758, 2809-10, 3121, 3126-27, 3131, 3133, 3174, 3180-90, 3863-3866, 3869-71, 3940, 3974-76, 4028-34, 4224-25, 4657, 4663, 4785, 5060, 5063, 5371, 5477-79, 5482-83, 6770-73, 9888, 10424, 10438-39, 10441-42, 10452, 12057, 12060-65, 12067, 12072-73, 12075, 12077-82, Bosque Integral Otonga, a lo largo del río Esmeraldas, 0.46333°S, 79.05027°W, QCAZ 7281-89; Bosque Integral Otonga, alrededores de la estación, 0.41933°S, 79.00336°W, 1,980 m, QCAZ 10697; Bosque Integral Otonga, arriba de la estación, 0.41478°S, 79.00073°W, QCAZ 3867-68; Bosque Integral Otonga, orillas del río Esmeraldas, 0.41932°S, 78.99396°W, 1,719 m, QCAZ 10393, 10395, 10399; Bosque Integral Otonga, sendero a la Estación, 0.41933°S, 79.00336°W, 1,646 m, QCAZ 10696; Cerca a Naranjito, 0.41944°S, 79.00333°W, QCAZ 7825; San Francisco de Las Pampas, 0.42371°S, 78.96765°W, 1,600-1,800 m, QCAZ 63, 68-70, 72-79, 3134-53, 3155, 3175; Vía a Otonga, 0.33183°S, 78.93791°W, 1,476-1,700 m, QCAZ 8412; *Imbabura*: 6 de Julio de Cuellaje, 0.4°N, 78.525°W, QCAZ 4346-47; 6 de Julio de Cuellaje, 0.40107°N, 78.5181°W, 1,886 m, QCAZ 4349; 6 de Julio de Cuellaje, 0.40102°N, 78.51779°W, 1,897 m, QCAZ 4350; 6 de Julio de Cuellaje, punto 8, 0.4°N, 78.525°W, QCAZ 4348; 6 de Julio de Cuellaje, San Antonio, Cordillera de Toisán, 0.45803°N, 78.54722°W, QCAZ 9450-53; Carretera nueva vía a Cuellaje, Sector de Santa Clara, Reserva Alto Choco, 0.37603°N, 78.45857°W, 2,062 m, QCAZ 4352-54; La Mina, Junín, 0.2754°N, 78.6603°W, 1,715 m, QCAZ 3071; Manduriaco, 0.277°N, 78.873°W, 1,330 m, QCAZ 5328; Manduriacu, 7.5 km NE of Bellavista, 0.31006°N, 78.85757°W, 1,177-1,227 m, QCAZ 11606, 12305-314; 12322, 12324, 12326, 12328, 12331; Reserva Siempre Verde, NE de Cotacachi, 0.37167°N, 78.42186°W, 2,468 m, QCAZ 8837; Reserva Alto Choco, Santa Rosa, 0.36939°N, 78.44942°W, 2,109 m, QCAZ 7330-31; *Pichincha*: 1-2 km oeste de Tandayapa, 0.004°S, 78.663°W, 2,000 m, QCAZ 2070-71; 2.9 km de Tandayapa, 0.00952°S, 78.65698°W, 1,820 m, QCAZ 406-10; 5 km E Tandayapa, 0.02°S, 78.651°W, 1,975 m, QCAZ 2066-69; A orillas del Río Chisinche, en la carretera a Conchacato, 0.448°S, 78.76423°W, 1,693 m, QCAZ 6884-89; 30 km E de Santo Domingo, hacia la Reserva de Bosque Integral Otonga, 0.3884°S, 78.92995°W, QCAZ 9769-70; 9775; Bosque Protector Mindo - Nambillo, refugio, 0.106°S, 78.687°W, 1,700 m, QCAZ 2910; Cooperativa El Porvenir, finca El Cedral, 0.114°N, 78.56993°W, 2,297 m, QCAZ 10501-502; Desviación a Mindo, 1-5 km de la intersección hacia abajo, 0.02853°S, 78.75861°W, 1,661 m, QCAZ 9724-31; Estación Científica Río Guajalito, 0.22676°S, 78.82171°W, 1,791-1,814 m, QCAZ 1330, 1333, 1500, 1645, 2682-84, 2786, 2813, 2815-16, 3040-45, 3056-57, 3373, 3385, 4123-25, 4210, 4214, 6413-14, 8859, 8864-65, 9974, 11404, 12088-101; Las Tolas, 0.72818°N, 78.77792°W, 1,200-1,600 m, QCAZ 11848-49; Manuel Cornejo Astorga (Tandapi), frente a la planta de agua potable "El Placer" vía a Conchacato, 0.42471°S, 78.78905°W, 1,500 m, QCAZ 6882; Manuel Cornejo Astorga (Tandapi), vía Atenas a 5 km de la carretera principal, 0.40625°S, 78.83621°W, 1,671 m, QCAZ 5365-70; Mindo, 1,342-1,560 m, QCAZ 12350-53, 12356, 12358, 12365, 12370, 12375-76; Mindo Biology Station, 0.07805°S, 78.73194°W, QCAZ 7518-20, 7522; Mindo, camino entre Mariposas de Mindo y Mindo Garden, 0.06753°S, 78.7535°W, 1,361 m, QCAZ 6851-53, 6858; Mindo Garden, 4 km de Mindo, 0.06901°S, 78.80166°W, QCAZ 2787; Mindo, El Monte, Road to Mindo Garden, 0.07805°S, 78.7319°W, QCAZ 7521; Mindo, Sachatamia Lodge, 0.02638°S, 78.75944°W, 1,700 m, QCAZ 11857-59; Nanegalito, Finca El Cedral, 0.1141°N, 78.57007°W, 2,272 m, QCAZ 9462-63; Pachijal, vía Nanegalito-Los Bancos, 0.13°S, 78.72644°W, 1,741 m, QCAZ 5494-500; Palmeras, 0.244°S, 78.794°W, 1,800 m, QCAZ 871, 881-83,1351-52, 2244, 3004-06; Recinto Chiriboga, Estación La Favorita , 0.21307°S, 78.78421°W, 1,680 m, QCAZ 5383-84; Reserva Ecológica Bosque Nublado "Santa Lucía," 0.11928°N, 78.59647°W, 1,624-1,927 m, QCAZ 10664, 11850-52, 11888-93,11897, 11899; Tandayapa, 0.00591°N, 78.67455°W, 1,670 m, QCAZ 4086. *Locality in error*: Pichincha, San Antonio de Pichincha, 0.00905°S, 78.44581°W, QCAZ 724.

Anolis otongae – Ecuador: *Cotopaxi*: Alrededores de San Francisco de Las Pampas, 0.42371°S, 78.96765°W, 1,800 m, QCAZ 2128; Bosque Integral Otonga , 0.41944°S, 79.00333°W, 1,900-2,300 m, QCAZ 1721, 2050-52, 3129, 3706, 3796, 3872-73, 4025, 4661, 5481, 6219, 11790-91, 12035, 12056, 12058, 12070-71; Los Libres, QCAZ 2781; Peñas Coloradas, 0.52343°S, 79.05908°W, QCAZ 1696; *Pichincha*: La Victoria, 0.47747°S, 79.05336°W, 2,104 m, QCAZ 6394-96.

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Reproduction and spawning behavior in the frog, *Engystomops pustulatus* (Shreve 1941)

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Abstract.—The study of reproductive strategies is central to understand the demography of populations and the energetic relationships of the species with their ecosystem. Documenting the reproductive natural history of the species is pressing in groups, like amphibians, that are threatened with extinction at a global scale. Herein, we describe the reproductive ecology and spawning behavior of the leptodactylid frog *Engystomops pustulatus*. In addition, we report observations that suggest the existence of an alternative mating strategy. Our results show that reproduction in *E. pustulatus* is characterized by high maternal investment (15% egg mass relative to body mass). We found evidence of size-assortative mating with a tendency of larger females to mate with larger males. Clutch size was correlated with female weight, female condition and male size. Larger females showed a tendency to lay larger foam nests and larger nests contained more eggs. At reproductive choruses, there was a male-biased operational sex ratio, indicative of high variance in male reproductive success. We observed an amplectant couple spawning while an additional male was embedded in the foam. We hypothesize that this behavior is evidence of an alternative mating strategy where a small non-amplectant male attempts to fertilize the eggs that are extruded by the amplectant female.

Resumen.—El estudio de las estrategias reproductivas es fundamental para entender la demografía de las poblaciones y las relaciones energéticas de las especies con su ecosistema. Documentar la historia natural reproductiva de las especies es apremiante en grupos, como los anfibios, que están amenazados con extinción a nivel mundial. Aquí, describimos la ecología reproductiva y el comportamiento de anidación en la rana leptodactílida *Engystomops pustulatus*. Además, reportamos observaciones que sugieren la existencia de una estrategia reproductiva alterna. Nuestros resultados indican que la reproducción en *E. pustulatus* está caracterizada por una alta inversión energética de la hembra (15% de masa de huevos en relación a la masa corporal). Se evidencia que el apareamiento es selectivo con respecto al tamaño, con una tendencia de hembras grandes a aparearse con machos grandes. El tamaño de la puesta estuvo correlacionado con el peso de la hembra, la condición de hembra y el tamaño del macho. Las hembras más grandes mostraron una tendencia de poner nidos de espuma más grandes y los nidos más grandes tuvieron un mayor número de huevos. En coros reproductivos, hubo una tasa sexual operativa sesgada hacia los machos, lo que indica una alta varianza en el éxito reproductivo de los machos. Se observó una pareja en amplexus construyento un nido mientras un macho adicional estaba incrustado en el nido de espuma. Hipotetizamos que este comportamiento evidencia una estrategia de apareamiento alterna en la que un macho pequeño intenta fertilizar huevos puestos por una hembra en amplexus con otro macho.

Key words. Alternative mating strategy, clutch size, clutch piracy, fertilization rates, nesting behavior, testis size

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Introduction

Understanding the natural history of reproduction is essential to characterize the ecological niche and the survival prospects of amphibians. Acquiring a better understanding of amphibian reproduction will assist conservation efforts in the vertebrate class with the higher number of species threatened with extinction (Chanson et al. 2008).

The deposition of eggs in foam nests characterizes the reproduction of most species of the Neotropical family Leptodactylidae, which has 201 species distributed from southern Texas to southern Chile (Frost 2014). In most species, males call in choruses that are visited by receptive females, which then actively choose a mate (Ryan 1985). Amplectant pairs build foam nests where hundreds of eggs are laid and fertilized (Crump 1974; Heyer 1969; Ryan 1985). The foam is formed when the male kicks the jelly surrounding the eggs while the female discharges them. The foam may protect the eggs from dehydration and/or predation (Duellman and Trueb 1994; Menin and Giaretta 2003) or from excessive heat (Gorzula 1977).

Foam nests may facilitate multiple paternity by retaining sperm (Kusano et al. 1991). There is a high proportion of foam-nesting species among known cases of multimale spawning in anurans, eight out of 15 (Byrne and Roberts 1999; Kaminsky 1997; Prado and Haddad 2003). Although several reproductive characteristics of Leptodactylidae should favor multiple male mating strategies, there are only two documented cases, *Leptodactylus chaquensis* and *L. podicipinus* (Prado and Haddad 2003). The paucity of records may be partly due to lack of studies. Although several leptodactylid species are abundant and live even in urban areas, little is known about its biology beyond brief accounts of its systematics and morphology. Such is the case of the widely distributed and abundant *Engystomops pustulatus* (Ron and Read 2012).

Herein, we describe the reproductive natural history of *Engystomops pustulatus* including fertilization rates, testis size, clutch size, and relative egg mass to explore factors that influence mate choice and reproductive output. We also describe its spawning behavior with observations suggest the existence of a secondary male mating strategy.

Materials and Methods

Study site and species

Engystomops pustulatus inhabits dry shrub, deciduous forest, and lowland moist forest below 300 m in western Ecuador. It can be relatively common during the rainy season, when they reproduce. They are explosive breeders that congregate around temporal pools. Males call from the water and amplectant pairs build foam nests to deposit their eggs (Ron and Read 2012). *Engystomops*

pustulatus should not be confused with *E. pustulosus*, a Central American species that has been a model for studies of behavioral ecology (e.g., Ryan 2005). For clarity, hereafter, we refer to *E. pustulosus* exclusively as "Túngara frog."

Operational sex ratio (e.g., the number of males relative to the number of females in breeding aggregations) in *Engystomops pustulatus* was assessed in western Ecuador at three localities: Reserva Cerro Blanco, (W 80.0214°, S 2.0264°, Provincia del Guayas; 19 March 2003), Patricia Pilar (Provincia Los Ríos; 21 February 2002), and the town of La Maná (Provincia de Cotopaxi; 28 December 2003). Reproductive output, nest size and size assortment were evaluated in La Maná (W 79.265°, S 0.943°, elevation 160 m) between 28 December 2003 and 08 February 2004 and Patricia Pilar (W 79.3707°, S 0.5372°, elevation 200 m) between 23 January and 20 April 2008 during the rainy season. At La Maná and Patricia Pilar, the vegetation is Evergreen Lowland Moist Forest (as defined by Sierra et al. 1999). Most of the forest in the region has been converted to pastures and agricultural lands. Field observations took place after dusk, between 19:00 and 3:00 h. Breeding occurred in small temporary ponds on the streets of the town. Some sites were under dim artificial light (street poles).

Fertilization rates and nest size

We estimated fertilization rates from amplectant pairs collected from the field. The amplectant pairs were placed in individual circular plastic containers (10 cm diameter) with water depth of one cm. Most pairs made a nest after few hours. Three or four days later, we washed the foam with a solution of chlorine and water and counted the number of hatched and undeveloped eggs (as described by Ryan 1983). We used this proportion as a proxy for fertilization rates. This methodology does not allow discriminating between undeveloped eggs as result of egg unviability or failed fertilization. Therefore, our methodology may slightly underestimate fertilization rates.

To estimate nest size, we measured (with digital calipers, to the nearest 0.01 mm) the length of the longest axis, width at the widest point perpendicular to the longest axis, and height of all nests laid in the containers. We estimated nest volume with the formula of ½ ellipsoid:

$$V = \frac{\pi}{12} abc$$

where a, b, and c are the length, width, and height, respectively. The measurements were taken while the nests were <1 day old.

Adult size and egg mass

Sex was determined by the presence of nuptial pads, vocal sac folds, and/or by gonad inspection. Snout-vent length (SVL) was measured with Fowler digital calipers (nearest 0.01 mm). Body mass was measured in the field

(before and after oviposition in females) with a digital balance (nearest 0.1 g). Relative egg mass (maternal investment) was calculated as 1—the ratio (female mass after oviposition/female mass before oviposition).

After being kept in the plastic containers to allow spawning, females were euthanized by immersion in chloretone, fixed in 10% formalin, and preserved in 70% ethanol. Egg mass and body mass were measured after preservation in females that did not spawn. Each female was weighted on a digital balance (to the nearest 0.001 g), after removing excess ethanol. Then, the remaining egg masses (including immature eggs and jelly) were removed from the abdomen and weighted. Relative egg mass was calculated by dividing total egg mass by non-gravid female mass. Estimates of relative egg mass could be influenced by preservation in ethanol. Therefore, comparisons with relative egg mass in non-preserved nesting females should be interpreted with caution. All preserved specimens are deposited at the amphibian collection of the Zoology Museum of Pontificia Universidad Católica del Ecuador.

Reproductive behavior

Behavioral observations were carried out at male choruses in La Maná, Ecuador. Spawning behavior was described from of a single nesting event at La Maná. Spawning was recorded in the field under infrared light with a digital camcorder SONY TRV70. The complete video is available at AmphibiaWeb (http://amphibiaweb. org).

Statistical analyses

For normally distributed variables, we tested the significance of relationship between them using linear regression ANOVAs; for non-normal variables, we tested relationship with Spearman's rank correlations. Differences between groups were tested with *t*-tests (assuming non-equal variances). Statistical tests were implemented in software JMP v.5.1 (SAS Institute, 2003).

Results

Reproductive output, fertilization rates, and nest size

Among 77 nests, the mean number of eggs was 320 (SD = 142.6, range 0–747). The average percentage of unfertilized eggs was 1.89% (SD = 3.3, 0–19.1, $n = 46$); Fig. 1A); ~1/5 of the nests had a fertilization rate of 100%. Snout-vent length difference between both parents was not correlated with the number of unfertilized eggs (Spearman's Rho = 0.098, $P = 0.524$) or the proportion of unfertilized eggs (Rho = 0.145, $P = 0.341$).

Mean nest volume was 37.0 cm3 (SD = 14.4, range 2.8–85.6, $n = 74$). Nest volume is correlated to the number of eggs (larger nests have more eggs; Table 1, Fig. 2A) and female size (larger females lay larger
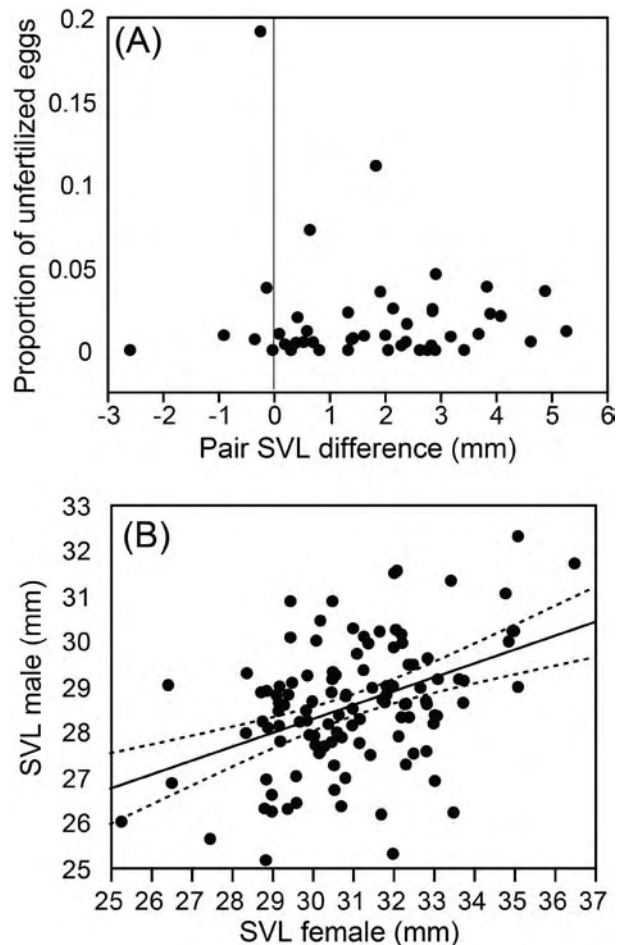
Fig. 1. Size and fecundity rates for amplectant pairs of *Engystomops pustulatus*. After collected in amplexus in the field, pairs were left in plastic containers where they could spawn. (A) Proportion of unfertilized eggs among pairs that successfully built a nest, (B) Female vs. male snout-vent length (SVL) with linear regression and 95% confidence intervals (dashed lines).

nests; Table 1, Fig. 2B). A multiple regression of number of eggs, female SVL, and male SVL explains 25% of the variation in nest volume ($F = 7.51$, df = 66, $P < 0.001$). However, only number of eggs is significant for the regression model ($F = 18.18$, $P < 0.001$).

Number of eggs was significantly correlated with male SVL (Fig. 2C) but not with female SVL. Number of eggs was correlated with female mass before and after oviposition and female condition (Table 1).

Non-spawning females had large masses of eggs in their abdomens (mean relative egg mass = 0.354, SD = 0.138, range 0.129–0.621, $n = 13$). Average maternal investment for spawning females was 15.2% of body weight (SD = 7.77, 1.8–39.4, $n = 42$).

Size assortment and spawning

We found size-assortative mating as male and female size of amplectant pairs was correlated (ANOVA's $F = 24.1$, $P < 0.001$, $R^2 = 0.176$; Fig. 1B). Overall, females were significantly larger than their mates ($n = 115$; mean female SVL = 31.0 mm, SD = 1.9, range 25.3–36.5; male
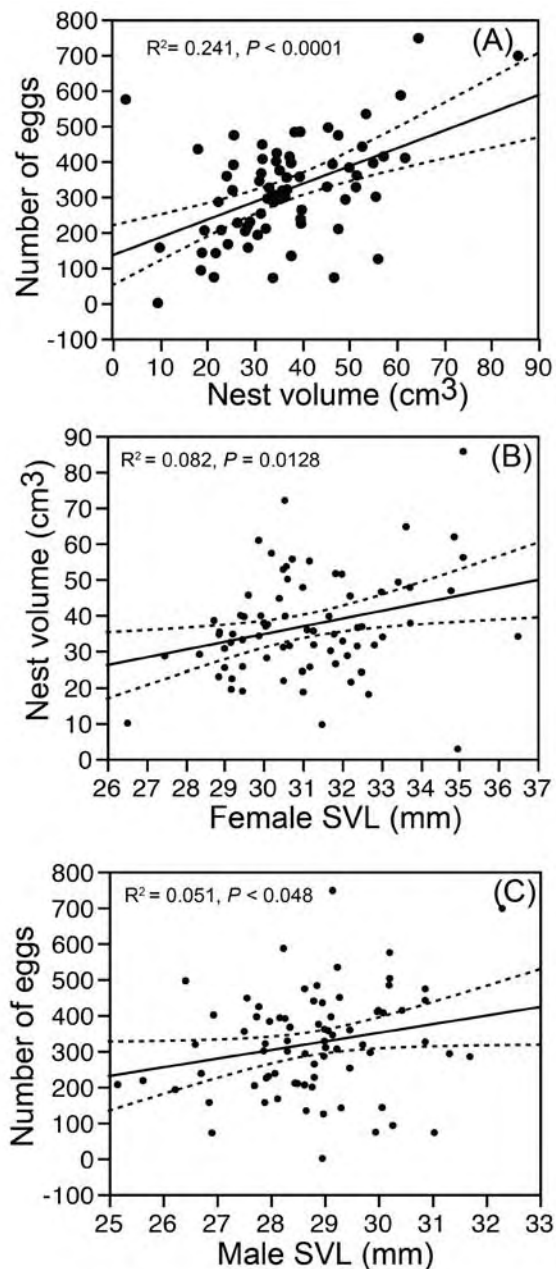
Fig. 2. Bivariate plots for (A) nest volume vs. number of eggs, (B) females size vs. nest volume, and (C) male size vs. number of eggs in *Engystomops pustulatus*. Linear regressions with 95% confidence intervals (dashed lines), determination coefficients (R^2), and ANOVA's *P* values are shown.

SVL = 28.5, SD = 1.3, 25.2–32.3; paired-*t* = 14.7, df = 104, *P* < 0.001). However, in 10 pairs (8.6%) the male was larger. Mean SVL difference between amplectant male and female was 2.5 mm (SD = 1.74, range -2.6–6.7, *n* = 105).

Reproductive behavior

Males began calling immediately after dusk. They called while floating in temporary ponds with water <10 cm deep. Male density at some choruses was high, resulting in some males calling a few centimeters away from

each other. Males defended calling sites and aggressive interactions ensued if another male approached within a radius of <10 cm. Aggressive behavior consisted of mew-like vocalizations and attempts to clasp the rival male. Amplexus and egg deposition occurred at the same ponds where choruses were calling. Amplexus is axillary.

Operational sex ratio at choruses was male-biased. During a survey at La Maná, we recorded 14 males but only one female; at Cerro Blanco, the ratio was 3:1 (*n* = 16 individuals); at Patricia Pilar, the ratio was 8.5:1 (*n* = 19). The average ratio is 8.5:1 (*n* = 3 surveys).

Spawning behavior.—Nests were built while in amplexus, on shallow water, next to vegetation or muddy banks. The following description is based on an amplectant pair found on 04 February 2004 at ~1:00 AM (male QCAZ 26672, SVL = 26.2 mm, hereafter referred as α-male; female QCAZ 26671, SVL = 31.7 mm) building a foam nest. At the beginning of the observation, the nest already had a diameter >50 mm. The male remained in amplexus until the couple left the nest (50 minutes later). To form the foam, the male kicked the egg mass while they were being extruded from the female's vent. Kicking occurred in regular bursts with intervening periods during which the couple was immobile. In a typical burst cycle, the male's legs move downward, presumably to place his feet next to the female vent. Then, the male's feet move up until they reach the posterior end of his dorsum. At that moment, usually one or two eggs become visible in the jelly matrix between the feet. This is followed by a series of ~20 rapid kicks on which his legs become partly extended backward and then distended forward until reaching the posterior end of his dorsum. During these kicks, his legs move simultaneously but in opposite directions (forward-backward) and feet momentarily touch medially. The burst ends with to 2–4 forceful kicks on which his legs are nearly completely extended posterolaterally, partly removing the foam that lies immediately behind the couple. Each male burst seems to be triggered by an abdominal movement of the female.

Each burst of kicking lasted on average 4.64 s (SD = 0.53, range 2.13–6.22, *n* = 215); the intervening immobile periods lasted 9.25 s (SD = 12.15, range 0.12–119, *n* = 215). Total duration of bursts was 16'30" during 50' of observation. The duration of each burst and the number of bursts decreased during the second half of the sequence (Fig. 3).

Multimale nesting behavior.—Multimale spawning was only observed once, during the spawning event described in the previous section (male QCAZ 26672, female QCAZ 26671). From the beginning of the observation, a peripheral adult male (QCAZ 26673; hereafter referred to as β-male) was sitting on the nest edge, directly opposite to the nesting couple and with the posterior ½ of its body embedded in the foam (Fig. 4). On at least five occasions its body moved slightly from side to side in sequences that lasted 3–4 s (Fig. 3). The movements were always in concert with the kicking bursts of the α-male.

Table 1. Pearson's correlation coefficients and ANOVA's *P* values for linear regressions. Body condition is defined as the residuals between SVL and mass. SVL = snout-vent length.

Variable 1	Variable 2	R²	*n*	*P*
Nest volume	Female size (SVL)	0.082	74	0.013*
Nest volume	Female mass (before oviposition)	0.020	56	0.287
Nest volume	Male size (SVL)	0.026	74	0.168
Nest volume	Male condition	<0.001	74	0.796
Nest volume	Number of eggs	0.241	70	<0.001*
Number of eggs	Male size (SVL)	0.051	76	0.049*
Number of eggs	Female size (SVL)	0.027	76	0.151
Number of eggs	Female mass (before oviposition)	0.111	62	0.008*
Number of eggs	Female mass (after oviposition)	0.128	58	0.006*
Number of eggs	Female condition	0.125	62	0.005*
Number of eggs	Male condition	0.011	76	0.352

*Significant at *P* <0.05
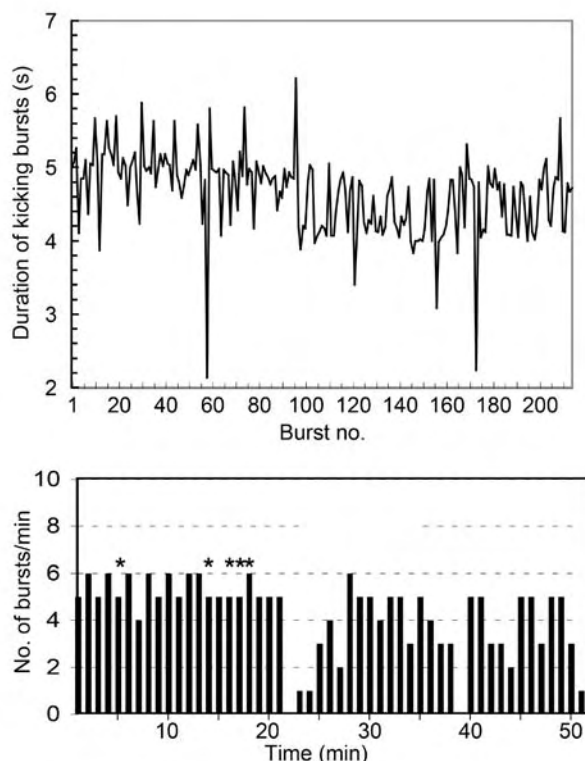
Fig. 3. Spawning of *Engystomops pustulatus* nesting couple (QCAZ 26671–72) and β-male (QCAZ 26673). Above: duration of kicking bursts. Below: number of bursts per minute; asterisks indicate β-male movements in the foam. Measurements are shown in sequence from the beginning of the observation until the couple left the nest. See text for details.

Most likely, the movements were generated by kicking bursts of the β-male legs (hidden below the foam). He left 23 min later, apparently following an amplectant couple (not collected) that approached at a distance of 10 cm from the nest (see below).

The β-male (SVL = 25.3 mm) was one of the smallest in the population. Out of 49 calling males measured during the same season, only three were smaller (mean SVL = 27.55 mm, SD = 1.23); out of 59 males found in amplexus, only one was smaller (mean SVL = 27.9 mm, SD = 1.20). Assuming a normal SVL distribution, the probability of drawing a male with equal or lower SVL by chance is 0.020 (*z*-score = –2.058). On a sample of seven males including the β-male, mean testes mass was 0.47% of total body mass (range 0.24–0.70%; mean body mass = 1.59 g, SD = 0.28). Contrary to our expectations, the β-male had the proportionally smallest testes.

Discussion

Clutch size, fertilization success, and parental investment

Number of eggs/clutch in *Engystomops pustulatus* is ~37% higher than in the túngara frog (Ryan 1985). In several anurans, clutch size is significantly correlated to body size (e.g., Crump 1974; Ryan 1985; Wells 2007). In *E. pustulatus*, such a relationship was significant for female condition and gravid and non-gravid female mass. However, the relationship was not significant for female SVL. Interestingly, we also found a significant correlation between number of eggs and male SVL suggesting that larger males have a higher reproductive success. This correlation could not be explained by indirect correlations with the other measured variables because they were either uncorrelated with male SVL (e.g., nest volume) or uncorrelated with number of eggs (e.g., female SVL).

Female SVL and number of eggs are correlated with nest volume. Nest foam also results from intense male physical activity. However, we were unable to find a relationship between nest volume and either male size or male body condition (Table 1).

We found size assortative mating as large females have a tendency to mate with large males. An adaptive explanation for size assortative mating states that it increases fertilization rates because it results in female and male vents being closer during amplexus (Licht 1976). Evidence for this scenario has been reported for the Túngara frog (Ryan 1985). *Engystomops pustulatus* lacks
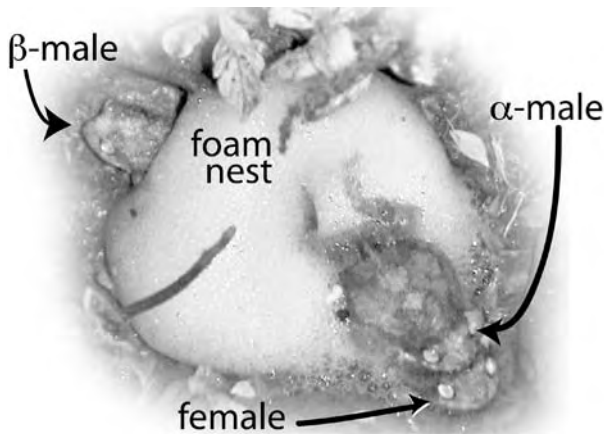
Fig. 4. *Engystomops pustulatus* nesting couple (QCAZ 26671–72) and β-male (QCAZ 26673). The couple builds the foam nest as the male kicks the egg masses extruded by the female. Frame from video (infra-red recording). See text for details.
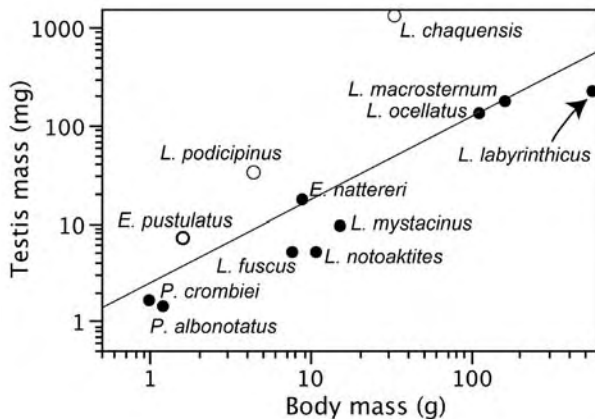
Fig. 5. Relationship (in log space) for body and testis mass among 11 species of Leptodactylinae frogs. Except for *Engystomops pustulatus*, data is from Prado and Haddad (2003). Open circles indicate species on which multimale spawning has been reported. Note that *E. pustulatus*, in which multi-male spawning apparently occurs, also has larger testis than other Leptodactylinae.

that relationship as demonstrated by couples with large differences in size (2–4 mm) showing high fertilization rates (Fig. 1A). The lack of influence of size difference on fertilization may be explained by our observation of spawning behavior because the male uses his feet to drag the eggs from the female's underside to his own vent. Therefore, the relative position of male and female vents may have a minor influence in the relative position of eggs and released sperm. Fertilization rates are generally high (more than 98% on average) suggesting that size differences between male and female have little influence in individual fitness. Similar results have been reported in other explosive breeding anurans like *Lithobates sylvaticus* (Howard and Kluge 1985) and Anaxyrus cognatus (Krupa 1988).

Size assortative mating could also result from non-adaptive interactions. If small males mating with large females are more easily displaced than large males mat-

ing with large females, a size correlation will result (e.g., Howard and Kluge 1985). This mechanism seems unlikely in *Engystomops*. During our fieldwork with *E. pustulatus* and with other species of *Engystomops* in the Chocó and the Amazon region, we never saw unmated males attempting to displace amplectant males. Attempts were rare in *E. pustulosus* and all of them were unsuccessful (Ryan 1985). Therefore, an explanation for size assortative mating in *E. pustulatus* and its sister species, *E. puyango* (reported by Ron et al. 2010) is pending.

Reproductive investment (or effort) is a measure of the allocation of energy in reproduction relative to total energy (Pianka 2011). Theory predicts that a high reproductive investment should be more adaptive if females are unlikely to survive to another reproduction event (Williams 1966). Our estimate of mean reproductive investment for *Engystomops pustulatus* (15.2%; egg mass relative to body mass) is relatively high in comparison to other anurans. For example, Crump (1974) and Prado and Haddad (2005) report investments ranging from 3.1 to 18.2% for 34 Neotropical species (including nine leptodactylids). The investment of *E. pustulatus*, however, is not the highest recorded for an anuran. For example, the myobatrachid *Crinia signifera* invests 25.9% of the gravid female mass in each spawning event (Lemckert and Shine 1993). This high investment was interpreted as resulting from a low probability of survival to additional spawning events (Lemckert and Shine 1993). Similarly, we hypothesize that the observed large investment in *E. pustulatus* could result from low survival rates.

Nesting behavior

Overall, nest building behavior was similar to that reported for the Túngara frog (Dalgetty and Kennedy 2010; Heyer and Rand 1977) and *Physalaemus ephippifer* (Hödl 1990). The kicking bursts observed in *E. pustulatus* are comparable to the "rotational movements" described in *P. ephippifer* except that the legs seem to extend further backwards in *E. pustulatus* (compared to figure 5 in Hödl 1990).

Nest building is an energetically costly task (Ryan 1985) and the observed decrease in the frequency of kicking bursts towards the end of spawning (Fig. 3) was also reported in the túngara frog (Ryan 1985) and *Physalaemus ephippifer* (Hödl 1990). As in *Leptodactylus labyrinthicus*, the Túngara frog, and *P. ephippifer*, kicking bursts seemed to be triggered by a female abdominal movement (Heyer and Rand 1977; Hödl 1990; Silva et al. 2005). The movie quality did not allow us to determine whether the decrease in burst frequency was male or female-driven in *E. pustulatus*.

Multimale mating behavior

Our observation of more than one male spawning with a female during oviposition suggests that multiple paternity and alternative reproductive strategies may exist in

Engystomops pustulatus. Although the β-male was not in amplexus, its movements were similar and in synchrony with those of the amplectant male, suggesting that it was attempting to fertilize eggs (clutch piracy). A similar reproductive behavior (with synchronic leg movements) has been reported in *Leptodactylus chaquensis* although with up to seven males in addition to the amplectant male (Prado and Haddad 2003). Egg fertilization by peripheral non-amplectant males has also been demonstrated in *Chiromantis xerampelina*, a foam-nesting rhacophorid (Jennions and Passmore 1993).

The evolution of multimale spawning should be facilitated in reproductive systems where: (1) the operational sex ratio is strongly male biased, (2) fertilization is external, (3) fecundity is high, and (4) eggs are spatially aggregated (Byrne and Roberts 2004; Shuster and Wade 2003). All this characteristics are part of the reproduction of *E. pustulatus*. Therefore, the occurrence of multimale spawning was probable. As previously reported in the Túngara frog (Ryan 1983), our data suggests that an individual male is frequently unable to fertilize all the eggs of a clutch, even in the absence of sperm competition. Although the presence of unfertilized eggs suggests the potential for fitness gain of a β-male sneaking into the nest of an amplectant pair, the proportion of unfertilized eggs was typically low (1.89% on average). Higher fitness gains for the β-male may result from sperm competition.

We could not determine the frequency of multimale spawning in the population. We observed monoandrous spawning frequently and multimale spawning was only recorded once, suggesting that it is relatively infrequent. This is consistent with observations across a variety of taxa showing that β-male strategies exist at a low frequency in natural populations (Shuster and Wade 2003; but see Byrne 2002; Jennions and Passmore 1993). The low number of reports of multimale spawning among leptodactylids is surprising because the characteristics of the reproductive system of Leptodactylidae should favor the evolution of secondary male mating strategies. The paucity of known cases may be, at least partly, a sampling artifact because the reproductive behavior has been described in only few species.

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High prevalence of *Batrachochytrium dendrobatidis* in an Andean frog community (Reserva Las Gralarias, Ecuador)

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Abstract.—We report patterns of infection of *Batrachochytrium dendrobatidis* (*Bd*) in a cloud forest amphibian community in the Andean Western Cordillera of Ecuador (Reserva Las Gralarias). Data were obtained during the rainy seasons of two consecutive years, using qPCR (year 2012) and end-point PCR (year 2013). We show that average *Bd* prevalence in this amphibian community is high (2012: 35–49%; 2013: 14–32%), but found no evidence of population declines or that *Bd* is negatively affecting host populations. We found a significant correlation between *Bd* prevalence and taxonomy, reproductive mode, and habitat, but no correlation between *Bd* infection intensity and the same three variables. Contrary to our expectations, frog species with aquatic reproductive modes (glassfrogs, treefrogs) showed lower *Bd* prevalence than direct-developing frogs (*Pristimantis* spp.). Although further monitoring is needed to determine long-term population trends, our two-year dataset on disease and population size support the hypothesis that frogs are tolerant to infection, a condition that could potentially have resulted from exposure to previous *Bd* epidemic outbreaks.

Resumen.—En este estudio reportamos datos sobre los patrones de infección de *Batrachochytrium dendrobatidis* (*Bd*) en una comunidad de anfibios en la Cordillera Occidental de los Andes del Ecuador (Reserva Las Gralarias). Los datos fueron obtenidos durante la estación lluviosa en dos años consecutivos, utilizando qPCR (año 2012) y PCR de punto final (año 2013). Los resultados muestran una alta prevalencia de *Bd* en la comunidad (2012: 35–49%; 2013: 14–32%); sin embargo, no se encontró evidencia de disminuciones poblacionales o de que *Bd* esté afectando negativamente a las especies de anfibios. Existe una relación significativa entre la prevalencia de *Bd* y la taxonomía, modo reproductivo y hábitat de los anfibios, pero no hubo correlación entre la intensidad de infección de *Bd* y las mismas tres variables. Contrario a nuestras predicciones, las especies de anuros con larvas acuáticas (ranas de cristal, ranas arbóreas) presentaron prevalencias de *Bd* más bajas que los anuros de desarrollo directo (*Pristimantis* spp.). A pesar de que se requiere de un monitoreo continuo para determinar las dinámicas poblacionales a largo plazo, los datos obtenidos hasta el momento apoyan un escenario donde las especies de ranas de la Reserva Las Gralarias parecen tolerar la infección de *Bd*, una condición posiblemente adquirida mediante la exposición a brotes epidémicos previos.

Key words. Chytridiomycosis, emerging disease, amphibian declines, Andes, conservation

Palabras claves. Quitridiomicosis, enfermedad emergente, declinación de anfibios, Andes, conservación

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Introduction

A third of global amphibian species are threatened with extinction (Stuart et al. 2004; Wake and Vredenburg 2008) and, most concerning, numerous local population declines and extinctions have occurred in relatively pristine areas, where anthropogenic habitat destruction is low (Lips 1998, 1999; Drost and Fellers 1996; La Marca et al. 2005). In the last two decades, several studies have attributed anuran mass mortality events to the emergence of the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*), a pathogen with widespread geographic and ecological distribution (Berger et al. 1998; Daszak et al. 1999, 2003; Lips et al. 2006; Becker and Zamudio 2011; Rodriguez et al. 2014). Alternative explanations to amphibian declines add a role to global warming and temperature variability (Pounds et al. 2006; Rohr and Raffel 2010; Menéndez-Guerrero and Graham 2013).

Batrachochytrium dendrobatidis infects the keratinized skin of amphibians and disrupts the regulatory functioning of the integument (Berger et al. 1998; Voyles et al. 2009). Infection inhibits host immune responses in some species (Fites et al. 2013) and in severe cases of infection, electrolyte depletion and osmotic imbalance may lead to mortality (Voyles et al. 2007, 2009). However, not all amphibian species are equally susceptible to the pathogen. For example, at Santa Fé, Panama, *Bd* has caused declines or local extinctions of most anurans in the original community, but six species of frogs and toads remain abundant, despite being infected by the fungus (Lips et al. 2006). In laboratory challenge experiments, amphibian mortality rates range from 0% to 100%, depending on the species, host age, pathogen genotype, and dosage (Berger et al. 2005a; Daszak et al. 2004; Longo et al. 2014). The reasons for host differences in susceptibility include immunogenic variation (Ellison et al. 2014; Savage et al. 2014), microhabitat use (Kriger and Hero 2007; Gründler et al. 2012), association with water as embryos, tadpoles, or adults (Lips et al. 2003), and host thermoregulatory behavior (Richards-Zawacki 2010). Because *Bd* transmission may happen through frog-frog contact, or through motile zoospore movement from one host to another, frogs and toads that spend more time in water are expected to have higher exposure and susceptibility to infection than species that are primarily terrestrial (i.e, direct developers; Lips et al. 2003; Kriger and Hero 2007).

In this study, we report data on infection patterns of *Bd* obtained during the rainy seasons of two consecutive years in the amphibian community of Reserva Las Gralarias, a cloud forest site in the Andean Western Cordillera of Ecuador. We found that *Bd* prevalence in all amphibian species is high, but found no evidence that *Bd* is negatively affecting amphibians (i.e., no apparent population declines, or records of clinical signs of chytridiomycosis). We report on infection prevalence and intensity dynamics for the two-year period, and examine patterns of *Bd* infection in species that vary in their taxonomy, reproductive mode, and habitat.

Materials and Methods

Study site: The study was conducted at Reserva Las Gralarias (0º01' S, 78º44' W; 1822–2400 m), a private reserve covering an area of 1,063 acres (425 ha) located on the Pacific slopes of the Andes, Pichincha Province, Mindo Parish, Ecuador. The study site has an elevational range of 1,825–2,400 m and includes primary and secondary forest, regenerating pasture, and numerous ephemeral and permanent streams and creeks (Hutter and Guayasamin 2012).

Amphibian taxonomy: For generic and suprageneric classification, we follow the taxonomic proposals of Hedges et al. (2008), Guayasamin et al. (2009), and Faivovich et al. (2005), Pyron and Wiens (2011), as summarized in Frost (2014).

Amphibian richness and abundance: During the rainy seasons of 2012 (23 January–29 March) and 2013 (14 March–22 April), we sampled trails of Reserva Las Gralarias during the night, including most of its habitat heterogeneity and elevational gradient, to record the species richness of the reserve. We placed eight transects, each with an area of 500 × 4 m (Appendix 1), to maximize species detection and to obtain a baseline dataset on population size and *Bd* prevalence. Each transect was sampled by two people for 3–4 hours during the night (generally starting at 8 pm); temperatures during sampling varied between 11–15 ºC. All detected amphibians were, when possible, photographed. Calling males were also reported and identified with the aid of photographic and acoustic guides (Arteaga et al. 2013; Centro Jambatu 2011–2014). We used a Student's *t*-test to quantify differences in population sizes in transects that were sampled multiple times during the rainy season of 2012 and 2013 (Lucy's Creek and Kathy's Creek); the normality of species abundance was assessed using a Shapiro-Wilk Test.

Diagnosis of Batrachochytrium dendrobatidis: We swabbed the ventral regions of all amphibians captured in our survey, following the standard procedures in Hyatt et al. (2007; Fig. 1); dry swabs were stored in -4 ºC until analysis. Testing for *Bd* was carried out using Real-Time PCR (q-PCR) for samples obtained during 2012 and end-point Polymerase Chain Reaction (PCR) for samples obtained in 2013; the use of these two methods was contingent on access to q-PCR (available during 2012). In both cases, DNA extractions were carried out using guanidinium thiocyanate. For samples obtained during 2012, we used a 1:10 dilution of the extract as template in Taqman q-PCR assays for the detection of *Bd* (Boyle et al. 2004). This assay uses *Bd*-specific primers ITS1-3 Chytr and 5.8S Chytr, in addition to the fluorescently-labeled probe Chytr MGB2, and amplifies the ITS-1 fragment of the
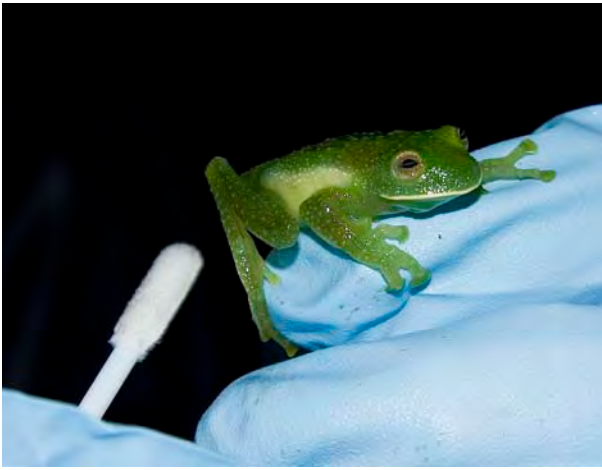
Fig. 1. Swab sample obtained from *Centrolene heloderma* at Reserva Las Gralarias, Ecuador.

Bd genome at the junction of the ITS-1 and 5.8S regions. We used a standard curve that included 1000, 100, 10, 1, and 0.1 zoospore genome equivalents, and followed qPCR conditions described in Boyle et al. (2004). For samples obtained during 2013, *Bd* presence was tested using the internal transcribed spacer regions (ITS-1, ITS-2) primers Bd1a (5'-CAGTGTGCCATATGTCACG-3') and Bd2a (5'-CATGGTTCATATCTGTCCAG-3') developed by Annis et al. (2004); the presence/absence of *Bd* was determined via the visualization of the amplified band in agarose gel electrophoresis. The two methods to detect *Bd* have different sensitivities; therefore, direct comparisons of *Bd* prevalence between years should be considered with caution. However, family and habitat correlates with infection status should not be biased by detection method, and qPCR offers the additional advantage of quantifying infection intensity (load).

Prevalence and correlates of *Batrachochytrium dendrobatidis* in amphibians: We estimated prevalence of

Bd within each anuran species as the number of frogs that tested positive for *Bd*, divided by the total number of sampled frogs for that particular species in a given year. We estimated the 95% confidence interval for prevalence in each species, (Wilson 1927; Newcomb 1998). We modeled *Bd* presence or absence in each individual by using a logistic regression. We tested for possible associations of *Bd* prevalence with the following variables: habitat (terrestrial, riparian, lentic), reproductive mode (aquatic, terrestrial), and taxonomy (family). Statistical significance of results was assessed with a chi-square test.

Infection intensity of *Batrachochytrium dendrobatidis* and correlates in amphibians: We tested for possible associations of *Bd* intensity (measured as zoospore genomic equivalents) with the following variables: habitat (terrestrial, riparian, lentic), reproductive mode (aquatic, terrestrial), and taxonomy (family, genus, species; Appendix 2). Given the strong right skew of infection load, we used the non-parametric Kruskal-Wallis test. All statistical analyses were performed using R v. 2.15.3 (R CoreTeam 2012).

Results

Species richness and abundance: During the two sampling periods, we recorded a total of 2,450 individuals of 28 species (Appendix 2). The abundance of species at Lucy's Creek and Kathy's Creek is summarized in Tables 1 and 2. Because most taxa were scarce, we restricted the comparisons between years to relatively abundant species (glassfrogs). Abundances of glassfrogs at Lucy's Creek and Kathy's Creek were not significantly different between years (Tables 1, 2).

Prevalence of *Batrachochytrium dendrobatidis* in amphibians: Swabs of 320 frogs were tested for *Bd*, and
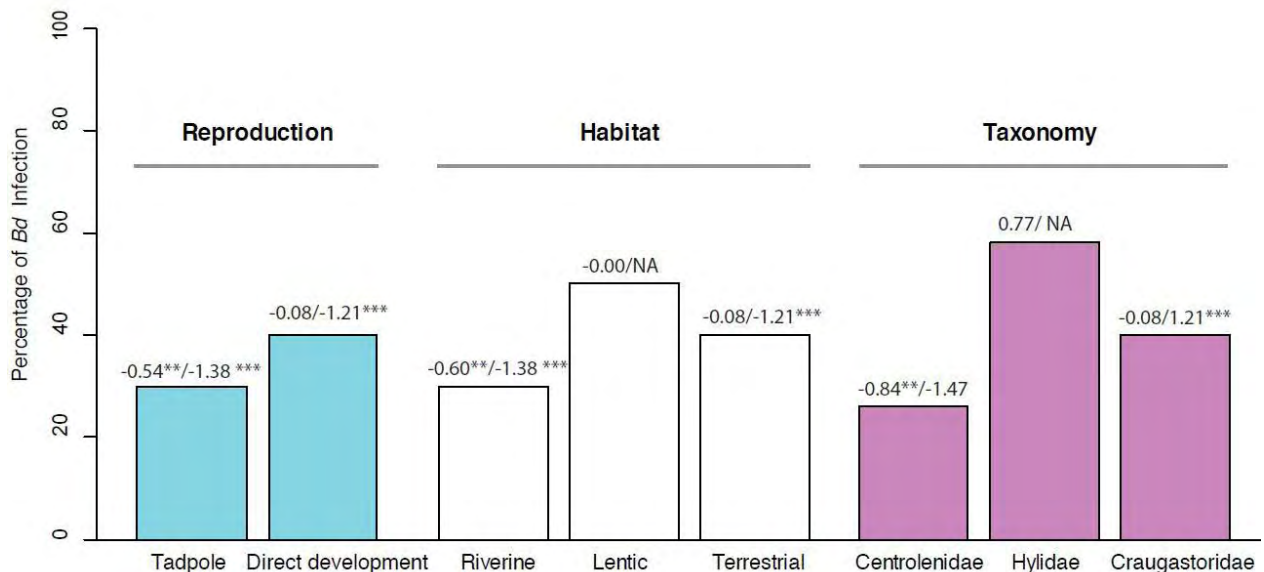
Fig. 2. Significant *Bd* infection differences in amphibians according to reproductive modes, habitat use, and taxonomy. *P* values are reported for 2012 and 2013; significance is noted by ** ($p < 0.01$) and *** ($p < 0.001$).

Table 1. Abundance of amphibian species at Lucy's Creek, Reserva Las Gralarias. Abundances are presented as minimum–maximum, followed, in parenthesis, by mean ± standard error. The Student's *t*-test was performed only in species with normally distributed abundances.

	Lucy's Creek		*t*-test (*p*)	Population trend
Year	**2012**	**2013**		
Number of nights sampled	**10**	**6**		
Family/Species				
Centrolenidae				
Centrolene lynchi	5–20 (13.1 ± 5.13)	2–18 (9.0 ± 6.54)	0.184	No difference
Centrolene peristictum	4–35 (20.7 ± 11.68)	6–26 (15.2 ± 7.00)	0.314	No difference
Nymphargus grandisonae	1–10 (6.2 ± 3.12)	0–8 (3.8 ± 2.93)	0.155	No difference
Hylidae				
Hyloscirtus alytolylax	3–7 (4.3 ± 1.42)	0–6 (3.3 ± 2.16)	0.295	No difference
Craugastoridae				
Pristimantis achatinus	0–2 (0.3 ± 0.67)	0–1 (0.2 ± 0.41)	—	—
Pristimantis appendiculatus	0–2 (0.8 ± 0.92)	0–1 (0.5 ± 0.55)	—	—
Pristimantis calcarulatus	0–2 (0.2 ± 0.63)	0–1 (0.2 ± 0.41)	—	—
Pristimantis eremitus	0	0–2 (0.5 ± 0.84)	—	—
Pristimantis eugeniae	0	0–2 (0.3 ± 0.82)	—	—
Pristimantis illotus	0–1 (0.1 ± 0.32)	0	—	—
Pristimantis parvillus	0–1 (0.1 ± 0.32)	0	—	—
Pristimantis sobetes	0	0–2 (0.3 ± 0.82)	—	—
Pristimantis w-nigrum	0–2 (0.6 ± 0.84)	0–2 (0.7 ± 0.82)	—	—

approximately a third of those were positive. In samples from 2012, prevalence of *Bd* was relatively high, with 42% of all frogs testing positive for *Bd* infection. During 2013, *Bd* prevalence was 22%. Differences in prevalence between the two years are likely caused by detection method. Most species infected in 2012 carried low *Bd* loads as determined by qPCR; the highest *Bd* load obtained was in *Centrolene ballux* with 22.5 genomic equivalents. Prevalence per species per year is summarized in Table 3.

The logistic regression shows a significant relationship (*p* < 0.001) of *Bd* infection with species reproductive mode, habitat, and taxonomy (Fig. 2). Frogs with a terrestrial reproductive mode (direct developers; i.e., genus *Pristimantis*; see Duellman and Trueb 1986) have a higher *Bd* prevalence than amphibians with aquatic reproduction (i.e., glassfrogs and treefrogs). Frog species that are dependent on riverine habitats for reproduction show significantly less infection than anurans that use terrestrial or lentic habitats for reproduction (*p* < 0.001). Also, species in the Centrolenidae family (glassfrogs) show a lower *Bd* prevalence than species in Craugastoridae and Hylidae (Table 3). Although, *Bd* prevalence during 2012 was significantly higher than in 2013 (probably as a result of higher sensitivity of qPCR), we found no significant interaction among sampling year and reproductive mode, habitat, or taxonomy.

Infection intensity of *Batrachochytrium dendrobatidis* and correlates in amphibians: We found no relationship between *Bd* infection intensity (*Bd* load, year 2012) and taxonomy, reproductive mode, or habitat.

Discussion

Our results show a relatively high mean prevalence of *Bd* (36%) across both years in the Andean frog community of Reserva Las Gralarias (see Hossack et al. 2010 for comparison). From a total of 20 species analyzed, only three (*Nymphargus griffithsi*, *Pristimantis illotus*, and *P. pteridophilus*) tested negative for *Bd*; however, sample sizes for non-infected species were low (5, 1, and 3 individuals, respectively). Infected species included frogs with very different reproductive modes, including taxa with terrestrial direct development (*Pristimantis* spp.), species that deposit eggs in ponds (*Dendropsophus carnifex*), and others that place their eggs on vegetation from where hatching tadpoles drop into streams (*Centrolene* spp., *Nymphargus* spp., *Hyloscirtus* spp.).

Because *Bd* is an aquatic pathogen (Berger et al. 2005b) we expected amphibian species with aquatic reproductive modes to show higher infection prevalence (Lips et al. 2005; Brem and Lips 2008). In fact, the most dramatic amphibian declines and extinctions in the Andes have occurred in species with aquatic larvae (La Marca et al. 2005; Bustamante et al. 2005; Merino-Viteri et al. 2005; Coloma et al. 2010). Our results indicate, surprisingly, a higher *Bd* prevalence in frogs with a terrestrial reproductive mode (*Pristimantis* spp.) than in those that

Table 2. Abundance of amphibian species at Kathy's Creek, Reserva Las Gralarias. Abundances are presented as minimum–maximum, followed, in parenthesis, by mean ± standard error. The Student's *t*-test was performed only in species with normally distributed abundances.

Family/Species	Kathy's Creek		*t*-test	Population trend
Year	**2012**	**2013**		
Number of nights sampled	**10**	**5**		
Centrolenidae				
Centrolene ballux	5–37 (22.7 ± 11.6)	3–25 (11.4 ± 8.67)	0.078	No difference
Centrolene peristictum	0–5 (2.1 ± 1.66)	0–5 (2.0 ± 1.87)	0.918	No difference
Nymphargus grandisonae	0–7 (3.7 ± 2.21)	0–6 (2.2 ± 2.28)	0.242	No difference
Nymphargus griffithsi	0–8 (2.3 ± 2.26)	0–3 (1.4 ± 1.34)	—	—
Nymphargus lasgralarias	3–28 (19.4 ± 8.54)	7–28 (15.0 ± 8.69)	0.366	No difference
Hylidae				
Hyloscirtus alytolylax	0–1 (0.1 ± 0.32)	0–1 (0.4 ± 0.59)	—	—
Craugastoridae				
Pristimantis achatinus	0–3 (0.3 ± 0.95)	0–1 (0.2 ± 0.45)	—	—
Pristimantis appendiculatus	0–7 (1.2 ± 2.10)	0–1 (0.4 ± 0.59)	—	—
Pristimantis calcarulatus	0–3 (1.1 ± 0.74)	0–3 (1.0 ± 1.23)	—	—
Pristimantis eremitus	0–1 (0.1 ± 0.32)	0–1 (0.2 ± 0.45)	—	—
Pristimantis eugeniae	0–1 (0.1 ± 0.32)	1–2 (0.8 ± 0.84)	—	—
Pristimantis sobetes	0–1 (0.2 ± 0.42)	0–2 (0.4 ± 0.89)	—	—
Pristimantis w-nigrum	0–1 (0.1 ± 0.32)	0–1 (0.4 ± 0.59)	—	—

reproduce in water (mainly glassfrogs; *Centrolene* spp., *Nymphargus* spp.). This finding supports the idea that even terrestrial breeders may serve as reservoirs for the pathogen in diverse amphibian communities (Longo et al. 2013). Higher prevalence in terrestrial frogs requires that *Bd* zoospores survive in terrestrial habitats. Johnson and Speare (2003) indicated that *Bd* can survive in moist soil for up to three months. Cloud forests in western Ecuador typically have near constant rain and high levels of humidity during the rainy season (Hutter and Guayasamin 2012; Arteaga et al. 2013), and this may extend zoospore survival in terrestrial environments at Las Gralarias. Higher *Bd* prevalence in terrestrial frogs compared to that in frogs with aquatic reproduction might also be related to intrinsic differences in, for example, the efficacy of immune responses (Rosenblum et al. 2009; Woodhams et al. 2007) or differences in anuran skin microbiota (Flechas et al. 2012).

Our results also show that *Bd* prevalence is significantly associated with taxonomy (i.e., family). Thus, glassfrogs (family Centrolenidae) might have immune responses or skin microbiota that work as better barriers to the pathogen than those in terrestrial (i.e., *Pristimantis*) frogs. The strong correlation of prevalence with taxonomy, habitat, and reproductive mode (Appendix 2) indicates that further studies need to focus on the specific effects of each of these factors; in other words, phylogeny (and taxonomy) correlates with reproductive mode and habitat use.

A second surprising finding of our study is that, although prevalence of *Bd* is high in most anuran species, we did not observe any sign of population declines or abrupt crashes, nor have we found dead or sick frogs during four years of intensive fieldwork (2010–2014; JMG pers. obs.). Thus, this frog community persists with an endemic pathogen and with relatively low loads (less than 10 zoospores, Table 3). The apparent increased resistance or tolerance of amphibians from Reserva Las Gralarias to *Bd* infection may be explained by one or several of the following mechanisms: (i) amphibian innate and/or acquired defense mechanisms (Savage and Zamudio 2012; Woodhams et al. 2007); (ii) skin bacterial commensals with anti-fungal properties (Harris et al. 2006); (iii) behavioral and ecological factors that reduce the likelihood of infection and disease (e.g., microhabitat selection, reproductive mode; Lips et al. 2003; Rowley and Alford, 2007), and/or (iv) variation in *Bd* virulence (Berger et al. 2005a; Fisher et al. 2009). Our amphibian monitoring took place during the rainy season when most species are active. Therefore, future studies should determine whether or not this apparent tolerance to *Bd* is stable through longer periods of time or if it fluctuates depending on environmental variables influencing host immunity, behavior, microbiota, or pathogenicity.

After the emergence of an infectious disease, surviving hosts can evolve tolerance or resistance (Retallick et al. 2004; Savage and Zamudio 2011). The earliest known record of *Bd* in Ecuador is in 1980, in the Harlequin frog *Atelopus bomolochos* (Ron and Merino-Viteri 2000), a species that is now probably extinct (Coloma et al. 2014). If *Bd* reached and spread in Ecuador during the early 1980s (Ron et al. 2003; Lips et al. 2008), we hy-

Table 3. Prevalence of *Batrachochytrium dendrobatidis* (*Bd*) in amphibians at Reserva Las Gralarias, Ecuador, during the rainy seasons of 2012 and 2013. *Bd* prevalence for each species is followed, in parenthesis, by a 95% confidence interval. *Bd* load summary data includes only samples that tested positive for *Bd*.

	2012				2013		
	N	Positive *Bd*	Prevalence	*Bd* Load (mean ± sd)	N	Positive *Bd*	Prevalence
Family: Centrolenidae							
Centrolene ballux	17	8	47% (24–71%)	6.5 ± 10.7	9	2	22% (39–59%)
Centrolene heloderma	6	1	17% (1–63%)	0.6	1	0	0% (0–95%)
Centrolene lynchi	6	1	17% (1–63%)	–	5	1	20% (1–70%)
Centrolene peristictum	21	6	29% (12–52%)	2.1 ± 1.5	16	3	19% (5–46%)
Nymphargus grandisonae	21	5	24% (9–48%)	4.7	–	–	–
Nymphargus griffithsi	3	0	0% (1–69%)	–	2	0	0% (0–80%)
Nymphargus lasgralarias	16	6	38% (16–64%)	6.5 ± 1.5	10	2	20% (4–56%)
Family: Hylidae							
Dendropsophus carnifex	10	5	50% (20–80%)	–	–	–	–
Hyloscirtus alytolylax	9	8	89% (51–99%)	2.4 ± 2.4	7	2	29% (5–70%)
Family: Craugastoridae							
Pristimantis achatinus	7	4	57% (20–88%)	–	–	–	–
Pristimantis appendiculatus	23	10	44% (24–65%)	1.9 ± 2.4	–	–	–
Pristimantis calcarulatus	15	2	13% (2–42%)	1.1	15	1	7% (4–34%)
Pristimantis eremitus	4	4	100% (40–100%)	0.9 ± 0.2	4	2	50% (9–91%)
Pristimantis eugeniae	18	12	66% (41–86%)	2.5	2	1	50% (3–97%)
Pristimantis hectus	8	2	25% (4–64%)	–	14	4	29% (10–58%)
Pristimantis illotus	–	–	–	–	1	0	0% (0–95%)
Pristimantis parvillus	9	4	44% (15–77%)	–	–	–	–
Pristimantis sobetes	8	3	38% (10–74%)	–	9	3	33% (9–69%)
Pristimantis pteridophilus	–	–	–	–	3	0	0% (0–69%)
Pristimantis w-nigrum	21	13	62% (39–81%)	1.5 ± 0.7	–	–	–
TOTAL	**222**	**94**	**42% (35–49%)**		**88**	**19**	**22% (14–32%)**

pothesize that many of the population declines observed in the country at that time (e.g., Coloma 1995, 2002; Coloma et al. 2000; Ron et al. 2003; Bustamante et al. 2005; La Marca et al. 2005; Lips et al. 2008; Coloma et al. 2010) could be attributable to chytridiomycosis. Thus, it is probable that most Andean amphibian communities have been exposed to *Bd* for more than three decades and that current sampling finds remnant species that are tolerant to *Bd* while the susceptible species are already extinct. Under this scenario, selection should have favored the persistence of amphibian species or specific populations that have developed defenses against *Bd*; therefore,

changing host composition of these communities right after pathogen emergence. We acknowledge, however, that this is a working hypothesis which assumptions depend on our knowledge of the historical distribution of the chytrid. For example, if new data shows that *Bd* was present in the Ecuadorian Andes before amphibian declines were noticed, such piece of information would support the endemic pathogen hypothesis, which states that environmental changes triggered *Bd* outbreaks (Rachowicz et al. 2005).

Reserva Las Gralarias is one of the most studied and species-rich area in the cloud forest of the tropical Andes, containing numerous species considered endangered by the IUCN (2014; see Appendix 2). However, the community (and surrounding areas) lacks at least three groups of species that were conspicuous in Ecuadorian cloud forests: marsupial frogs (*Gastrotheca plumbea*, *G. guentheri*), harlequin frogs (*Atelopus longirostris*, *A. mindoensis*), and dendrobatid frogs (*Hyloxalus lehmani*, *H. maquipucuna*) (Coloma et al. 2011–2014; Arteaga et et al. al. 2013). Marsupial and harlequin frogs are particularly susceptible to *Bd* (Lips et al. 2003; Flechas et al. 2012; Ellison et al. 2014; DiRenzo et al. 2014) and are the primary species that suffered population declines and extinctions in Ecuador (Lips et al. 2002; La Marca 2005) even in pristine areas. The absence of these lineages at Reserva Las Gralarias supports to the hypothesis that this is a post-decline amphibian community. Understanding the long-term effects of pathogens (eg., chytrid) and temperature variability in such a community is essential for the continued effective management of endangered species in the Andean cloud forests.

Considering Ecuador's high diversity of amphibian species, life history modes, and evolutionary history, our study provides a baseline to study the evolution of defense strategies against *Bd*. We recommend further research to determine the mechanisms driving the observed differences in pathogen exposure among hosts differing in reproductive modes, habitat, and taxonomy.

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Appendix 1. Transects sampled at Reserva Las Gralarias. Each transect has an area of 500 × 4 m. Latitude and longitude are in decimal degrees.

Transect	Elevation (m)	Latitude	Longitude	Habitat
Lucy's creek	1822–1858	At start: -0.00492 At end: -0.00342	At start: -78.73344 At end: -78.74051	Riverine vegetation along creek
Kathy's creek	2041–2066	At start: -0.01696 At end: -0.0156	At start: -78.7314 At end: -78.73386	Riverine vegetation along creek
Santa Rosa river	1884–1882	At start: -0.0133 At end: -0.01054	At start: -78.72368 At end: -78.7211	Riverine vegetation along river
Waterfall trail	1897–2107	At start: -0.0135 At end: -0.01379	At start: -78.72461 At end: -78.7269	Primary and secondary terra firme forest
Five-Frog creek	2141–2156	At start: -0.03166 At end: -0.03098	At start: -78.70421 At end: -78.70853	Riverine vegetation along creek
Osoverde & Guarumo trail	2141–2156	At start: -0.03166 At end: -0.03098	At start: -78.70421 At end: -78.70853	Primary and secondary terra firme forest
Puma trail	1923–2031	At start: -0.00954 At end: -0.00708	At start: -78.7346 At end: -78.73662	Primary and secondary terra firme forest
TKA trail	2192–2216	At start: -0.0275 At end: -0.02516	At start: -78.70477 At end: -78.70353	Primary and secondary terra firme forest
Peccary trail	1803–1896	At start: -0.00750 At end: -0.0076	At start: -78.72635 At end: -78.72862	Primary and secondary terra firme forest

Appendix 2. Amphibians at Reserva Las Gralarias, with corresponding IUCN (2014) conservation status. The list includes three potential new species (*Pristimantis* sp. 1, *Pristimantis* sp. 2, and *Pristimantis* sp. 3). Reproductive modes are sensu Haddad and Prado (2005).

Species	Reproductive mode	Habitat for reproduction	Conservation status
Family: Centrolenidae (7 spp.)	Mode 25: Eggs hatching into exotrophic tadpoles that drop in lotic water		
Centrolene ballux	Mode 25	Vegetation along fast-flowing streams	Critically Endangered
Centrolene heloderma	Mode 25	Vegetation along fast-flowing streams	Critically Endangered
Centrolene lynchi	Mode 25	Vegetation along fast-flowing streams	Endangered
Centrolene peristictum	Mode 25	Vegetation along fast-flowing streams	Vulnerable
Nymphargus griffithsi	Mode 25	Vegetation along fast-flowing streams	Vulnerable
Nymphargus grandisonae	Mode 25	Vegetation along fast-flowing streams	Least Concern
Nymphargus lasgralarias	Mode 25	Vegetation along fast-flowing streams	Data Deficient

Appendix 2 (continued). Amphibians at Reserva Las Gralarias, with corresponding IUCN (2014) conservation status. The list includes three potential new species (*Pristimantis* sp. 1, *Pristimantis* sp. 2, and *Pristimantis* sp. 3). Reproductive modes are sensu Haddad and Prado (2005).

Species	Reproductive mode	Habitat for reproduction	Conservation status
Family: Craugastoridae (16 spp.)	Mode 23: Direct development of terrestrial eggs		
Pristimantis achatinus	Mode 23	Terrestrial, mainly in pastures and modified environments	Least Concern
Pristimantis appendiculatus	Mode 23	Terrestrial, mainly primary and secondary forests	Least Concern
Pristimantis calcarulatus	Mode 23	Terrestrial, mainly primary and secondary forests	Vulnerable
Pristimantis crenunguis	Mode 23	Terrestrial, mainly primary and secondary forests	Endangered
Pristimantis eremitus	Mode 23	Terrestrial, mainly primary and secondary forests	Vulnerable
Pristimantis eugeniae	Mode 23	Terrestrial, mainly primary and secondary forests	Endangered
Pristimantis hectus	Mode 23	Terrestrial, mainly primary and secondary forests	Data deficient
Pristimantis illotus	Mode 23	Terrestrial, mainly primary and secondary forests	Near Threatened
Pristimantis parvillus	Mode 23	Terrestrial, mainly primary and secondary forests	Least Concern
Pristimantis pteridophilus	Mode 23	Terrestrial, mainly primary and secondary forests	Endangered
Pristimantis sobetes	Mode 23	Terrestrial, mainly primary and secondary forests	Endangered
Pristimantis verecundus	Mode 23	Terrestrial, mainly primary and secondary forests	Vulnerable
Pristimantis w-nigrum	Mode 23	Terrestrial, mainly primary and secondary forests	Least Concern
Pristimantis sp. 1	Mode 23	Terrestrial, mainly primary and secondary forests	Not evaluated
Pristimantis sp. 2	Mode 23	Terrestrial, mainly primary and secondary forests	Not evaluated
Pristimantis sp. 3	Mode 23	Terrestrial, mainly primary and secondary forests	Not evaluated
Family: Hylidae (3 spp)			
Dendropsophus carnifex	Mode 1: Eggs and exotrophic tadpoles in lentic water	Ponds	Least Concern
Hyloscirtus alytolylax	Mode 25	Vegetation along fast-flowing streams	Near Threatened
Hyloscirtus criptico	Mode 25	Vegetation along fast-flowing streams	Not evaluated
Family: Caeciliidae (1 sp.)			
Caecilia buckleyi	Unknown	Unknown	Not evaluated
Family: Rhinatrematidae (1 sp.)			
Epicrionops bicolor	Unknown	Unknown	Least Concern

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Anolis podocarpus. Photo by A. Almendáriz.

Overview of the herpetofauna of the unexplored Cordillera del Cóndor of Ecuador

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Abstract.—The Cordillera del Cóndor is an area rich in unique vegetation assemblages and endemic faunal elements; the herpetofauna is especially diverse, particularly the anurans. The montane forest and sandstone tepuis, located atop large andesite and quartz formations, provide a variety of habitats and microhabitats in which the herpetofauna finds food, shelter, and reproductive sites, such as terrestrial and arboreal bromeliads and a soil type termed "bamba" that is covered with mosses and roots. Information compiled from publications and recent studies has revealed the presence of 120 species of amphibians and 59 species of reptiles, including 41 probable new species (36 amphibians and five reptiles) in the genera *Centrolene*, *Dendrobates*, *Pristimantis*, *Lynchius*, *Chiasmocleis*, *Bolitoglossa*, *Anolis*, *Erythrolamprus*, *Tantilla*, and *Dipsas*.

Resumen.—La Cordillera del Cóndor es un área rica en formaciones vegetales únicas y elementos faunísticos endémicos; presenta una singular diversidad herpetofaunística, particularmente de la anurofauna. Los bosques montanos y los de "tepuy," asentados sobre piedras grandes de andesita y cuarzo crean variedad de hábitats y microhábitats, en donde la herpetofauna encuentra alimento, refugio y lugares para la reproducción, como por ejemplo las bromelias terrestres y arbóreas y un suelo denominado "bamba" que está cubierto de musgos y raíces. La información recopilada de material publicado y de los estudios realizados en los últimos años revela la presencia de 120 especies de anfibios y 59 especies de reptiles. Los resultados incluyen 41 especies posiblemente nuevas (36 anfibios y cinco reptiles) de los géneros: *Centrolene*, *Dendrobates*, *Pristimantis*, *Lynchius*, *Chismocleis*, *Bolitoglossa*, *Anolis*, *Erythrolamprus*, *Tantilla*, y *Dipsas*.

Key words. Ecuador, Cordillera del Cóndor, amphibian, reptile

Introduction

The fauna of Ecuador, in general, has not been extensively studied, despite a notable increase in research activity in recent years (Albuja et al. 2012). In particular, the Cordillera del Cóndor region, in southern Ecuador along the border with Peru (Figure 1), is a very poorly known area. The purpose of this paper is to summarize and review herpetofaunal studies of the Cordillera del Cóndor region. Studies of the avian and mammalian fauna have been published elsewhere (e.g., Albuja and Patterson 1996; Brito and Arguero 2012; Freile et al. 2014).

The long-running border conflicts between Ecuador and Peru and the difficulty in accessing the region have maintained the ecosystems of the Cordillera del Cóndor almost intact. It has only been since the end of the conflicts known as the Pasquisha War (which ended in February 1981) and the Alto Cenepa War (which ended in February 1995), that roads into the area have begun to open, which has resulted in incipient colonization and an awakened interest in mineral prospection in the region. Nevertheless, there are still some parts of the Cordillera del Cóndor that remain unaltered.

The Cordillera del Cóndor is part of a biologically diverse, discontinuous, sub-Andean cordillera that has several characteristics that distinguish it from the rest of the Andes. Whereas the main Andes are of metamorphic and igneous origin, the Cordillera del Cóndor is sedimentary, composed largely of limestone and sandstone (Schulenberg and Awbrey 1997). The region is dominated by

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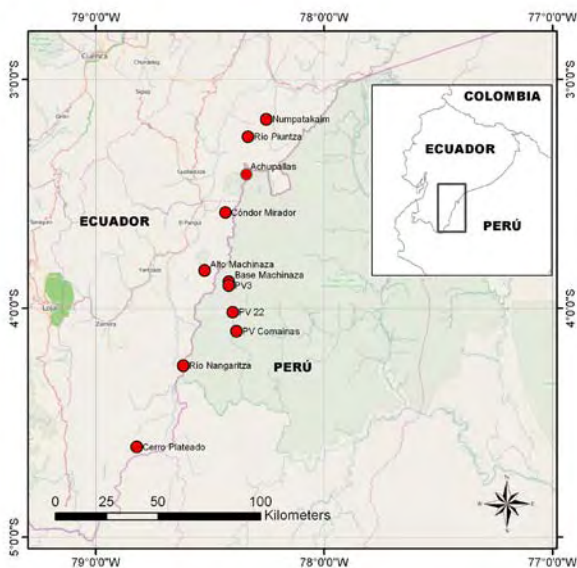
Fig. 1. Map of the Cordillera del Cóndor region.

geologically complex mountains topped with sandstone plateaus at elevations of 300 m to almost 3,000 m that support habitats similar to the sandstone mountains of the Guyana Shield (Figure 2); many of the plateaus have vegetation similar to that of the tepuis. Due to its location just northeast of the Huancabamba Depression, the lowest point in the Andes (Duellman 1999), the Cordillera del Cóndor receives moisture from both the Atlantic and the Pacific sides of the Andes—moisture from the Western slopes of the Andes as well as moisture moving east across the Amazon basin drops over the Cordillera del Cóndor, providing frequent, year-round precipitation (Schulenberg and Awbrey 1997).

The first systematic botanical studies of the region were carried out in 1990 and 1991 in the Río Nangaritza basin (in the southern region of the Cordillera del Cóndor), under the auspices of the Proyecto Promobot and the Tratado de Cooperación Amazónica, with the participation of both Ecuadorian and foreign scientists. These explorations were limited to areas below about 1,700 m

in altitude. In 1993, A. H. Gentry collected plants on one of the highest points of the mountain range (2,100 m) as part of a Rapid Assessment Program (RAP) survey, organized by Conservation International (CI, a non-governmental organization). Gentry found that the vegetation structure and families of plants were similar to those of the sandstone tepuis of the Guiana Highlands (Schulenberg and Awbrey 1997). In recent years, D. Neill and his collaborators have surveyed the flora at several points in the cordillera, publishing descriptions of new species and studying the environmental heterogeneity associated with variable types of soils (Neill 2005; Neill and Asanza 2012; Neill and Ulloa 2011; Riina et al. 2014; Ulloa et al. 2012). The diversity of plant assemblages on the sandstone plateaus produces a variety of microhabitats that provide food, shelter, and reproductive sites for the herpetofauna, particularly terrestrial and arboreal bromeliads; the "bamba" soils of many of these tepuis is thickly covered with mosses and roots, and serves to filter the tannins that darken the turbid water in creeks and streams (Figure 3).

According to the ecosystem classification for continental Ecuador (Ministerio del Ambiente del Ecuador 2012), the following ecosystems have been identified in the Cordillera del Cóndor:

- Evergreen piedmont forest in the Cóndor-Kutukú ranges
- Evergreen forest on the sandstone plateaus of the Cóndor range in the lower Ecuadorian Amazon

Fig. 3. Vegetation in the interior of a tepui forest. *Photo by A. Almendáriz.*

Fig. 2. Alto Paquisha, 2,400 m. *Photo by A. Almendáriz.*

- Lower montane evergreen forest in the Cóndor-Kutukú ranges
- Evergreen piedmont forest on the sandstone mesas of the Cóndor-Kutukú ranges
- Evergreen lower montane forests on the sandstone mesas of the Cóndor-Kutukú ranges
- Montane humid shrub in the Cóndor range
- Evergreen montane forests on the sandstone mesas of the Cóndor-Kutukú ranges
- Montane humid shrub with herbaceous rosette thickets (herbazales) in the Cóndor range

Materials and Methods

Herpetological surveys of the region have been few and limited (Figure 1). The information presented below is drawn from an extensive survey of the literature and recent field work. The majority of the studies have employed the Rapid Ecological Assessment strategy or RAP developed by CI (Sayre et al. 2002), in habitats where the presence of herpetofaunal elements was anticipated.

The northern zone of the Cordillera del Cóndor is known from three studies. The first was carried out in 1972 in conjunction with a privately funded orchid collecting expedition (accompanied by personnel from the Missouri Botanical Garden and the University of Kansas Museum of Natural History), at elevations of 870–2,000 m at the headwaters of the Río Piuntza, Río Chuchumbleza, Río Numpatacaimi, and Río Santa Agueda in Morona Santiago Province (Duellman and Simmons 1988). The second survey was a RAP assessment conducted by CI, the Escuela Politécnica Nacional, Fundación Fedima, and the Universidad Nacional Mayor de San Marcos. The areas surveyed included the Ecuadorian flank of the Cordillera del Cóndor (Coangos and Achupallas in Morona Santiago Province [Figure 4], Miazi and Shaimi in Zamora Chinchipe Province). The Peruvian flank of the Cordillera del Condor was surveyed at the base of Cerro Machinaza, Alfonso Ugarte-PV3, Falso Paquisha-PV22, and Puesto de Vigilancia Comainas. Subsequently, a third survey was carried out by Fundación Natura (FN 2000) to establish the Parque El Cóndor, which inventoried the Comunidad Numpatakaime and confluence of the Río Tsuirim and the Río Coangos.

Another survey, conducted as part of the Proyecto Paz y Conservación Binacional en la Cordillera del Cóndor Ecuador-Perú by the Organización Internacional de las Maderas Tropicales, Conservation International, Fundación Natura, and the Instituto Nacional de Recursos Naturales (INRENA) in 2005 (Organización Internacional de las Maderas Tropicales and Fundación Natura y Conservación Internacional 2005), collected data from several localities in the southern sector on the Ecuadorian flank of the Cordillera del Cóndor, including Cóndor Mirador and Herradura. The corresponding Peruvian flank survey was focused on the Zona Reservada Santiago Comaina.

Between March 2008 and July 2012, the Escuela Politécnica Nacional team, under a contract with the Cardno-Entrix Corporation, carried out 16 expeditions to survey the herpetofauna of Alto Manchinaza. In 2009, a CI RAP survey was conducted by personnel from the Pontificia Universidad Católica del Ecuador, Louisiana State University, and Fundación Ecológica Arcoiris, with support from Secretaría Nacional de Ciencia y Tecnología del Ecuador (SENACYT) of the tepuyes of the upper basin of the Río Nangaritza (Guayasamín et al. 2011). In 2012, the Fundación Naturaleza y Cultura Internacional and the Universidad Estatal Amazónica organized an expedition to Cerro Plateado (Figure 5), the southern point of the Cordillera del Cóndor, which included researchers from the Escuela Politécnica Nacional.

Results

The 1972 survey of the northern zone of the Cordillera del Cóndor resulted in the capture of 30 species, including nine new species (e.g., Duellman and Simmons 1988, Lynch 1974, 1976, 1979; Lynch and Duellman 1980). Specimens and additional records from this survey are deposited in the Biodiversity Research Institute at the University of Kansas, along with additional related specimens accounting for 47 species total (Reynolds 1997; Schulenberg and Awbrey 1997).

Fig. 4. Achupallas sector, 2,100 m. *Photo by A. Almendáriz.*

Fig. 5. Peak of Cerrro Plateado, 2,900 m. *Photo by V. Carvajal.*

Fig. 6. *Pristimantis* sp. *Photo by A. Almendáriz.*

Fig. 7. *Centrolene condor. Photo by A. Almendáriz.*

Fig. 8. *Excidobates condor. Photo by A. Almendáriz.*

Fig. 9. *Enyalioides rubrigularis* (female). *Photo by A. Almendáriz.*

Fig. 10. *Hyloscirtus condor. Photo by J. Brito.*

The second survey of the northern Zone of the Cordillera del Cóndor recorded a total of 34 species—27 amphibians and seven reptiles (Almendáriz 1997a, 1997b). Although the survey was conducted under adverse environmental conditions, geographic range extensions for several species were recorded. The Peruvian flank surveys recorded 58 species (35 anurans and 23 reptiles); the data from the Peruvian surveys was collected by personnel from the Museo de Historia Natural of the Universidad Nacional Mayor de San Marcos in 1987 (Reynolds and Icochea 1997a, 1997b) and expanded the ranges of two species for Peru, *Rhinella festae* and *Hemiphractus bubalus*. The Parque El Cóndor survey lists a total of 36 species (22 anurans, eight lizards, and six snakes), including nine species new to the Cordillera del Cóndor herpetofauna, and makes reference to the difficulty of identifying some of the material. The report summarizes information known up to the year 2000, and lists a total of 95 species for the region (56 amphibians and 39 reptiles).

The survey of the southern sector of the Cordillera del Cóndor collected specimens that were not identified to the species level of the genus *Pristimantis* (Figure 6), and a glass frog provisionally identified as *Centrolene* cf. *croceopodes* that in 2008 was named as *Centrolene condor* (Cisneros-Heredia and Morales-Mite 2008; Figure 7).

The surveys of Alto Manchinaza recorded 70 species of amphibians and 43 species of reptiles (Almendáriz et al. *in prep.*). The results of these surveys revealed the

presence of several little known or new species, including a new species of highland poison dart frog, *Excidobates condor* (Almendáriz et al. 2012; Figure 8) and geographic range extensions for *Phyllomedusa ecuatoriana*, *Centrolene condor*, *Chimerella mariaelenae*, *Hyloxalus mystax*, and *Enyalioides rubrigularis* (Figure 9), among others. In addition, ecological data and information on reproduction, vocalizations, and other aspects of the life history for these species was collected (Almendáriz and Batallas 2012a, 2012b; Batallas and Brito 2014; Brito et al. *in prep.*); at least 28 probably new species from different genera (*Centrolene*, *Bolitoglossa*, *Pristimantis*, *Anolis*, *Atractus*, *Erythrolamprus*, *Tantilla*, and *Dipsas*) were obtained, which are in the process of being described. This project included environmental education and community outreach work (Almendáriz 2012).

The 2009 Pontificia Universidad Católica del Ecuador survey recorded 27 species of amphibians and 17 reptiles, including a new species of anuran, *Pristimantis minimus* (Terán-Valdez and Guayasamín 2010). This project included the publication of a field guide to plants and animals of the tepuis of Nangaritza (Almendáriz 2010; Freile et al. 2010).

The 2012 expedition to Cerro Plateado (Almendáriz and Brito 2013) recorded 19 species. Of these, 14 were anurans and salamanders, including nine species of the family Craugastoridae (most were members of the genus *Pristimantis*). Based on the ecology of the area, it is assumed that more species occur at this locality, including members of the family Centrolenidae. A new species of torrent frog, *Hyloscirtus condor* (Figure 10), was described based on specimens obtained on this expedition (Almendáriz et al. 2014), and at least eight new species in the genera *Lynchius*, *Pristimantis*, and *Bolitoglossa* were obtained that will be described in the future.

Discussion

The Cordillera del Cóndor is of particular importance due to its high biodiversity and the presence of several unique ecosystems (e.g., the sandstone formations similar to tepuis). The Cóndor region, with its diverse range of habitats, contains numerous species that correspond to three faunal components: (1) Amazonian lowlands; (2) eastern flanks of the Andes, and (3) an endemic fauna limited to the southern part of Ecuador (Duellman and Lynch 1988). As shown in Figure 11, the Baja Amazonia herpetofaunal assemblage contains more reptile species (63%) than amphibian species (28%). In the herpetofaunal assemblages associated with the eastern slopes of the Andes there are slightly more amphibian species (34% and 32%). The percentages relative to the endemic component of the Cordillera del Cóndor are notably higher in amphibians than in reptiles (41% and 7%).

A summary of the herpetofaunal diversity of the region is provided in Tables 1 and 2; a comparison of known species diversity and predicted species diversity
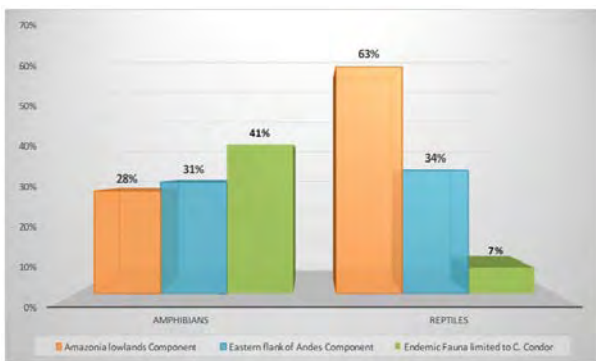
Fig. 11. Herpetofaunal assemblages and endemics from the Cordillera del Cóndor.
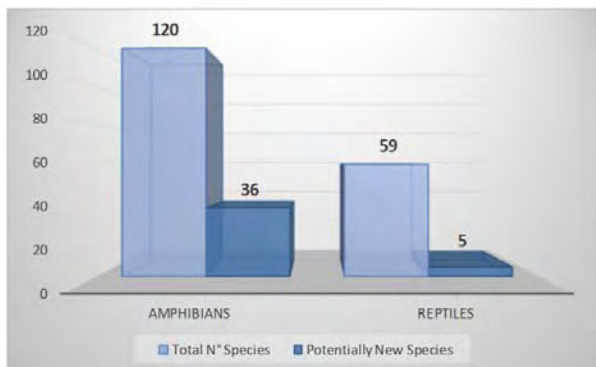
Fig. 12. Documented and predicted species diversity in the Cordillera del Cóndor.

Fig. 13. *Lynchius* sp. *Photo by J. Brito.*

Fig. 14. *Pristimantis muscosus. Photo by A. Almendáriz.*

Fig. 15. *Cercosaura dicra. Photo by G. Gallardo.*

Fig. 16. *Erythrolamprus* sp. *Photo by A. Almendáriz.*

Fig. 17. *Tantilla* sp. *Photo by A. Almendáriz.*

Fig. 18. *Anolis podocarpus. Photo by A. Almendáriz.*

for the region is provided in Figure 12. Some of the more distinctive species found in the region include frogs of the genera *Lynchius* (Craugastoridae; Figure 13) and *Pristimantis* (Craugastoridae; Figure 14), the gymnophthalmid lizard *Cercosaura dicra* (Figure 15), and the colubrid snakes *Erythrolamprus* (Figure 16) and *Tantilla* (Figure 17).

During the last five years, the following species have been described based on material from the Cordillera del Cóndor: *Enyalioides rubrigularis* (Torres-Carvajal et al. 2009; Figure 9), *Anolis podocarpus* (Ayala-Varela and Torres-Carvajal 2010; Figure 18), *Pristimantis minimus* (Terán-Valdez and Guayasamín 2010), *Excidobates condor* (Almendáriz et al. 2012; Figure 8), *Hyloscirtus condor* (Almendáriz et al. 2014; Figure 10), and *Siphlophis ayauma* (Sheehy et al. 2014). The work has expanded the known geographic distribution of *Anolis soini* (Ayala-Varela et al. 2011; Figure 19) and revealed new distribu-

tion and natural history information for two other species of the genera *Centrolene* and *Hyloxalus* (Almendáriz and Batallas 2012a, 2012b). It is also noteworthy that several new species are in the process of being described in the genera *Pristimantis* and *Chiasmocleis* (Almendáriz et al. *in prep.*). A detailed publication about the herpetofauna of the Cóndor region is in preparation (Almendáriz et al. *in prep.*).

Based on information published in the most recent studies conducted in the Cordillera del Cóndor, there are a total of 120 amphibian species (11 families, 31 genera), and 59 reptile species (nine families, 28 genera), not including those found at elevations below 850 m (Figure 20). In addition, based on the specimens discussed above, the area contains approximately 41 potentially new species (36 amphibians and five reptiles; see Figure 12). These numbers indicate that the region has significant endemic diversity (see Table 1, Table 2, and Figure 11).

Conclusion

Within the Cordillera del Cóndor, four areas protected by the Sistema Nacional de Areas Protegidas have been established: (1) Reserva Biológica El Cóndor; (2) Reserva Biológica El Quimi; (3) Reserva Biológica Cerro Plateado; and (4) Refugio de Vida Silvestre El Zarza. On the eastern flank, the Peruvian government has concentrated its efforts to create Parque Nacional Ichigkat Muja-Cordillera del Cóndor (SERNANP 2012). Nevertheless, the ecosystems in the Cordillera del Cóndor are threatened by imminent human colonization and settlement, the introduction of agriculture and livestock, and mining; the latter activity poses the greatest threat to the conservation of the tepui-like forests and the health of the aquatic ecosystems, which are the reproductive habitats of many species of anurans, including hylids and centrolenids. On the other hand, the fact the amphibians of the Andean region have limited distribution makes them susceptible to extinction, and in some cases, the protection of their habitat does not improve their chance of survival (Guayasamín et al. 2011). This situation warrants intensified research and conservation studies of these vertebrates, especially in little explored areas as in the case of the Cordillera del Cóndor. The preliminary results of surveys of Alto Machinaza and Cerro Plateado have revealed the presence of possibly new species in these areas, indicating that future interventions in these areas should comply strictly with the measures to protect ecosystems, environmental mitigation, and management plans.

Fig. 19. *Anolis soini. Photo by J. Vaca G.*
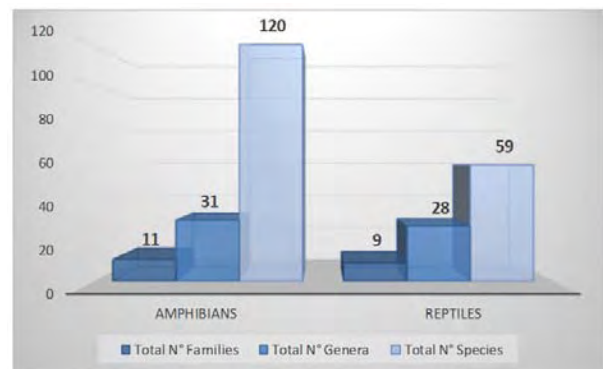
Fig. 20. Familial, generic, and specific diversity of amphibians and reptiles in the Cordillera del Cóndor.

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Table 1. Species of amphibians recorded from the Cordillera del Cóndor.

Location	Alto Machinaza		Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Numpatkaim / ríos Tsuirim y Coangos	RAP 7	RAP 7	Río Puintza	Base de Datos MEPN
Authority	Almendáriz et al., in prep.	Almendáriz et al., in prep.	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación, 2005 (Ecuador)	Proy. Paz y Conservación, 2005 (Perú)	Fundación Natura 2000	Almendáriz 1997	Reynolds & Icochea 1997	Duellman & Lynch 1988	Registros Adicionales
Elevation	1300–1850 msnm	1850–2400 msnm	1600–2860 msnm	950–1850 msnm	1700 msnm	850–1200 msnm	930–1050 msnm	900–2200 msnm	665–1750 msnm	1550–1830 msnm	800–1700 msnm
ANURA											
Aromobatidae											
Allobates kingsburyi	X			X							
Bufonidae											
Atelopus boulengeri										X	
Atelopus pulcher						X					
Atelopus spumarius							X		X		
Atelopus cf. palmatus				X							
Rhinella festae	X	X	X	X	X	X	X		X	X	
Rhinella marina	X				X	X	X	X	X		
Rhinella margaritifera	X	X		X	X						
Centrolenidae											
Centrolene audax	X			X							
Centrolene condor		X			X						
Chimerella mariaelenae	X										
Hyalinobatrachium pellucidum				X							
Nymphargus chancas				X							
Nymphargus cochranae	X	X		X							
Nymphargus posadae	X										
Rulyrana flavopunctata	X										X
Rulyrana mediarmidi	X										
Centrolene cf. wiley	X										X

Location	Alto Machinaza	Alto Machinaza	Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Numpatkaim / ríos Tsuirim y Coangos	RAP 7	RAP 7	Río Puintza	Base de Datos MEPN
Authority	Almendáriz et al., *in prep.*	Almendáriz et al., *in prep.*	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación, 2005 (Ecuador)	Proy. Paz y Conservación, 2005 (Perú)	Fundación Natura 2000	Almendáriz 1997	Reynolds & Icochea 1997	Duellman & Lynch 1988	Registros Adicionales
Elevation	1300-1850 msnm	1850-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	930-1050 msnm	900-2200 msnm	665-1750 msnm	1550-1830 msnm	800-1700 msnm
Centrolene sp. nov.	X										
Craugastoridae											
Noblella lochites	X							X		X	
Lynchius simmonsi			X	X			X			X	
Lynchius sp. nov.			X								
Pristimantis achuar	X						X	X			
Pristimantis altamazonicus	X	X						X			
Pristimantis cf. *bromeliaceus*	X	X						X			
Pristimantis condor	X	X	X		X			X	X	X	
Pristimantis croceoinguinis					X					X	
Pristimantis diadematus	X			X							
Pristimantis exoristus	X										
Pristimantis galdi	X		X		X			X		X	
Pristimantis incomptus	X										
Pristimantis martiae		X				X					
Pristimantis minimus				X							
Pristimantis muscosus		X									
Pristimantis pecki	X									X	
Pristimantis peruvianus	X	X		X			X	X	X	X	
Pristimantis prolatus	X	X									
Pristimantis proserpens	X	X						X			
Pristimantis quaquaversus	X	X			X			X		X	
Pristimantis rhodostichus	X	X									

Location	Alto Machinaza	Alto Machinaza	Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Numpatkaim / ríos Tsuirim y Coangos	RAP 7	RAP 7	Río Puintza	Base de Datos MEPN
Authority	Almendáriz et al., *in prep.*	Almendáriz et al., *in prep.*	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación, 2005 (Ecuador)	Proy. Paz y Conservación, 2005 (Perú)	Fundación Natura 2000	Almendáriz 1997	Reynolds & Icochea 1997	Duellman & Lynch 1988	Registros Adicionales
Elevation	1300-1850 msnm	1850-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	930-1050 msnm	900-2200 msnm	665-1750 msnm	1550-1830 msnm	800-1700 msnm
Pristimantis cf. *schultei*	X										
Pristimantis spinosus	X							X		X	
Pristimantis trachyblepharis				X			X				
Pristimantis ventrimarmoratus				X							
Pristimantis cf. *atratus*	X										
Pristimantis cf. *serendipitus*	X	X									
Pristimantis cf. *versicolor*	X										
Pristimantis sp. A				X							
Pristimantis sp. B				X							
Pristimantis sp. 1		X									
Pristimantis sp. 2		X									
Pristimantis sp. 3		X									
Pristimantis sp. 4		X									
Pristimantis sp. 5		X									
Pristimantis sp. 6		X									
Pristimantis sp. 7		X									
Pristimantis sp. 8		X									
Pristimantis sp. 9	X										
Pristimantis sp. 10	X										
Pristimantis sp. 11		X									
Pristimantis sp. 12		X									
Pristimantis sp. 13		X									
Pristimantis sp. 14	X										

Location	Alto Machinaza	Alto Machinaza	Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Numpatkaim / ríos Tsuirim y Coangos	RAP 7	RAP 7	Río Puintza	Base de Datos MEPN
Authority	Almendáriz et al., *in prep.*	Almendáriz et al., *in prep.*	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación, 2005 (Ecuador)	Proy. Paz y Conservación, 2005 (Perú)	Fundación Natura 2000	Almendáriz 1997	Reynolds & Icochea 1997	Duellman & Lynch 1988	Registros Adicionales
Elevation	1300-1850 msnm	1850-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	930-1050 msnm	900-2200 msnm	665-1750 msnm	1550-1830 msnm	800-1700 msnm
Pristimantis sp. 15	X										
Pristimantis sp. 16	X										
Pristimantis sp. 17		X									
Pristimantis sp. 18	X										
Pristimantis sp. 19	X										
Pristimantis sp. 20		X									
Pristimantis sp. 21								X			
Pristimantis sp. 22								X			
Pristimantis sp. 23			X								
Pristimantis sp. 24			X								
Pristimantis sp. 25			X								
Pristimantis sp. 26			X								
Pristimantis sp. 27			X								
Pristimantis sp. 28			X								
Dendrobatidae											
Colostethus fugax											X
Dendrobates sp.				X							
Excidobates condor		X									
Hyloxalus exasperatus										X	
Hyloxalus mystax		X	X								
Hyloxalus shuar											X
Ranitomeya variabilis							X				

Almendáriz et al.

Species	Alto Machinaza (Almendáriz et al., in prep.) 1300–1850 msnm	Alto Machinaza (Almendáriz et al., in prep.) 1850–2400 msnm	Cerro Plateado (Almendáriz and Brito 2013) 1600–2860 msnm	Alto Nangaritza (Guayasamín et al. 2011) 950–1850 msnm	Condor Mirador / La Herradura (Proy. Paz y Conservación, 2005 Ecuador) 1700 msnm	Quebrada Shinganatza (Proy. Paz y Conservación, 2005 Perú) 850–1200 msnm	Numpatkaim / ríos Tsuirim y Coangos (Fundación Natura 2000) 930–1050 msnm	RAP 7 (Almendáriz 1997) 900–2200 msnm	RAP 7 (Reynolds & Icochea 1997) 665–1750 msnm	Río Puintza (Duellman & Lynch 1988) 1550–1830 msnm	Base de Datos MEPN (Registros Adicionales) 800–1700 msnm
Hemiphractidae											
Gastrotheca testudinea	X										
Gastrotheca weinlandii								X		X	
Hemiphractus bubalus	X								X	X	
Hemiphractus proboscideus				X							
Hemiphractus scutatus	X									X	
Hylidae											
Dendropsophus bifurcus											
Dendropsophus rhodopeplus					X		X	X			
Dendropsophus sarayacuensis				X							
Dendropsophus minutus	X	X		X	X						
Hyloscirtus condor			X								
Hyloscirtus phyllognathus	X	X		X							
Hypsiboas almendarizae	X			X							
Hypsiboas boans						X		X	X		
Hypsiboas cinerascens	X					X					
Hypsiboas fasciatus	X			X	X		X	X			
Hypsiboas geographicus								X			
Hypsiboas lanciformis	X			X	X	X	X	X			
Osteocephalus buckleyi										X	
Osteocephalus festae	X		X					X			
Osteocephalus taurinus											
Osteocephalus sp. A				X							

Location	Alto Machinaza	Alto Machinaza	Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Numpatkaim / ríos Tsuirim y Coangos	RAP 7	RAP 7	Río Puintza	Base de Datos MEPN
Authority	Almendáriz et al., *in prep.*	Almendáriz et al., *in prep.*	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación, 2005 (Ecuador)	Proy. Paz y Conservación, 2005 (Perú)	Fundación Natura 2000	Al-mendáriz 1997	Reynolds & Icochea 1997	Duellman & Lynch 1988	Registros Adicionales
Elevation	1300-1850 msnm	1850-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	930-1050 msnm	900-2200 msnm	665-1750 msnm	1550-1830 msnm	800-1700 msnm
Phyllomedusa ecuatoriana	X	X									
Scinax garbei											X
Leptodactylidae											
Adenomera hylaedactylus											X
Leptodactylus leptodactyloides	X										
Leptodactylus pentadactylus					X			X			
Leptodactylus wagneri	X								X		
Lithodytes lineatus							X		X		
Microhylidae											
Chiasmocleis antenori				X				X			
Chiasmocleis sp. nov.	X										
CAUDATA											
Plethodontidae											
Bolitoglossa sp. 1								X			
Bolitoglossa sp. 2		X	X								
Bolitoglossa sp. 3		X									
Bolitoglossa sp. 4				X							
GYMNOPHIONA											
Caeciliidae											
Caecilia crassisquama		X									

Table 2. Species of amphibians recorded from the Cordillera del Cóndor.

Location	Alto Machinaza	Alto Machinaza	Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Quebrada Shin-ganatza	RAP 7	RAP 7
Authority	Almendáriz et al., *in prep.*	Almendáriz et al., *in prep.*	Almendáriz and Brito 2013	Guayasamín et al. 2011	Proy. Paz y Conservación 2005 (Ecuador)	Proy. Paz y Conservación 2005 (Perú)	Proy. Paz y Conservación 2005 (Perú)	Almendáriz 1997	Reynolds & Icochea 1997
Elevation	1300-1800 msnm	1800-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	850-1200 msnm	900-2200 msnm	665-1750 msnm
SAURIA									
Gymnophthalmidae									
Alopoglossus atriventis				X					
Alopoglossus buckleyi	X	X	X	X					X
Cercosaura argula	X								
Cercosaura dichra	X						X		
Potamites cochranae	X			X	X		X	X	
Potamites ecpleopus	X								
Potamites strangulatus	X			X					
Riama cf. anatolorus	X			X					X
Pholidobolus macbrydei			X						
Iguanidae									
Enyalioides cofanorum	X					X			
Enyalioides praestabilis				X			X		
Enyalioides rubrigularis				X					
Enyalioides sp. A				X					
Anolis cf. fitchi		X							
Anolis fuscoauratus	X			X			X		
Anolis ortonii							X	X	X
Anolis podocarpus	X	X							
Anolis punctatus	X					X			
Anolis soinii	X								
Anolis scypheus	X	X							
Sphaerodactylidae									
Lepidoblepharis festae							X		
Teiidae									
Kentropyx altamazonica								X	

	Alto Machinaza		Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Quebrada Shinganatza	RAP 7	RAP 7
Location									
Authority	Almendáriz et al., *in prep.*		Almendáriz and Brito 2013	Guayasamin et al. 2011	Proy. Paz y Conservación 2005 (Ecuador)	Proy. Paz y Conservación 2005 (Perú)	Proy. Paz y Conservación 2005 (Perú)	Almendáriz 1997	Reynolds & Icochea 1997
Elevation	1300-1800 msnm	1800-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	850-1200 msnm	900-2200 msnm	665-1750 msnm
SERPENTES									
Boidae									
Epicrates cenchria cenchria	X								X
Colubridae									
Atractus major	X								
Atractus sp.		X							
Chironius carinatus	X	X	X	X					X
Chironius monticola	X	X	X	X			X	X	
Chironius scurrulus	X		X	X					
Clelia clelia	X						X		X
Dipsas catesbyi	X						X	X	
Dipsas indica	X			X					
Dipsas pavonina	X								
Dipsas peruana	X	X							
Dipsas vermiculata	X	X							X
Dipsas cf. *peruana*	X			X					
Erythrolamprus cobella	X						X	X	
Erythrolamprus festae	X			X			X		
Erythrolamprus mimus micrurus	X								
Erythrolamprus regina	X								
Erythrolamprus sp. 1	X	X		X					X
Imantodes cenchoa	X	X		X					X
Imantodes lentiferus	X								X
Leptodeira annulata	X	X							X
Oxyrhopus melanogenys	X								
Oxyrhopus petolarius	X								
Siphlophis ayauma	X								

Location	Alto Machinaza		Cerro Plateado	Alto Nangaritza	Condor Mirador / La Herradura	Quebrada Shinganatza	Quebrada Shin-ganatza	RAP 7	RAP 7
Authority	Almendáriz et al., in prep.		Almendáriz and Brito 2013	Guayasamin et al. 2011	Proy. Paz y Conservación 2005 (Ecuador)	Proy. Paz y Conservación 2005 (Perú)	Proy. Paz y Conservación 2005 (Perú)	Almendáriz 1997	Reynolds & Icochea 1997
Elevation	1300-1800 msnm	1800-2400 msnm	1600-2860 msnm	950-1850 msnm	1700 msnm	850-1200 msnm	850-1200 msnm	900-2200 msnm	665-1750 msnm
Synophis bicolor	X			X					
Tantilla sp.		X							
Xenodon rhabdocephalus							X		
Xenodon severus									X
Elapidae									
Micrurus peruvianus	X								
Micrurus petersi	X								
Micrurus steindachneri steindachneri	X								
Micrurus cf. peruvianus	X								
Tropidophiidae									
Tropidophis taczanowski			X						
Viperidae									
Bothrocophias microphthalmus	X			X	X				
Bothrops atrox	X	X		X				X	X
Bothrops pulchra	X	X							
Bothrops taeniata					X				

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Herpetofaunal community of a high canopy tank bromeliad (*Aechmea zebrina*) in the Yasuní Biosphere Reserve of Amazonian Ecuador, with comments on the use of "arboreal" in the herpetological literature

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Abstract.—Tank bromeliads provide microhabitat that supports a high diversity of organisms in the harsh environment of tropical forest canopies. Most studies of organisms occupying tank bromeliads have focused on invertebrates found within bromeliads near or at ground level. Few investigations of vertebrate communities utilizing this keystone resource are available. We describe the amphibian and reptile community occupying the high canopy tank bromeliad, *Aechmea zebrina*, in lowland rainforest of the Yasuní Biosphere Reserve in the Amazon Basin of Ecuador. We used single-rope climbing techniques to sample a total of 160 *A. zebrina* bromeliads from 32 trees, at heights of 18.3 to 45.5 m above ground. We collected 10 metamorphosed anuran species, one gecko, one snake, and observed two species of lizard within bromeliads. Summary statistics for a suite of environmental factors associated with herpetofauna in *A. zebrina* bromeliads are reported. We estimated the density of anurans occupying *A. zebrina* communities and contrast these estimates with anuran densities from tropical forest floor anuran studies. Finally, we discuss the use of the term "arboreal" within the herpetological literature, and make recommendations for terminology used to describe the vertical space occupied by a species or assemblage.

**Key words.** Amphibian, anuran, epiphyte, forest, microhabitat, rainforest, reptile

Introduction

Forest canopies provide habitat for approximately 50% of terrestrial species, yet there are few studies specific to canopy herpetofauna (Stewart 1985; Vitt and Zani 1996; Kays and Allison 2001; Guayasamin et al. 2006; McCracken and Forstner 2008; Lowman and Schowalter 2012; Scheffers et al. 2013; McCracken and Forstner 2014). Basic ecological knowledge of arboreality (tree-living) and utilization of high canopy microhabitats by amphibians and reptiles remain depauperate in the literature (Moffett 2000; Kays and Allison 2001; Lehr et al. 2007). A canopy microhabitat frequently used by herpetofauna in tropical forests are epiphytes, and in particular epiphytic tank bromeliads that are phytotelms capable of holding relatively large volumes of water (Lowman and Rinker 2004; McCracken and Forstner 2008). In lowland Neotropical rainforest, canopy tank bromeliads typically reside in the overstory and emergent canopy trees at vertical heights of 5–45+ meters with ~5 to >150 individuals on a single tree (McCracken and Forstner 2006). These arboreal bromeliad communities create a three-dimen-

sional "wetland in the sky" that have been estimated to impound up to 50,000 liters of water per hectare (Kitching 2000; McCracken and Forstner 2006). Tank bromeliads function as a "keystone resource" in the harsh forest canopy environment where the atmosphere meets and interacts with 90% of Earth's terrestrial biomass; providing a climate-buffered refuge, water source, and food source for canopy herpetofauna (Nadkarni 1994; Ozanne et al. 2003; Cardelús and Chazdon 2005).

Kays and Allison (2001) found only 4% of 752 articles published between 1988 and 1998 on tropical forest arboreal vertebrates focused on reptiles and amphibians. Many species of herpetofauna are described as being arboreal regardless of whether they are restricted to the vertical stratum a few centimeters to a few meters above ground, or solely inhabit the high forest canopy at 20 or more meters vertical height (Chaparro et al. 2007; McCracken et al. 2007; Guayasamin and Funk 2009). Forest structure is associated with vertical partitioning or stratification of the component plant community (e.g., trees, shrubs, lianas) and accentuates vertical patterns followed by other organisms (Moffett 2000; Lowman and Rinker

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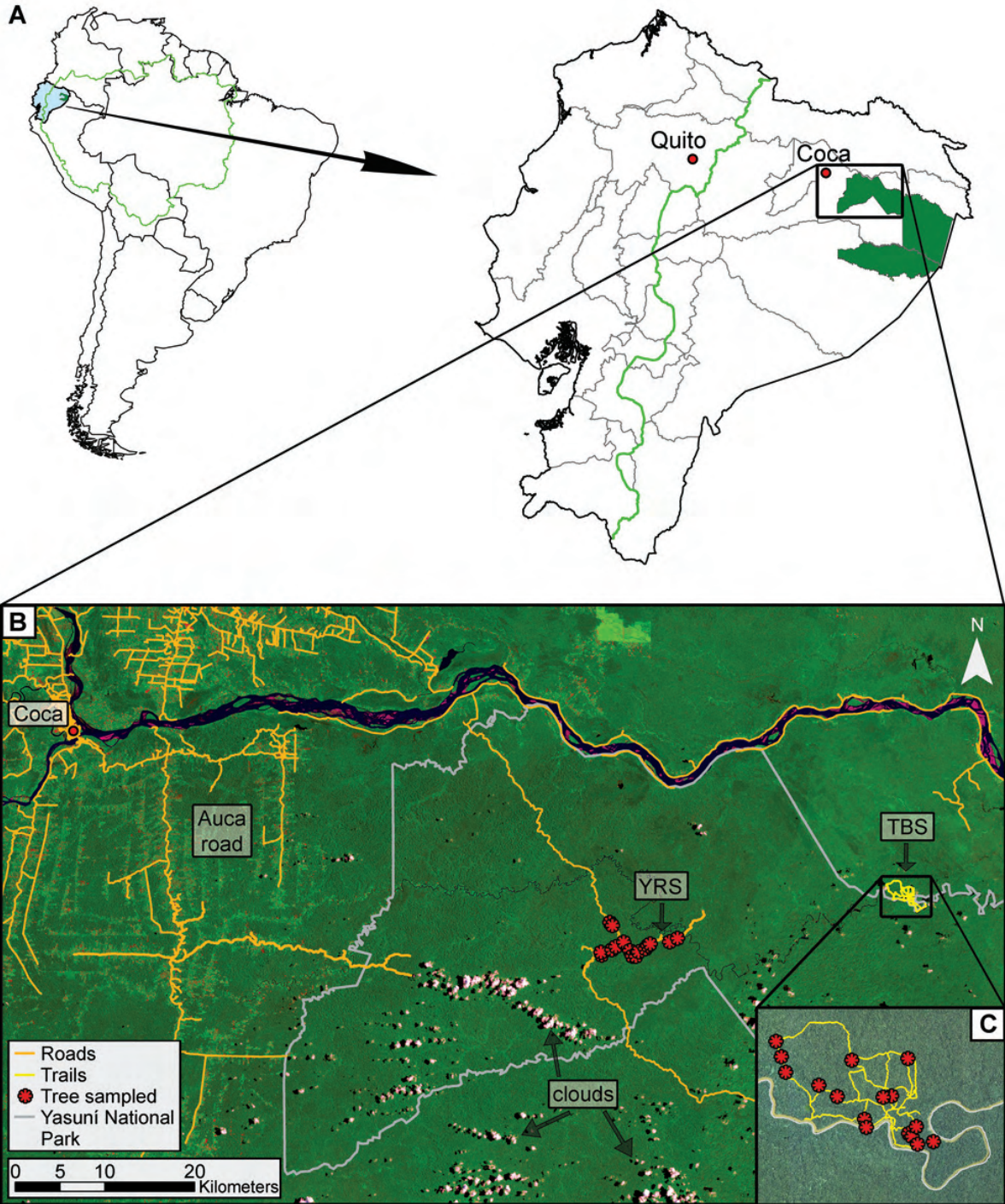
Fig. 1. (A) Map of South America with Ecuador (shaded light blue) and Yasuní National Park (solid dark green) highlighted. The Amazon ecoregion is outlined with light green line. (B) Northeastern section of Yasuní National Park (light gray line) and surrounding region where trees were sampled for *Aechmea zebrina* bromeliads within the vicinity of the Tiputini Biodiversity Station – Universidad San Francisco de Quito (TBS) and the Yasuní Research Station – Pontificia Universidad Católica del Ecuador (YRS). (C) Detail of TBS where trees were sampled for *A. zebrina* bromeliads. Note: Map is modified from Figure 2 in McCracken and Forstner (2014) and used under the Creative Commons Attribution license.

2004). Spatial patterns of forest cohabitants, such as tank bromeliads and their inhabitants, are likewise strongly influenced by forest structure as a result of the fundamental organization of resources and space (Lowman and Rinker 2004). Identifying the vertical space occupied by a particular amphibian or reptile species in its given habitat will allow greater insight to their ecological role in the system.

Herein, we describe amphibians and reptiles occupying the high canopy tank bromeliad, *Aechmea zebrina*, in lowland rainforest of the Yasuní Biosphere Reserve in the Amazon Basin of Ecuador. We report a suite of environ-
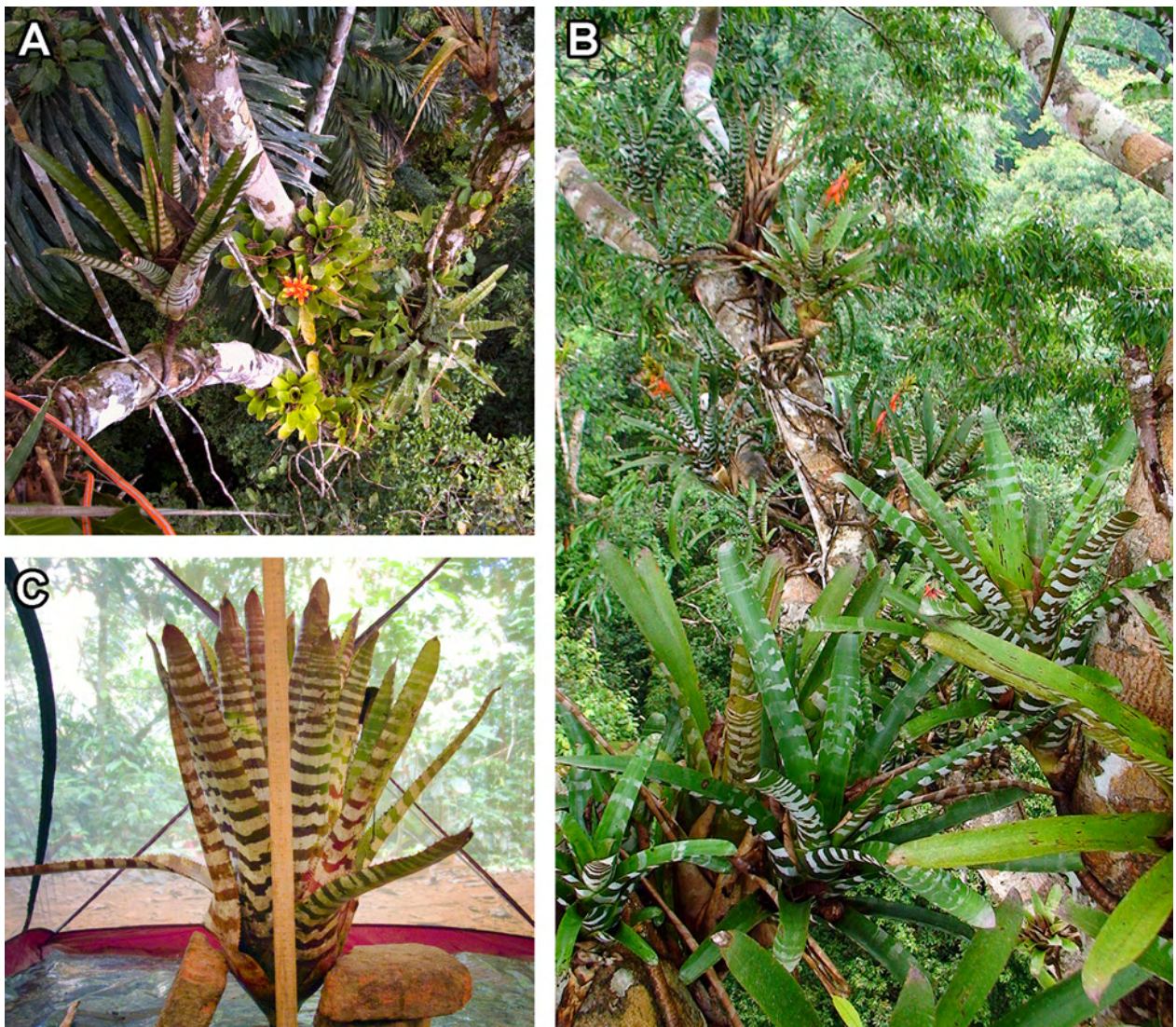
Fig. 2. (A) A downward vertical view (*in situ*) of *Aechmea zebrina* (foreground center left, and at lower elevation in upper right and center right) and a cluster of *Aechmea tessmannii* (center, with one in bloom) bromeliads in the tree canopy from ~34 m. (B) A community of *A. zebrina* bromeliads at ~38 m (*in situ*). (C) An *A. zebrina* bromeliad (*ex situ*) inside screen tent being measured and prepared for dismantling, collected from ~44 m in the canopy. Notice the more upright leaves and reddish color because of increased sun exposure due to high canopy location.

mental factors associated with herpetofauna in *A. zebrina* bromeliads. We estimate the density of anurans occupying mean *A. zebrina* community sizes in two tree size classes, representative of our shortest and tallest trees in the study. We then compared these with anuran densities from tropical forest floor anuran studies by calculating the two-dimensional area (m²) of the tree crowns for the two tree size classes. In completing our review, we feel it is important to discuss the use of the term "arboreal" within the herpetological literature and make recommendations for the incorporation of additional terminology to provide a more informative description of the vertical space utilized by a species or assemblage.

Materials and Methods

The study was conducted in the northwestern portion of the Yasuní Biosphere Reserve (Yasuní) located in Orel-

lana Province, Ecuador. The reserve includes Yasuní National Park, Waorani Ethnic Reserve, and their respective buffer and transition zones (Finer et al. 2009). Yasuní is part of the Napo Moist Forest terrestrial ecoregion covering approximately 1.7 million ha of the upper Amazon Basin (Finer et al. 2009; Bass et al. 2010). Yasuní has an elevation range of 190–400 m above sea level; the northwestern region averages 2,425–3,145 mm of rainfall per year with no less than 100 mm per month, temperature averages 25 °C (15 °–38 °C), and humidity averages 88% (Blandin 1976; Duellman 1978; Balslev et al. 1987; Bass et al. 2010). Yasuní holds world record species diversity for several taxa, including the highest documented landscape scale (lowland tropical rainforest) herpetofauna diversity with 150 species of amphibians and 121 species of reptiles (Bass et al. 2010). Collections were made in the vicinity of two research stations, the Tiputini Biodiversity Station (TBS) (0°38'14"S, 76°08'60"W) operated by
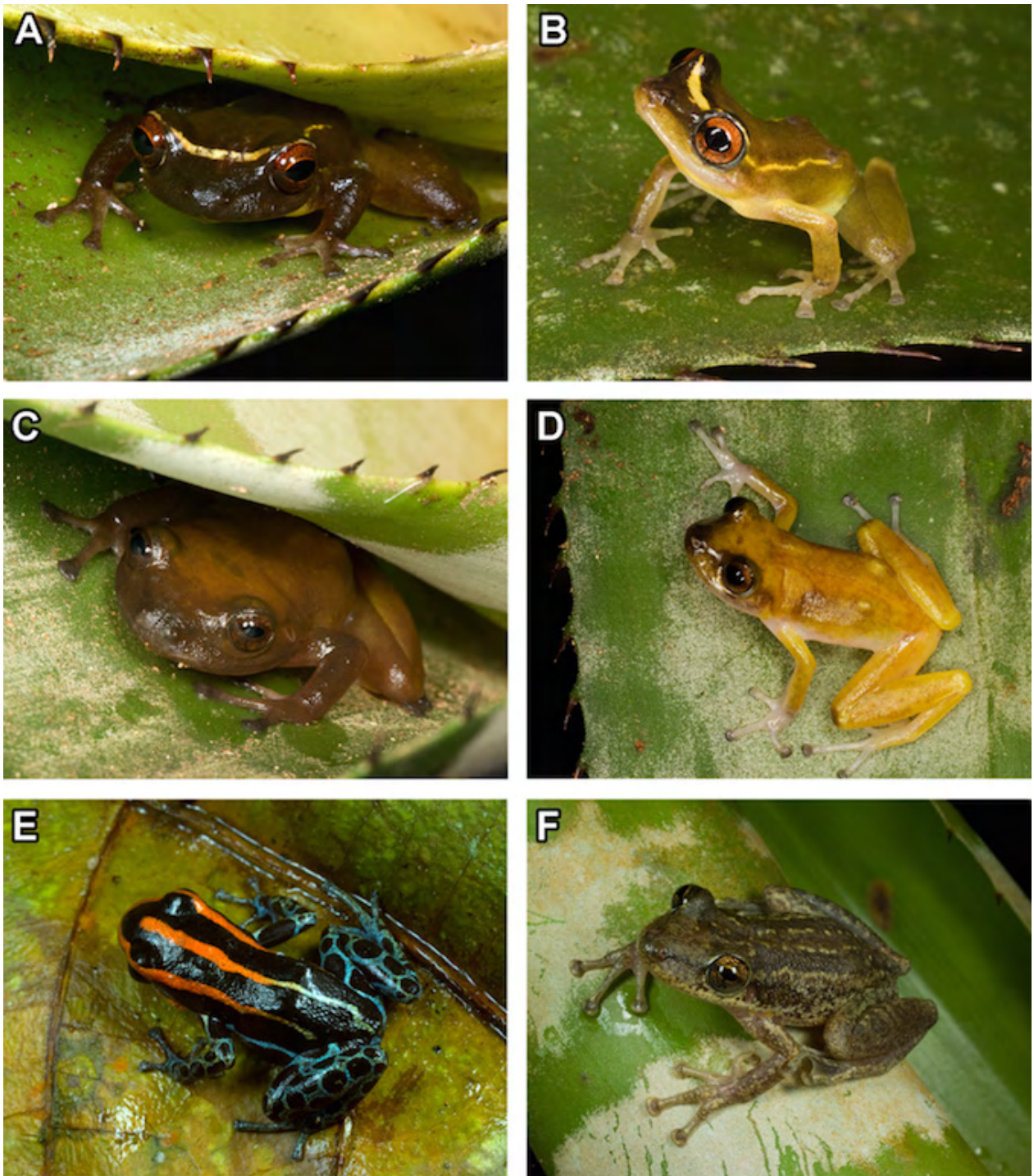
Fig. 3. A collection of anurans collected from *Aechmea zebrina* bromeliads. (A) *Pristimantis aureolineatus* hiding in leaf axil, and (B) on a leaf of *A. zebrina*. (C) *Pristimantis waoranii* emerging from leaf axil, and (D) on a leaf of *A. zebrina*. (E) *Ranitomeya ventrimaculata* and (F) *Scinax ruber* collected from *A. zebrina* bromeliads.

the Universidad San Francisco de Quito and the Yasuní Research Station (YRS) (0°40'27"S, 76°23'51"W) operated by the Pontificia Universidad Católica del Ecuador (Fig. 1). Tiputini Biodiversity Station is only accessible by river and surrounded by undisturbed primary lowland rainforest, and YRS is located approximately 27 km west on an oil pipeline road (Maxus road) that has been expe-riencing forest disturbance within its vicinity but is still surrounded by large tracts of undisturbed forest. Sampling of *A. zebrina* bromeliads took place between 0800 and 1800 hours from April to November of 2008.

We focused our sampling on a single large epiphytic tank bromeliad species, *Aechmea zebrina*, that is native to the Amazon regions of Ecuador and southeastern Co-

lombia (Smith 1953). *Aechmea zebrina* occupy vertical heights of approximately 18–45+ m in the overstory and emergent canopy trees, and range between 1 to >150 individuals on a single host tree (SFM, unpublished data). The leaves are upright and arranged in a spiral with their leaf axils tightly overlapping to form water-holding reservoirs (Fig. 2). These cavities provide a critical refuge and food source for invertebrate and vertebrate species in the harsh canopy climate (Nadkarni 1994).

Sampling methodology for *A. zebrina* bromeliads followed our previously published methods (McCracken and Forstner 2008). Single-rope technique (SRT) was used to climb trees for canopy access, and five bromeliads were collected haphazardly from each tree at estimated even vertical intervals between one another (Perry 1978). Before each bromeliad removal, we checked for active amphibians or reptiles, we recorded the bromeliads elevation, measured the air temperature adjacent to the bromeliad, and the temperature and pH of water held in one of the outer leaf axils. Ideally, when the bromeliad is disturbed the response of most animals is a retreat into the bromeliads leaf bracts and thus prevents loss of specimens (McCracken and Forstner 2008). Bromeliads were removed and sealed in a 55-gallon (208 L) plastic bag and then lowered to the ground. After bromeliad collections we counted the number of *A. zebrina* inhabiting the tree and measured tree height. Bromeliads were transported back to camp where we processed them in a screened tent to prevent escape of animals. We first poured all water from the bromeliads through a 1 mm sieve to separate arthropods, leaf litter, and detritus. We then measured the water volume with a graduated cylinder and the pH of the homogenized solution. We counted the number of mature leaves (used as a size metric) and measured the height of bromeliads to nearest centimeter (from base of plant to highest vertical leaf tip). Bromeliads were then dismantled leaf-by-leaf to collect all herpetofauna.

We identified and counted all metamorphosed anurans and reptiles to species level for each bromeliad. Larval anurans were also collected and counted, with the majority identified to genus or species. In an attempt to better identify larval anurans we maintained individual tadpoles outdoors in 12 oz. plastic cups with water and detritus collected from bromeliads. Once tadpoles began to metamorphose the cups were covered with window screen to prevent escape. Upon sufficient development to allow identification the froglets were euthanized and preserved. All herpetofauna were handled and preserved following the guidelines compiled by the American Society of Ichthyologists and Herpetologists (ASIH), and in compliance to the rules overseen by the Texas State University Animal Care and Use Committee (Permit #: 0721-0530-7, 05-05C38ADFDB, and 06-01C694AF). Additionally, we report the herpetofauna species observed active amongst *A. zebrina* bromeliads but not collected. We calculated summary statistics of recorded habitat variables for each species and report raw data for

Fig. 4. The Banded cat-eyed snake, *Leptodeira annulata*, collected in an *Aechmea zebrina* bromeliad at 43.5 m above the forest floor.

singletons and doubletons. Summary statistics were calculated for recorded habitat variables across all bromeliads sampled, bromeliads occupied by ≥1 metamorphosed anurans, and bromeliads not occupied by anurans.

We then compared an estimated number of anuran individuals in *A. zebrina* bromeliads per 100 m² of tree crown area to other published work of tropical frog assemblages collected at or near ground level. No other studies were available to provide canopy estimates. Mean anuran abundance per tree was calculated by taking the mean number of metamorphosed anurans per *A. zebrina* bromeliad ($\overline{x} = 0.6$) and multiplying by the mean number of bromeliads per tree ($\overline{x} = 66$). Based on tree crown diameter measurements by Asner et al. (2002) in lowland rainforest of eastern Amazonia we calculated the number of anurans per 100 m² of a typical tree crown area for the two largest tree size classes. The two largest classes had mean tree heights of 25.3 m (Dominant) and 46.1 m (Super dominant) with a mean crown diameter of 11.6 m and 19.9 m, respectively. Mean tree crown diameters were used to calculate the area of a circle. These two tree size classes were used as we did not measure individual crown diameters and consider these two as representative of the shortest (28 m) and tallest (49 m) trees in our study. We then divided the mean number of anurans per tree
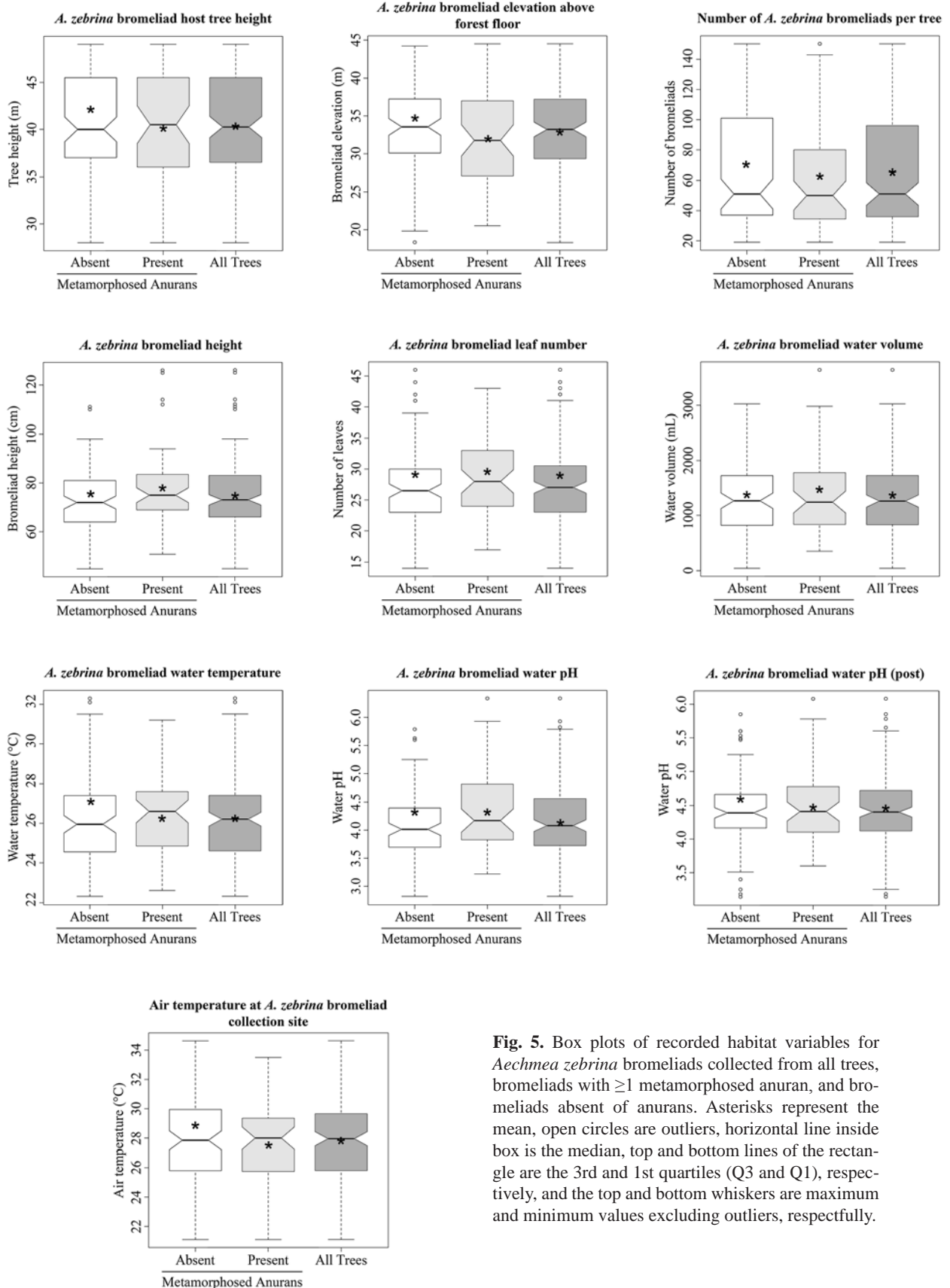
Fig. 5. Box plots of recorded habitat variables for *Aechmea zebrina* bromeliads collected from all trees, bromeliads with ≥1 metamorphosed anuran, and bromeliads absent of anurans. Asterisks represent the mean, open circles are outliers, horizontal line inside box is the median, top and bottom lines of the rectangle are the 3rd and 1st quartiles (Q3 and Q1), respectively, and the top and bottom whiskers are maximum and minimum values excluding outliers, respectfully.

in our study by the tree size class crown area calculated from Asner et al. (2002) and multiplied by 100 to generate an estimated density of individuals per 100 m^2.

All calculations and statistics based on counts of metamorphosed anurans collected (not larval anurans) and conducted in the R statistical software (version 3.0.1) (R Development Core Team 2013).

Results

We sampled five bromeliads from each of 32 trees for a total of 160 *A. zebrina* bromeliads sampled. We collected 10 metamorphosed anuran species (Fig. 3), one gecko, one snake (Fig. 4), and two species of lizard were observed amongst bromeliad leaves but not collected (Table 1). A total of 95 metamorphosed anurans ($\overline{x} = 0.6$ per bromeliad) were collected from 56 of the 160 bromeliads (35%) sampled. Between one and five individuals ($\overline{x} = 1.7$), and up to two species were observed in single *A. zebrina* bromeliads occupied by metamorphosed anurans. The species found together include (number of bromeliads with species together): *Pristimantis aureolineatus* and *P. waoranii* (7), *P. waoranii* and *P. acuminatus* (1), *P. waoranii* and *P. orphnolaimus* (1), *P. aureolineatus* and *Ranitomeya ventrimaculata* (1). We also collected a minimum of four larval amphibian species from the water-filled leaf axils of *A. zebrina* bromeliads including *Osteocephalus fuscifacies*, *O. planiceps*, *Ranitomeya variabilis*, and *R. ventrimaculatus*. A total of 271 larval anurans were collected from 35 of the 160 bromeliads (21.9%) sampled, with 14 of the 35 larval occupied bromeliads (40%) also occupied by ≥1 metamorphosed anurans. *Osteocephalus* spp. tadpoles account for 60.5% ($n = 164$) of confirmed species identifications for all larval anurans, and these were collected from five bromeliads.

A single *O. fuscifacies* and a single *O. planiceps* (both adults) were each found in separate bromeliads with larvae of same species (identified after rearing). The gecko, *Thecadactylus solimoensis* (formerly *T. rapicauda*), was found in an *A. zebrina* bromeliad amongst the outer leaf axils at 31.5 m above the forest floor in a tree 46.0 m tall (Bergmann and Russell 2007). The Banded cat-eyed snake, *Leptodeira annulata*, was found in a central leaf axil of an *A. zebrina* bromeliad at 43.5 m above the forest floor in a tree 45.5 m tall (Fig. 4). *Anolis transversalis* was observed twice amongst the leaves of *A. zebrina* bromeliads during collections; once on a bromeliad at ~27 m above the forest floor (36 m tall tree) and in another tree at ~35 m above the forest floor (41 m tall tree). A single male *Anolis ortonii* was observed displaying his dewlap on an outer leaf of an *A. zebrina* bromeliad at ~20 m above the forest floor in a tree 28 m tall. Summary statistics for all species reported in Table 1.

Trees sampled for *A. zebrina* bromeliads were 28 to 49 m in height ($\overline{x} = 40.4$ m ± 5.5, $n = 32$), and 28 to 49 m in height ($\overline{x} = 40.2$ m ± 5.8, $n = 27$) for trees with ≥1 bromeliad occupied by metamorphosed anurans. *Aechmea zebrina* bromeliads were collected at above ground elevations of 18.3 to 44.5 m ($\overline{x} = 32.9$ m ± 5.6, $n = 160$), and bromeliads occupied by ≥1 metamorphosed anurans occurred at elevations of 20.5 to 44.5 m ($\overline{x} = 32.1$ m ± 6.3, $n = 56$). The number of *A. zebrina* bromeliads per host tree was 19 to 150 individuals ($\overline{x} = 66 ± 40$, $n = 32$), and 19 to 150 individuals ($\overline{x} = 63 ± 38$, n = 27) for trees with ≥1 bromeliads occupied by metamorphosed anurans. *Aechmea zebrina* bromeliads were 45 to 126 cm in height ($\overline{x} = 75 ± 14$, $n = 160$), and 51 to 125 cm in height ($\overline{x} = 78 ± 15$, $n = 56$) for bromeliads occupied by ≥1 metamorphosed anurans. The number of mature leaves per *A. zebrina* was 14 to 46 ($\overline{x} = 28 ± 6$, $n = 160$), and 17 to 43 ($\overline{x} = 29 ± 6$, $n = 56$) for bromeliads occupied

Table 1. Amphibians and reptiles collected or observed within *Aechmea zebrina* bromeliads. For each species the number observed, height range (bromeliad in tree), and mean height are provided. Only metamorphed anurans at time of collection included.

Species	Number observed	Height range (m)	Mean height (m)
Osteocephalus fuscifacies	3	24.3-28.1	25.6
Osteocephalus planiceps	1	31.5	–
Osteocephalus taurinus	1	30.6	–
Pristimantis acuminatus	1	40.4	–
Pristimantis aureolineatus	36	22-44.5	35.7
Pristimantis orphnolaimus	2	31.5-38.3	34.9
Pristimantis waoranii	35	21.2-43.9	31.9
Ranitomeya ventrimaculata	1	36.5	–
Ranitomeya variabilis	9	25.7-35.2	30.9
Scinax ruber	6	33.8-35	34.8
Anolis ortonii	1	20	–
Anolis transversalis	2	27-35	31
Thecadactylus solimoensis	1	31.5	–
Leptodeira annulata	1	43.5	–

by ≥1 metamorphosed anurans. The water volume of *A. zebrina* bromeliads was 42 to 3645 mL ($\bar{x}$ = 1343 ± 656, *n* = 160), and 355 to 3645 mL ($\bar{x}$ = 1428 ± 726, *n* = 56) for bromeliads occupied by ≥1 metamorphosed anurans. Water temperature within an outer leaf axil of *A. zebrina* bromeliads at time of collection was 22.3 to 32.3 °C ($\bar{x}$ = 26.2 ± 2.1, *n* = 160), and 22.6 to 31.2 °C ($\bar{x}$ = 26.2 ± 1.9, *n* = 56) for bromeliads occupied by ≥1 metamorphosed anurans. Water pH within an outer leaf axil of *A. zebrina* bromeliads at time of collection was 2.82 to 6.34 ($\bar{x}$ = 4.18 ± 0.66, *n* = 160), and 3.22 to 6.34 ($\bar{x}$ = 4.34 ± 0.73, *n* = 56) for bromeliads occupied by ≥1 metamorphosed anurans. Water pH of sieved homogenized water for each *A. zebrina* bromeliad was 3.14 to 6.08 ($\bar{x}$ = 4.44 ± 0.53, *n* = 160), and 3.60 to 6.08 ($\bar{x}$ = 4.48 ± 0.55, *n* = 56) for bromeliads occupied by ≥1 metamorphosed anurans. Air temperature adjacent to bromeliads at time of collection was 21.1 to 34.6 °C ($\bar{x}$ = 27.8 ± 2.8, *n* = 160), and 21.1 to 33.5 °C ($\bar{x}$ = 27.6 ± 2.7, *n* = 56) for bromeliads occupied by ≥1 metamorphosed anurans. Summary statistics for bromeliads absent of anurans are contrasted with those given above in Fig. 5.

By taking the mean number of metamorphosed anurans per bromeliad ($\bar{x}$ = 0.6) and multiplying by the mean number of *A. zebrina* bromeliads per tree ($\bar{x}$ = 66), we calculated an estimated mean of 39.6 metamorphosed anurans occupying the *A. zebrina* bromeliads of an average tree in our study. The Dominant class tree crown area from Asner et al. (2002) was 105.7 m² (25.3 m tall) with a calculated 37.5 anurans per 100 m², and the Super dominant class tree crown area was 311 m² (46.1 m tall) with a calculated 12.7 anurans per 100 m².

Discussion

Our study identified 14 species of herpetofauna (10 anurans and four reptiles) utilizing the tank bromeliad *Aechmea zebrina* as habitat in the high canopy environment of the northwestern Amazon Basin. A range of 1–5 metamorphosed anurans per bromeliad, with up to two species occupying a single bromeliad, were detected in over one-third of the bromeliads sampled. The observation of larval, metamorphs, and adults of *Osteocephalus fuscifacies* confirm that this species is a phytotelm breeder as proposed by Jungfer et al. (2013). The observation of larval, metamorphs, and an adult *Ranitomeya ventrimaculata* confirm that this species does deposit tadpoles in high canopy bromeliads as proposed by Brown et al. (2011). Our detection of the gecko *Thecadactylus solimoensis* at 31.5 m vertical height within the leaf axil of an *A. zebrina* bromeliad confirms this species use of bromeliads in the high canopy (Vitt and Zani 1997; Bergmann and Russell 2007). Our observation of the snake *Leptodeira annulata* within the leaf axils of an *A. zebrina* bromeliad at 43.5 m in the canopy is the highest recorded vertical height to our knowledge; *L. annulata* is described as terrestrial to

semi-arboreal with a previous maximum observed vertical height of 6 m above ground (Duellman 1978; Vitt 1996; Kacoliris 2006; Ávila and Morais 2007).

In McCracken and Forstner (2014) we analyzed the habitat data for differences among forest disturbance treatments and found no differences in habitat variables between treatments and no relationships between habitat variables and anuran occupancy or abundance. Also, we found differences between forest disturbance treatments for anuran abundance and occupancy; but report the summary statistics of the habitat data here as a resource characterizing the habitat occupied by canopy tank bromeliad dwelling herpetofauna. Of particular interest in this study was the mildly acidic mean water pH (4.18 in situ in leaf axils, 4.34 in sieved homogenized water) in *A. zebrina* bromeliads; as this is within the range reported to affect development of embryonic and larval anurans (Beattie and Tyler-Jones 1992). However, bromeliads are a known breeding site for amphibians and we observed an abundance of aquatic invertebrates and larval anurans in our collections (Benzing 2000).

Using the two largest tree size classes of Amazonian trees from Asner et al. (2002) as representative crown area for the shortest (28 m) and tallest (49 m) trees in our study, we calculated an estimate of 12.7–37.5 anurans per 100 m² of crown area for an average tree in our study. We consider this estimated range of canopy anuran density to be conservative because 1) the height of trees for the tree size classes used from Asner et al. (2002) are shorter than our shortest and tallest trees; 2) it is calculated on the two-dimensional space of the tree crown and does not include the vertical space occupied by a tree; 3) anurans were only collected from *A. zebrina* bromeliads and not other available habitat; and 4) the mean anuran abundance per tree in our study was used for calculations of both tree size classes, not accounting for the range of tree heights and number of bromeliads per tree. Regardless of these constraints, the estimated high anuran density of 37.5 anurans/100 m² is the greatest of any reported density for tropical frog assemblages from comparable studies (e.g., 36.1 anurans/100 m2 at La Selva, Costa Rica [Lieberman 1986]; 15.5 anurans/100 m² at Rio Llullapichis, Peru [Toft 1980]; see also Allmon 1991 and Rocha et al. 2007 for compiled sites comparison). The low estimate of 12.7 anurans/100 m² is still amongst the highest densities of reported studies, particularly in South America (Allmon 1991; Rocha et al. 2007). A limitation of this comparison is that these studies rely on the method of quadrat surveys for density calculations, where the majority of observed anurans are going to be leaf-litter inhabitants or those that are within arms reach (~2 m vertical height). Achieving a more accurate canopy anuran density will require research sampling all available canopy microhabitats and recording crown measurements for all sampled trees.

Within the herpetological community the use of the term "arboreal" has deviated from its recognized defini-

tion of "inhabiting or frequenting trees" and taken on a broader meaning in reference to vertical habitat use by amphibians and reptiles to simply mean living above ground level (Merriam-Webster.com. 2014. Merriam-Webster Dictionary. Available from http://www.merriam-webster. com [Accessed 27 April 2014]). While this definition suffices to distinguish these species (arboreal) from those occupying fossorial and ground level habitat, it does not adequately clarify the above ground vertical space utilized by a particular species. As an example, Doan (2003) reports the visual encounter survey (VES) method as the best way to sample for arboreal herpetofauna in rainforests. The VES method only allows the researcher access to habitat within arms reach (~2 m vertical height) and fails entirely at observing animals within the other ~40+ m of vertical habitat above in many rainforests. Arboreal herpetofauna may occur at vertical heights between >0 m to 88 m on vegetation and/or trees; simply referring to a species as arboreal provides no information about its occupied vertical range (Spickler et al. 2006). To alleviate confusion and accurately represent the vertical space occupied by a species or assemblage we propose two alternatives to be used separately or preferably together. First, basic descriptors delineating vertical zones for a defined forest type could be used to accompany "arboreal" (e.g., "arboreal within the understory" where "understory" has been defined as "near-ground nondominant vegetation") (Dial et al. 2004). Second, authors should specify vertical height ranges when describing or discussing "arboreal" anurans (e.g., "the arboreal frog *Pristimantis waoranii* is found in the overstory at 20.5–44 m" where "overstory" has been defined as "high, dominant foliage") (Dial et al. 2004). Providing vertical range data or descriptions is critical to understanding the many aspects of natural history for a species.

Conclusion

The canopy of tropical forests are among the most species-rich terrestrial habitats on Earth, yet remain a relatively unexplored biotic frontier (Basset et al. 2003; Lowman and Schowalter 2012). Our research has shown the tank bromeliad *Aechmea zebrina* to support a diverse and abundant herpetofauna community in the harsh equatorial tree canopy environment of the Yasuní Biosphere Reserve in the Amazon Basin of Ecuador. Additionally, our canopy work has contributed to the description of two new species of bromeliad-inhabiting anurans (*Pristimantis aureolineatus* [Guayasamin et al. 2006] and *P. waoranii* [McCracken et al. 2007]), the detection of *Batrachochytrium dendrobatidis* (Chytrid fungus) on anurans from the forest floor to the canopy in Amazonia (McCracken et al. 2009), and identified the use of high canopy bromeliads by the anuran *Scinax ruber* (McCracken and Forstner 2014). While canopy surveys of tank bromeliads are labor intensive, they provide a very

effective technique for collecting data on canopy inhabiting organisms and associated microhabitat factors.

Our estimates of canopy anuran densities, based on collections from a single species of bromeliad, demonstrate the potential ecological importance and current lack of knowledge on the canopy herpetofauna component in tropical systems. Typical inventories of herpetofauna in tropical forests are conducted at ground level (~2 m vertical height stratum) where microclimatic variables are more stable (Guayasamin et al. 2006). Sampling such shallow strata within the strongly vertical structure of these forests has likely served to bias metrics of herpetofauna assemblages by focusing on a narrow environmental space and neglecting the large available habitat above into the canopy (Guayasamin et al. 2006; Scheffers et al. 2014). Future inventory studies should routinely include canopy surveys to properly represent the herpetofauna of forested habitat.

Use of the term "arboreal" in the herpetofauna literature does not adequately define the vertical range of a species or assemblage. This serves to limit compilation and synthesis from the literature for the ecology of many of these tropical reptiles and amphibians. Our proposed amendments to accompany the description of arboreality in herpetofauna functionally serve to give scale and provide a better understanding of the vertical habitat utilized by a species or assemblage. As research on canopy herpetofauna continues to expand, knowledge of the vertical space occupied will be essential to answering hypothesis-driven research questions and enacting sufficient conservation measures to protect all species.

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A new species of *Pholidobolus* (Squamata: Gymnophthalmidae) from the Andes of southern Ecuador

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Abstract.—We describe a new species of *Pholidobolus* lizard from the Amazonian slopes of the Andes of southern Ecuador. Among other characters, the new species differs from other species of *Pholidobolus* in having a distinct diagonal white stripe extending from the fourth genial scale to the fore limb. We present a phylogeny based on mitochondrial DNA sequence data as additional evidence supporting delimitation of the new species, which is sister to all other species of *Pholidobolus*. Our phylogeny further supports the south-to-north speciation hypothesis proposed for other lizard clades from the northern Andes.

Key words. Clade *Pholidobolus*, DNA, lizard, phylogeny, South America, systematics

Introduction

The gymnophthalmid lizard clade *Pholidobolus* was recently defined by Torres-Carvajal and Mafla-Endara (2013) as the largest crown clade containing *Pholidobolus montium* Peters, 1863, but not *Macropholidus ruthveni* Noble, 1921. This phylogenetic definition (de Queiroz and Gauthier 1994) is based on a phylogenetic tree obtained from analyses of mitochondrial DNA nucleotide sequence data (Torres-Carvajal and Mafla-Endara 2013), and is in conflict with previous non-phylogenetic definitions of both *Pholidobolus* and *Macropholidus* (Montanucci 1973; Reeder 1996) based on morphological data. As defined by Torres-Carvajal and Mafla-Endara (2013), *Pholidobolus* contains four species—*P. affinis*, *P. macbrydei*, *P. montium*, and *P. prefrontalis*. Contrary to previous taxonomic arrangements (Montanucci 1973; Reeder 1996), "*P.*" *annectens* was shown to be part of the clade (traditionally ranked as a genus) *Macropholidus*. In addition, Torres-Carvajal and Mafla-Endara (2013) concluded that the controversial generic allocation of *P. anomalus* from southern Peru (Montanucci 1973; Reeder 1996) still remains to be established.

Pholidobolus lizards occur between 1,800 and 4,100 m along the southern part of the northern Andes (i.e., Ecuador and southern Colombia). Only one species, *P. macbrydei*, occurs also in the Huancabamba Depression in extreme southern Ecuador and possibly northern Peru.
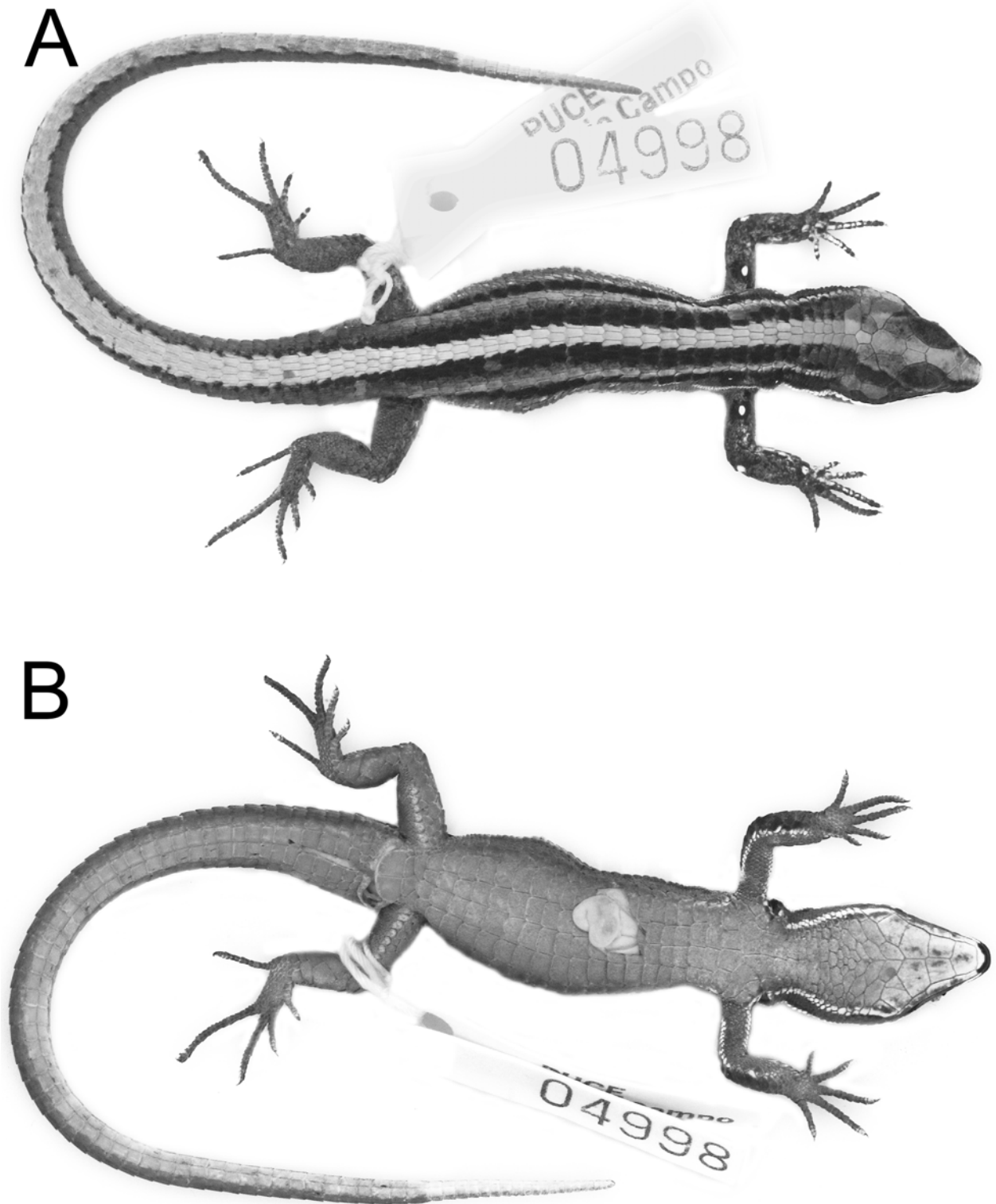
Herein, we describe a new species of *Pholidobolus* from the Andes in southern Ecuador using data on morphology and color pattern. We also present molecular evidence supporting recognition of the new species by performing phylogenetic analyses of nucleotide sequence data.

Methods

Morphological data: Type specimens and additional specimens examined (Appendix 1) were deposited in the herpetological collection at Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ). The following measurements were taken with a digital caliper and recorded to the nearest 0.1 mm, except for tail length, 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), snout-vent length (SVL), and tail length (TL). Sex was determined by dissection or by noting the presence of everted hemipenes. We follow the terminology of Reeder (1996) for description of the holotype and scale counts. Data for other species of *Pholidobolus* were taken from Montanucci (1973).

The left hemipenis of two type specimens of the new species (QCAZ 4998 and 4999) were prepared following the procedures of Manzani and Abe (1988), as modified by Pesantes (1994) and Zaher (1999), where the retractor

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Fig. 1. Holotype (QCAZ 4998; SVL = 45.52 mm) of *Pholidobolus hillisi* sp. nov. in dorsal (**A**) and ventral (**B**) views. *Photographs by OTC.*

muscle is manually separated and the everted organ is filled with stained petroleum jelly and paraffin. In addition, the hemipenial calcareous structures were stained in an alcoholic solution of Alizarin Red, following the adaptation of the procedures of Uzzell (1973) proposed by Nunes et al. (2012). Description of the hemipenes follows the terminology of Dowling and Savage (1960), Savage (1997), Myers and Donnelly (2001, 2008), and Nunes et al. (2012).

DNA sequence data: Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Tissue samples were first mixed with Proteinase K and a lysis buffer and digested overnight prior to extraction. DNA samples were quantified using a Nanodrop® ND-1000 (NanoDrop Technologies, Inc), re-suspended and diluted to 25 ng/ul in ddH2O prior to amplification.

Using primers and amplification protocols from the literature (Pellegrino et al. 2001; Torres-Carvajal and Mafla-Endara 2013) we obtained 1,573 nucleotides (nt) representing mitochondrial genes 12S (344 nt), 16S (549 nt), and ND4 (680 nt) from three individuals of the new species described herein (GenBank accession numbers KP090167-KP090175).

Chronophylogenetic analyses: We added the three sequences generated in this study to the mtDNA dataset of Torres-Carvajal and Mafla-Endara (2013). Editing, assembly, and alignment of sequences were performed with Geneious Pro™ 5.3 (Biomatters Ltd. 2010). Genes were combined into a single dataset with three partitions, one per gene. The model of evolution for each partition was obtained in jModeltest 2 (Darriba et al. 2012) under the Akaike information criterion. Chronophylogenetic analyses were performed in Beast 2.1.3 (Bouckaert et al. 2014) as described in Torres-Carvajal and Mafla-Endara (2013), except that we performed four independent 108 generation runs with random starting trees, sampling every 10,000 generations. The resultant 36,000 trees were used to calculate posterior probabilities (PP) for each bipartition in a maximum clade credibility tree in TreeAnnotator 2.1.2 (Rambaut and Drummond 2014).

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

Pholidobolus hillisi sp. nov.

urn:lsid:zoobank.org:act:EB5A9DDD-742C-456F-B5C9-6E57EDEEE698

Proposed standard English name: **Cuilanes of Hillis**
Proposed standard Spanish name: **Cuilanes de Hillis**

Holotype: QCAZ 4998 (Figs. 1, 2), adult male, Ecuador, Provincia Zamora-Chinchipe, near San Francisco Research Station on Loja-Zamora road, 3°57'57"S, 79°4'45"W, WGS84, 1,840 m, 21 July 2012, collected by Santiago R. Ron, Andrés Merino, Fernando Ayala, Teresa Camacho, and Martin Cohen.

Paratypes (5): ECUADOR: Provincia Zamora-Chinchipe: QCAZ 4999 (adult male), 5000 (juvenile female), same data as holotype; QCAZ 6840 (adult female), 6842 (adult female), 6844 (adult male), San Francisco Research Station, 3°58'14"S, 79°4'41"W, WGS84, 1,840 m, 29 October 2004, 9 June 2005, and 29 September 2005, respectively, collected by Kristin Roos, Alban Pfeiffer, Andy Fries, Ulf Soltau, and Florian Werner.
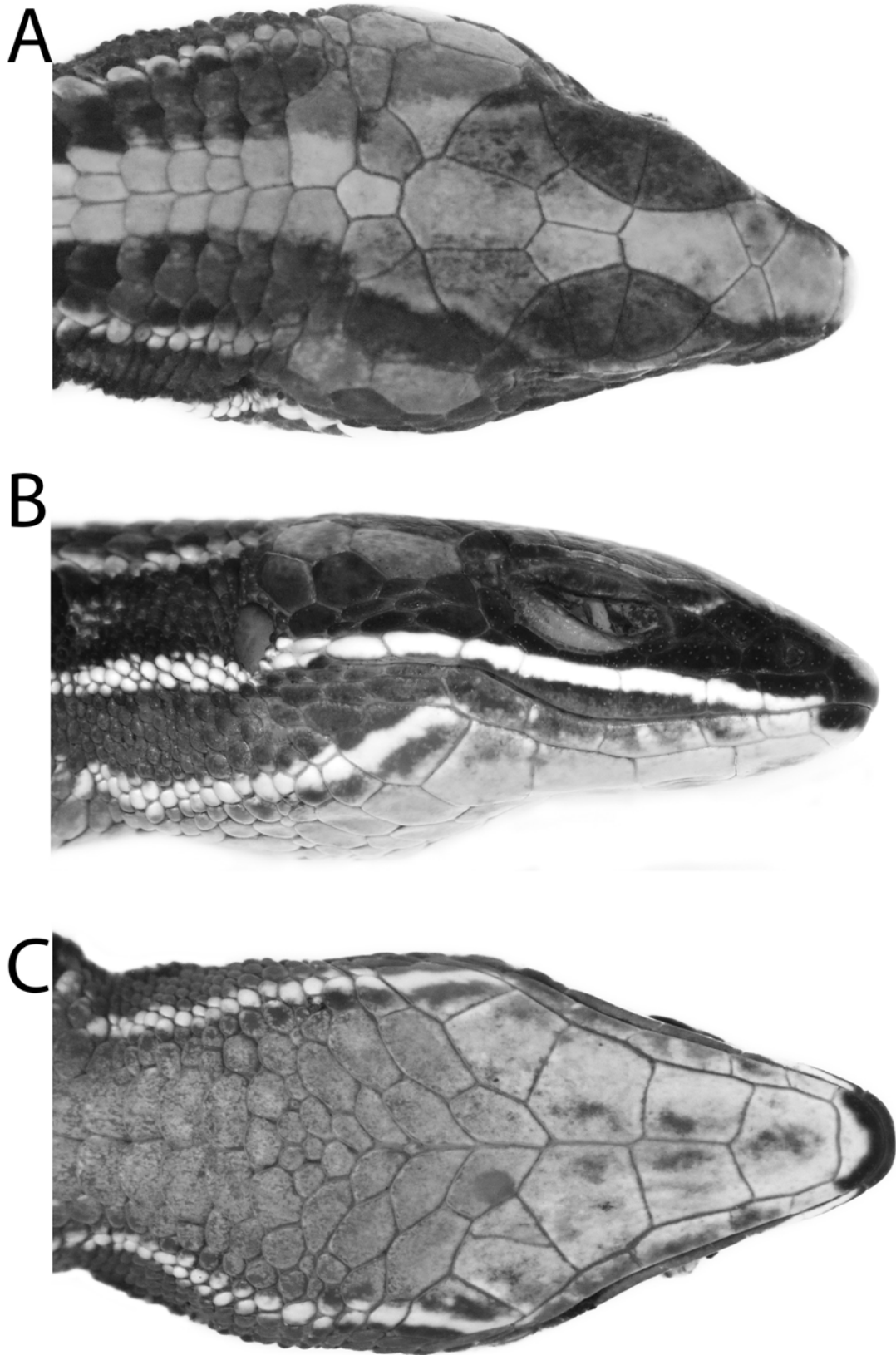
Diagnosis: *Pholidobolus hillisi* is unique among species of *Pholidobolus* in having a distinct diagonal white stripe on each side of the chin, extending from the fourth genial to the fore limb (Fig. 3). It further differs from all species of *Pholidobolus*, except *P. affinis*, in having three supraoculars (two in *P. macbrydei*, *P. montium*, and *P. prefrontalis*). *Pholidobolus affinis* differs from the new species by having flanks with black reticulations on a reddish orange ground color (flanks brown in *P. hillisi*; Fig. 4).

The new species also can be distinguished from *P. montium* and *P. macbrydei* by the presence of prefrontal scales (absent in the last two species). While *P. hillisi* shares with *P. affinis* and *P. prefrontalis* the presence of prefrontal scales, it differs from them in having a dark brown dorsum with a conspicuous light brown vertebral stripe (dorsum pale brown without a vertebral stripe in *P. affinis* and *P. prefrontalis*; Fig. 4). Furthermore, *P. hillisi* has fewer dorsal scales in transverse rows (28–31) than *P. affinis* (45–55), *P. montium* (35–50), *P. prefrontalis* (37–46), and P. *macbrydei* (31–43).

Pholidobolus hillisi shares with all other recognized species of *Pholidobolus* the absence of a single transparent palpebral disc and the presence of a ventrolateral fold between fore and hind limbs. These characters distinguish members of *Pholidobolus* from members of its sister clade *Macropholidus* (Torres-Carvajal and Mafla-Endara 2013).

Characterization: (1) Three supraoculars, anteriormost larger than posterior one; (2) prefrontals present; (3) femoral pores present in both sexes; (4) two to five opaque lower eyelid scales; (5) scales on dorsal surface of neck striated, becoming keeled from fore limbs to tail; (6) two or four rows of lateral granules at midbody; (7) 28–31 dorsal scales between occipital and posterior margin of hind limb; (8) lateral body fold present; (9) keeled ventrolateral scales on each side absent; (10) dorsum dark brown with a conspicuous narrow, pale brown, vertebral stripe that becomes grayish brown towards the tail; (11) labial stripe white; (12) sides of body dark brown;

Fig. 2. Head of the holotype (QCAZ 4998) of *Pholidobolus hillisi* sp. nov. in dorsal (**A**), lateral (**B**), and ventral (**C**) views. *Photographs by OTC.*
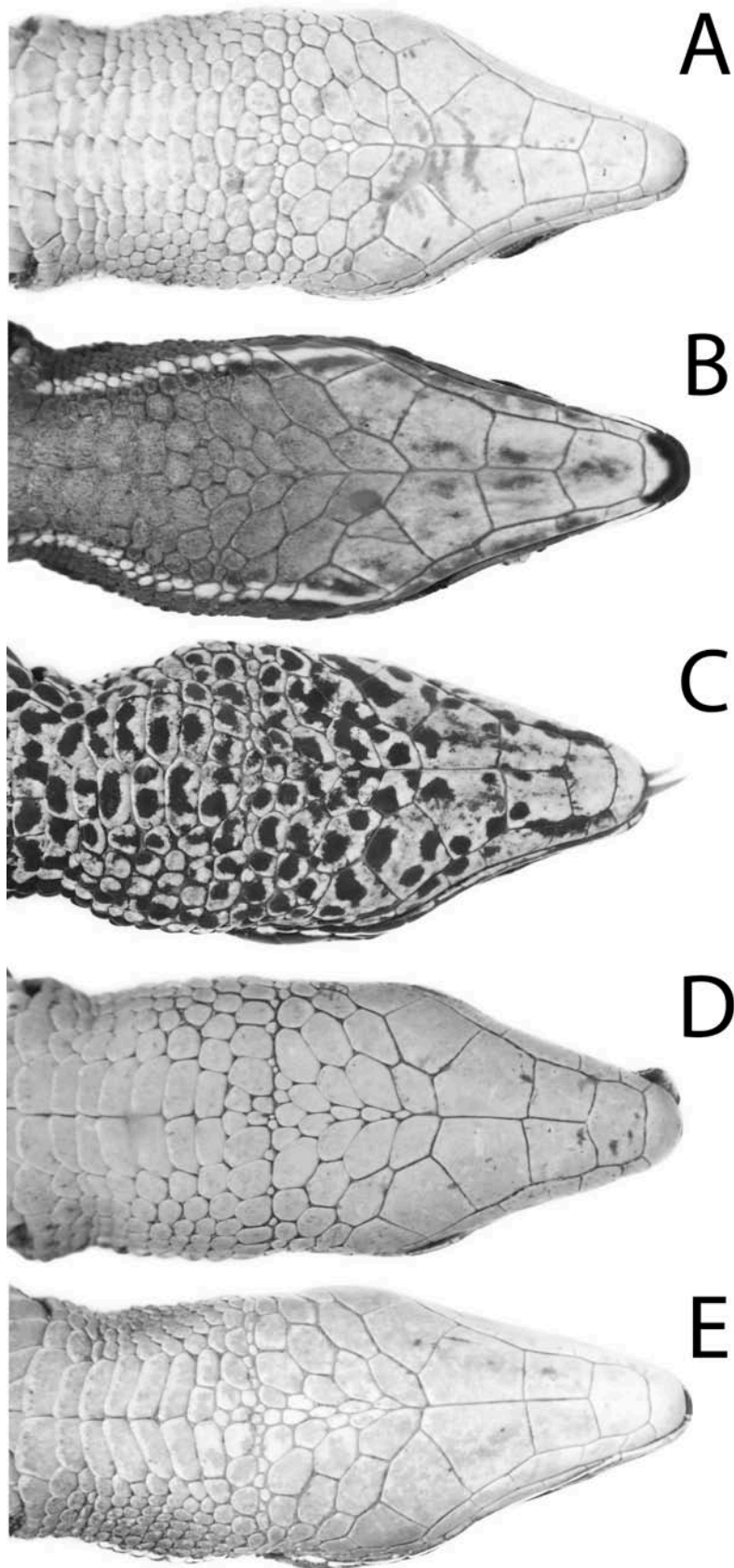
Fig. 3. Head of five species of *Pholidobolus* in ventral view. (A) *P. affinis*; (B) *P. hillisi* sp. nov.; (C) *P. macbrydei*; (D) *P. montium*; (E) *P. prefrontalis*. *Photographs by OTC.*

(13) white stripe along fore limb present; (14) a distinct diagonal white stripe on each side of the chin, extending from the fourth genial to the fore limb; (15) adult males with red flecks and ocelli (black with white center) dorsal to insertion of fore and hind limbs.

Description of holotype: Adult male (QCAZ 4998); snout-vent length 45.52 mm; tail length 104 mm; dorsal and lateral head scales juxtaposed, finely wrinkled; rostral hexagonal, 2.09 times as wide as high; frontonasal pentagonal, wider than long, laterally in contact with nasal, smaller than frontal; prefrontals pentagonal, nearly as wide as long, with medial suture, laterally in contact with loreal and first superciliary; frontal hexagonal, longer than wide, slightly wider anteriorly, in contact with the prefrontals and supraoculars I and II on each side; frontoparietals pentagonal, longer than wide, with medial suture, each in contact laterally with supraoculars II and III; interparietal roughly hexagonal, lateral borders parallel to each other; parietals slightly smaller than interparietal, tetragonal and positioned anterolaterally to interparietal, each in contact laterally with supraocular III 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 hexagonal, juxtaposed, smooth; two large supratemporal scales, smooth; nasal divided, irregularly pentagonal, longer than wide, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally, loreal posterodorsally and frenocular posteroventrally; nostril on ventral aspect of nasal, directed lateroposteriorly, piercing nasal suture; loreal rectangular; frenocular enlarged, in contact with nasal, separating loreal from supralabials; supraoculars three, with the first being the largest; four elongate superciliaries, first one enlarged, in contact with loreal; palpebral disk divided into two scales, pigmented; suboculars three, elongated and similar in size; three postoculars, medial 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 four pairs of genials, the anterior two in contact medially and the posterior two 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 four scales, the medial two distinctly widened.

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; scales on dorsal surface of neck striated, becoming keeled from fore limbs to the tail; number of dorsal scales between occipital and posterior margin of hind limbs 28; dorsal scale rows in a transverse line at midbody 30; one row of smooth, enlarged

ventrolateral scales on each side; dorsals separated from ventrals by three rows of small scales at the level of the 13th row of ventrals; lateral body fold present; ventrals smooth, wider than long, arranged in 20 transverse rows between the 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 fore limb striated, imbricate; scales on ventral surface of fore limb 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, 9/9 on III, 11/12 on IV, 8/8 on V; subdigital lamellae of fore limb single, 5/5 on finger I, 8/9 on II, 13/13 on III, 14/14 on IV, 8/9 on V; subdigital lamellae on toes I and II single, on toe III paired on the distal half, on toe IV all paired, on toe V paired at the base; number of subdigital lamellae (pairs when applicable) 6/5 on toe I, 9/9 on II, 13/14 on III, 19/20 on IV, 12/12 on V; groin region with small, imbricate scales; scales on dorsal surface of hind limbs striated and imbricated; scales on ventral surface of hind limbs smooth; scales on posterior surface of hind limbs granular; six femoral pores on each leg; preanal pores absent; cloacal plate paired, bordered by four scales anteriorly, of which the two medialmost are enlarged.

Measurements (mm) and proportions of the holotype: HL 12.6; HW 9.3; ShL 5.2; AGD 24.6; TL/SVL 1.72; HL/SVL 0.25; HW/SVL 0.18; ShL/SVL 0.10; AGD/SVL 0.48.

Hemipenial morphology (Fig. 5): Both organs extend along approximately nine millimeters in length. The lobes of the organs are fully everted and each hemipenis is fully expanded.

The hemipenial body is roughly conical in shape, with the base distinctly thinner than the rest of the organ, ending in two small lobes with apical folds in the apex. The sulcus spermaticus is central in position, originating at the base of the organ, which bears a fleshy fold partially overlapping the sulcus spermaticus. From this point on, the sulcus proceeds in a straight line towards the lobes, and acquires an S-shape at the first third of the body. The sulcus becomes broader at halfway the length of the hemipenial body, and returns to its regular width at the apical region; it gets divided in two branches at the lobular crotch. Just before the crotch, the central region of the sulcus bears a tiny fleshy fold, which is not part of the sulcus division. From this point on, the two branches of the sulcus run on the medial regions of the lobes among conspicuous lobular folds. The sulcate face of the hemipenial body presents two nude areas, parallel to the sulcus spermaticus, which run throughout the hemipenial body, getting thinner and encircling the base of the lobes.

The lateral and asulcate faces of the hemipenial body are ornamented with 28–30 rows of roughly equidistant flounces with calcareous spinules. The first four rows are
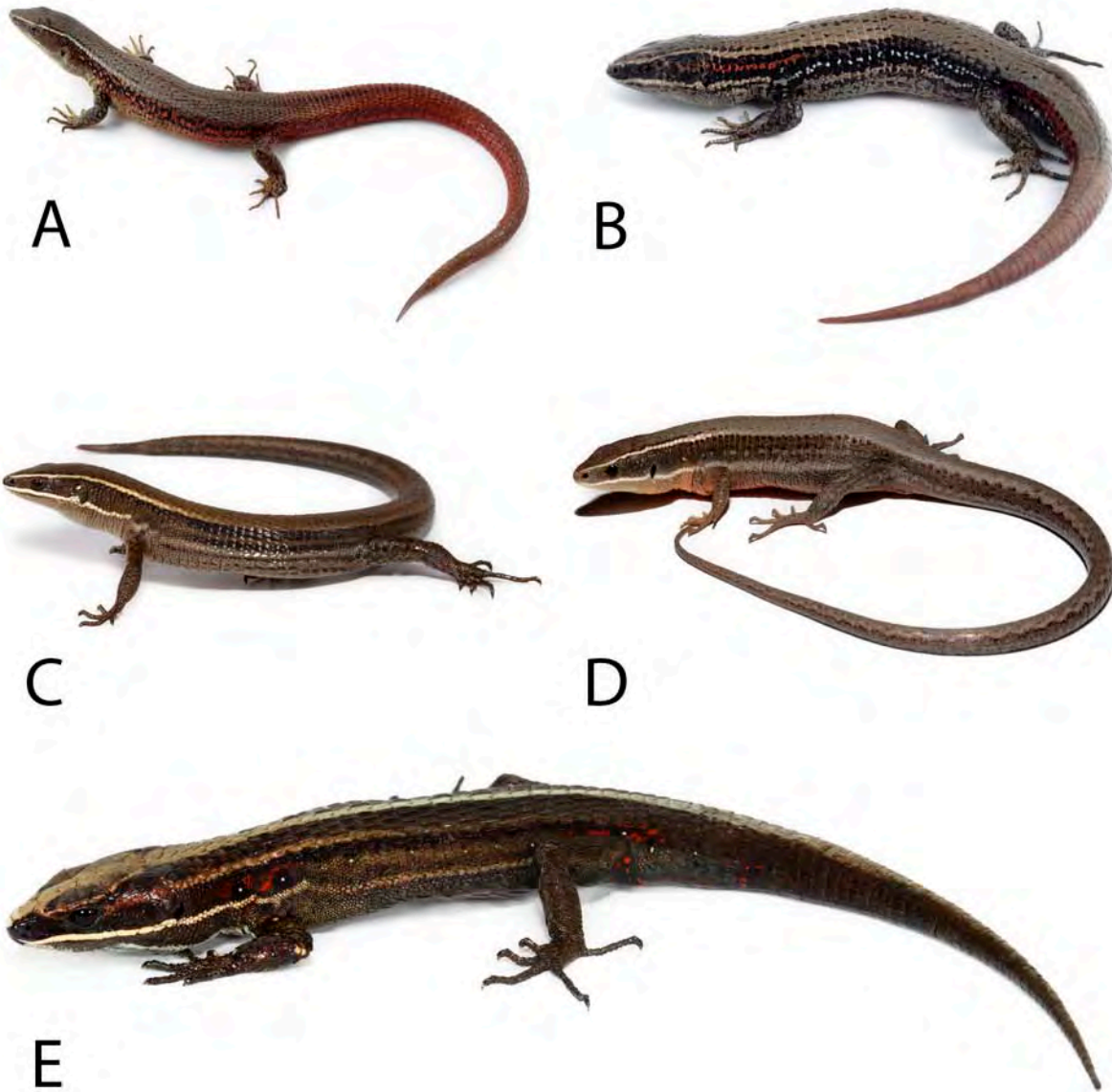
Fig. 4. Five species of *Pholidobolus* from Ecuador. (**A**) *P. affinis*; (**B**) *P. macbrydei*; (**C**) *P. montium*; (**D**) *P. prefrontalis*; (**E**) *P. hillisi* sp. nov. *Photographs by OTC (A, B, C, D) and S. R. Ron (E).*

straight, with a large series of spinules on the central aspect of the asulcate face, and small isolated series of 5-6 spinules bordering the nude areas parallel to the sulcus spermaticus. A V-shaped nude area at the central asulcate face of the body separates the remaining flounces. The fifth and sixth flounces are also interrupted laterally by an extension of the basal nude area. From the seventh to the apical-most one, the flounces cross the lateral aspects of the organ from the sulcus to the asulcate face, initially in roughly straight lines, gradually assuming chevron-shapes and getting reduced in length towards the apex of the organ.

The region between the asulcate and the lateral surfaces is marked by a conspicuous unevenness forming a bulge, which is shared by closely related species, such as *Macropholidus annectens*, *M. huancabambae*, *M. ruth-*

veni, *Pholidobolus affinis*, *P. macbrydei*, *P. montium*, and *P. prefrontalis* (Nunes, 2011).

Color of holotype in preservative: Dorsal background uniformly dark brown with a narrow light brown vertebral stripe extending from occiput onto tail; vertebral stripe slightly wider anteriorly; dorsal surface of head light brown medially (rostral, frontonasal, prefrontals, frontal and frontoparietals) and dark brown laterally (including supraoculars); white supralabial longitudinal stripe extending from first supralabial to fore limb; lateral aspect of neck dark brown with a dorsolateral light brown stripe that extends posteriorly along the flanks to the hind limbs; ventrolateral aspect of head and neck with a longitudinal white stripe extending posteriorly from fourth genial to insertion of fore limb and then laterally along

Fig. 5. Left hemipenis of *Pholidobolus hillisi* sp. nov. (QCAZ 4999) in sulcate (left), lateral (middle), and asulcate (right) views. *Photographs by P. Nunes.*

upper arm; fore limbs with scattered ocelli (black with white center); flanks grayish brown with two dorsolateral stripes, the dorsal one light brown and the ventral one dark brown; tail light brown dorsally and dark brown on the sides; two and three well-defined, small ocelli (black with white center) dorsal to insertion of fore and hind limbs, respectively; ventral surface of head gray, with dirty cream genials and scattered brown marks; chest, belly and ventral surface of limbs and tail dark gray.

Variation: Measurements and scale counts of *Pholidobolus hillisi* are presented in Table 1. Superciliaries usually four, five in QCAZ 6840; supralabials usually seven (eight of left side of specimen QCAZ 6840). Rows of lateral granules at midbody two (QCAZ 4999, 6844) to four (QCAZ 6842). Three specimens including the holotype, with a ventrolateral row of smooth enlarged scales (QCAZ 4999, 6840). Specimen QCAZ 6842 has a tiny

scale separating the cloacal scales posteriorly; all four scales bordering the cloacal plate anteriorly are similar in size in two specimens (QCAZ 4999, 6844), whereas the lateralmost scales overlap the cloacal scales in one specimen (QCAZ 6840).

No variation was observed in color pattern in preservative among adult males. They can be distinguished from females by the presence of ocelli and pale flecks around insertion of fore and hind limbs. Moreover, the characteristic diagonal white stripe on each side of the chin that extends from the fourth genial to the forearm is more conspicuous in males than in females. Females are larger (maximum SVL 55.7 mm, n=3) than males (maximum SVL 51.1 mm, n=3).

Coloration in life of an adult male paratype (QCAZ 4999) was similar to the holotype's coloration in preservative described above, except that specimen QCAZ 4999 had small red flecks both at insertion of fore limbs

Table 1. Sexual variation in lepidosis and measurements of *Pholidobolus hillisi* sp. nov. Range followed by mean ± standard deviation are given.

| Character	Males (*n*=3)	Females (*n*=3)
Dorsal scales between occipital and posterior margin of hind limb	28-30 (29±1)	29-31 (30±1)
Dorsal scale rows in a transverse line at midbody	27-34 (30.33±3.51)	29-35 (31±3.46)
Ventral scales between collar fold and preanals	18-20 (20.33±1.15)	18-19 (18.67±0.58)
Ventral scale rows in a transverse line at midbody	6-7 (6.67±0.58)	6
Subdigital lamellae on Finger IV	14-15 (14.33±43.0)	13-15 (13.67±1.15)
Subdigital lamellae on Toe IV	19-20 (19.33±0.58)	19
Femoral pores	5-8 (6.33±1.52)	2-5 (3.5) (*n*=2)
Maximum SVL	51.1	55.7
TL/SVL	1.86 (*n*=1)	1.84-2.14 (1.99) (*n*=2)
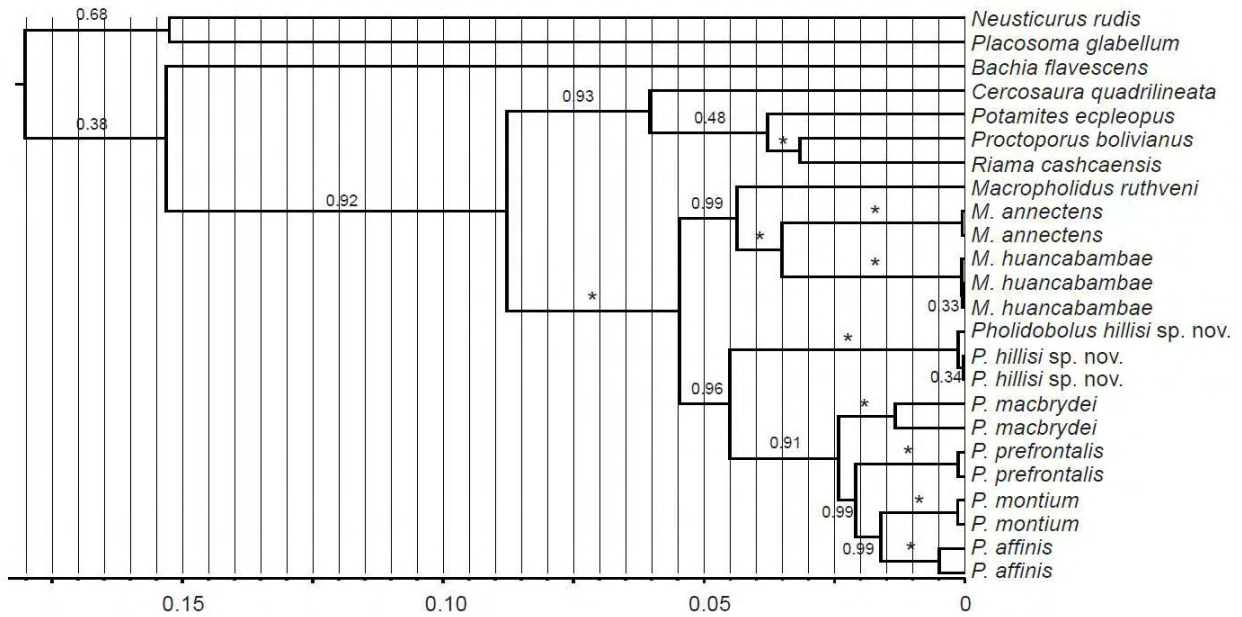
Fig. 6. Maximum clade credibility tree inferred from the analysis of a dataset containing three mitochondrial genes under uncorrelated, log normally distributed rates; branch lengths are in substitutions per site. Posterior probability values are shown above branches; asterisks correspond to values of 1.

extending onto sides of neck and at insertion of hind limbs extending onto base of tail. In addition, the lateral white stripe that starts on first supralabial extends further posteriorly along flanks in specimen QCAZ 4999 (Fig. 4).

Phylogenetic relationships: The maximum clade credibility tree resulting from the chronophylogenetic analysis supports inclusion of the new species within the *Pholidobolus* clade (Torres-Carvajal and Mafla-Endara 2013) with strong support (PP = 0.96; Fig. 6). Phylogenetic relationships among other species of *Pholidobolus* and species of *Macropholidus* are identical to those obtained by Torres-Carvajal and Mafla-Endara (2013). *Macropholidus ruthveni* is sister (PP = 0.99) to a clade containing both *M. annectens* and *M. huancabambae* (PP = 1). *Pholidobolus macbrydei* is sister (PP = 0.91) to a clade with the three remaining species of *Pholidobolus*; the latter clade included *P. prefrontalis* as sister (PP = 0.99) to a clade containing *P. affinis* and *P. montium* as sister taxa (PP = 0.99). In contrast to the results reported by Torres-Carvajal and Mafla-Endara (2013), the chronophylogenetic tree inferred in this paper suggests that the diversification of the clades *Macropholidus* and *Pholidobolus* occurred at about the same time (Fig. 6).

Distribution and ecology: *Pholidobolus hillisi* inhabits low montane forests in the eastern slopes of the Andes of southern Ecuador. This area represents a weather divide between the humid Amazon and the dry Inter-Andean regions (Beck et al. 2008). The new species is known from Provincia Zamora-Chinchipe, at 1,840 m (Fig. 7), in the deep valley of the Zamora river. The only gym-
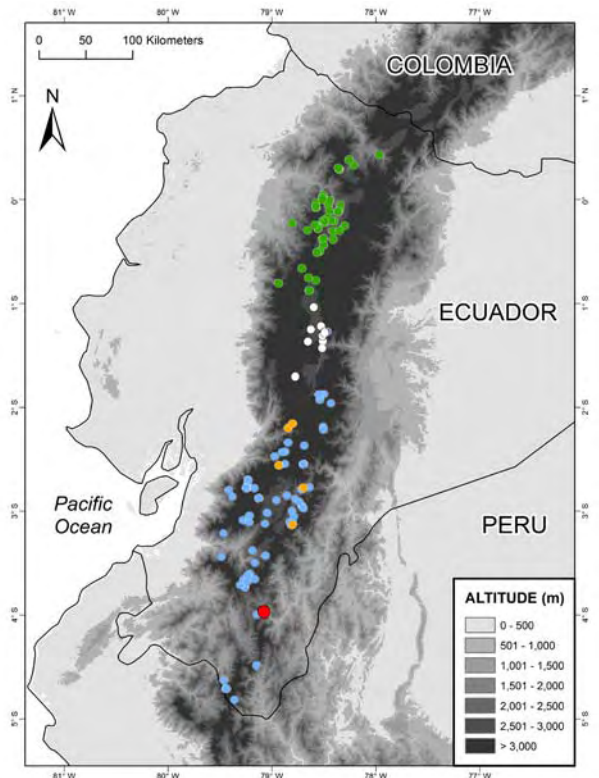
Fig. 7. Distribution of *Pholidobolus* in Ecuador. *P. affinis* (white circles); *P. macbrydei* (blue circles); *P. montium* (green circles); *P. prefrontalis* (orange circles); *P. hillisi* sp. nov (red circle).

nophthalmid species known to occur in sympatry with *P. hillisi* is *Alopoglossus buckleyi*, although *P. macbrydei* is parapatrically distributed (Fig. 7). Two specimens (QCAZ 4998, 4999) were found under logs and rocks next to the Zamora river between 1130 hrs and 1145 hrs,

whereas another specimen (QCAZ 5000) was basking on a rock next to the road at 1200 hrs. Other specimens (QCAZ 6840, 6842, 6844) were found and captured by a domestic cat around the San Francisco Research Station in pasture with interspersed shrubs.

Etymology: The specific epithet *hillisi* is a noun in the genitive case and is a patronym for David M. Hillis, who has had a great impact in the development of the field of molecular systematics (e.g., Hillis et al. 1996). In particular, he published a classic paper on evolutionary genetics of *Pholidobolus* lizards, where he compared some phylogenetic tree reconstruction techniques and emphasized the importance of phylogenetics in biogeography (Hillis 1985).

Remarks: The Andes of southern Ecuador and northern Peru between 4°S and 7°S consist of relatively low-elevation mountains that create a mixture of environments. This region, known as the Huancabamba Depression, has long been recognized as a major biogeographic barrier for Andean organisms (e.g., Cadle 1991; Duellman 1979; Vuilleumier 1969). Although all species of *Pholidobolus*, except *P. macbrydei*, are restricted to the southern part of the northern Andes (i.e., Ecuador and southern Colombia), the new species described herein occurs on the northern limit of the Huancabamba Depression.

The Huancabamba Depression seems to have influenced the radiation of several Andean lizard clades, such as *Stenocercus*, *Riama*, *Macropholidus*, and *Pholidobolus* (Doan 2003; Torres-Carvajal 2007; Torres-Carvajal and Mafla-Endara 2013). Except for *Macropholidus*, these clades have diversified along the northern Andes, suggesting that common geological or climatic events have influenced these radiations. The phylogenetic tree presented in this paper further supports the idea of a south-to-north sequence of speciation events (Doan 2003; Torres-Carvajal 2007) which is congruent with the recent south-to-north uplift of the northern Andes (Simpson 1979; Aleman and Ramos 2000).

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Appendix 1

Additional specimens examined

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: 6 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 Mucubí 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 Píllaro, 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 macbrydei.—ECUADOR: Provincia Azuay: 10 km S Cutchil, 3°8'2"S, 78°48'47"W, 2,900 m, QCAZ 823-24;1.2 km E Osorancho, 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; 7 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", 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; Ozogoche, 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 5597-9;. 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, 3348 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 Chinchipe: 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: 2 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; Cotacahi, 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; 5 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, 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 Chimborazo: Alausí, 2°11'54"S, 78°50'42"W, 2359 m, QCAZ 9907-9911; Tixán, 2°9'22"S, 78°48'3"W, 2,908 m, QCAZ 9951-54.

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Paola Mafla-Endara graduated in Biological Sciences from Pontificia Universidad Católica del Ecuador (PUCE) in 2011. Her undergraduate thesis entitled "Phylogeography of Andean lizards *Pholidobolus* (Squamata: Gymnophthalmidae) in Ecuador" provided her a gratifying knowledge about phylogenetics systematics, evolution, statistics, and biogeography. Since this time, she has developed a deep interest in molecular biology. Currently she works mostly in systematics and ecology of fungi. She is convinced that the same knowledge can be useful to solve similar questions in different subjects. This manuscript represents the second lizard species description coauthored by Paola. Others are in preparation.

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Citations

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Early development of the glass frogs *Hyalinobatrachium fleischmanni* and *Espadarana callistomma* (Anura: Centrolenidae) from cleavage to tadpole hatching

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Abstract.—We report the characteristics of embryonic development from cleavage to tadpole hatching in two species of glass frogs, *Hyalinobatrachium fleischmanni* and *Espadarana callistomma* (Anura: Centrolenidae). This analysis of embryonic development in centrolenid frogs enhances comparative studies of frog early development and contributes baseline information for the conservation and management of Ecuadorian frogs. These frogs reproduced in captivity and their embryos were fixed for developmental analysis. The morphology of embryos was evaluated in whole mounts, bisections, thick sections, and fluorescent staining of cell nuclei. Egg clutches contained an average of 23 and 35 eggs for *H. fleischmanni* and *E. callistomma*, respectively. The eggs of both frogs measured approximately 2.1 mm in diameter. The eggs of *H. fleischmanni* were uniformly pale green. In contrast, the animal hemisphere of *E. callistomma* eggs was dark brown and the vegetal hemisphere was light brown. The developmental time of *H. fleischmanni* and *E. callistomma* under laboratory conditions was 6 and 12 days, respectively from the 32–cell stage until tadpole hatching. Differences in environmental conditions may be associated with the time differences of early development observed in these frogs. The development of glass frogs from egg deposition to tadpole hatching was staged into 25 standard stages according to the generalized table of frog development. Archenteron elongation began in the early gastrula and notochord elongation began in mid to late gastrula, as in *X. laevis*. Development of the gastrocoel roof plate (grp) was precocious in the two centrolenid frogs. The grp was detected in the late gastrula of both species; whereas the grp was detected in neurula stages of *X. laevis*. The presence of the grp in embryos of these frogs suggests that the mechanisms of left-right asymmetry, found in *X. laevis* and other amphibians, may be shared by these centrolenid frogs. The development of *H. fleischmanni* and *E. callistomma* resembles the pattern found in frogs with rapid development such as the aquatic eggs of *X. laevis* and the development in floating foam-nests in the genus *Engystomops* (Leptodactylidae). Differences in egg pigmentation were particularly significant in connection with the divergent reproductive strategies of these glass frogs.

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Introduction

Centrolenid frogs are commonly known as glass frogs because the internal organs of the adult are visible through the transparent ventral body wall. This transparent region varies in size among species (Fig. 1A–B, D–E) (Cisneros-Heredia and McDiarmid 2007). Glass frogs are endemic to the tropical regions of South America from Venezuela to northern Argentina and south-eastern Brazil (AmphibiaWeb 2014) and are particularly diverse in the cloud forests of Colombia and Ecuador (Delia et al. 2010; Guayasamin and Trueb 2007; Ospina-Sarria et al. 2010). These arboreal frogs deposit their eggs in gelatinous masses on the upper or lower surface of plant leaves bordering stream banks. After hatching, the tadpoles drop into the underlying streams. Tadpoles are fossorial and live in the substrate along the shoreline (Delia et al. 2010; Duellman and Trueb 1986).

We studied the early development of the glass frogs *Hyalinobatrachium fleischmanni* and *Espadarana callistomma* (Anura: Centrolenidae) to compare their development with frogs that exemplify different reproductive modes and to contribute to the knowledge of frogs from Ecuador. Development of these centrolenid frogs was compared with the embryogenesis of Túngara frogs, *Engystomops* (Leptodactylidae). Túngara frogs construct foam nests that float in the water (Romero-Carvajal et al. 2009). In addition, this comparison was extended to the terrestrial embryos of poison arrow frogs (Dendrobatidae), embryos of the Marsupial frog, *Gastrotheca riobambae* (Hemiphractidae), and the aquatic embryos of *Xenopus laevis* (Pipidae) and *Ceratophrys stolzmanni* (Ceratophryidae) (Elinson and del Pino 2012; Nieuwkoop and Faber 1994; del Pino et al. 2004) (Table 1). The analysis of *H. fleischmanni* and *E. callistomma* early development was feasible because of the recent successful reproduction of centrolenid frogs in captivity at the Balsa de los Sapos, Centre of Amphibian Investigation and Conservation (CICA), Pontificia Universidad Católica del Ecuador (PUCE).

Hyalinobatrachium fleischmanni (Fig. 1A–C) occurs from southern Mexico to northern South America, including Ecuador. The egg clutches consist of 20–40 pale-green eggs, attached to the underside of plant leaves (Fig. 1C). Parental care of the egg clutch is provided by the male to maintain the needed humidity. The male prevents predation by katydids, wasps, ants, and other insects by kicking with his limbs at the predatory insect (Delia et al. 2010; Greer and Wells 1980; Savage 2002).

Espadarana callistomma (Guayasamin and Trueb 2007) (Fig. 1D–F) occurs in the lowlands of northeastern Ecuador and southern Colombia (Guayasamin and Trueb 2007; Ospina-Sarria et al. 2010). Darkly pigmented eggs are deposited on the upper surface of plant leaves (Guayasamin and Trueb 2007) (Fig. 1F). Egg predation by insects has not been reported for this species.

The left-right asymmetric location of organs, such as the liver and the heart is established in the *X. laevis* gastrocoel roof plate (grp) of the neurula by fluid-flow towards the left side, guided by the clockwise rotation of cilia (Blum et al. 2009; Schweickert et al. 2010). The rotation of cilia in the frog grp, or in equivalent structures of other vertebrates, determines the asymmetric expression of the gene Nodal in the grp left side (Blum et al. 2014b). The grp of *X. laevis* derives from the superficial prospective mesoderm of the early gastrula that becomes internalized during gastrulation, and ends up in the dorsal roof of the primitive gut. The grp can be detected by the presence of exposed mesoderm corresponding to the notochord and some paraxial mesoderm in the dorsal roof of the primitive gut, and it is bordered by the lateral endodermal crests (lec). As development advances, the lec close to the midline and the primitive gut cavity becomes totally lined with endoderm (Blum et al. 2009). The left-right asymmetry is determined by fluid flow guided by cilia rotation in the grp of frogs and other vertebrates. However, a comparable structure to the grp has not been reported for the chick and pig, and the left-right symmetry breakage in these vertebrates may depend on a modified mechanism (Blum et al. 2014a, b; Sáenz-Ponce et al. 2012b). We analyzed the presence of the grp in the gastrula and neurula of glass frogs to provide additional comparison.

We characterized the embryos of these glass frogs from cleavage to hatching of the tadpoles. We found that in glass frogs, gastrulation overlapped with body elongation, as in frogs with rapid embryonic development. The grp was detected in the late gastrula of both species of glass frogs. Its presence suggests that the mechanisms of left-right asymmetry, found in *X. laevis* and other amphibians, may be shared by these centrolenid frogs. The reproductive mode of these glass frogs is associated with rapid development. The strategy of egg deposition in the underside or upper surface of leaves is associated with differences in developmental time and pigmentation of embryos and tadpoles.

Materials and Methods

Locality of collection and staging of embryos. *Hyalinobatrachium fleischmanni* and *Espadarana callistomma* were collected from Esmeraldas Province, San Lorenzo, Durango, along the banks of the Río Durango and its tributaries in northwest Ecuador. The altitude of this site is 243 m above sea level, and the geographic coordinates are W 78.62405, N 1.04186. Frogs of both species were collected on 04 October 2009 by Elicio Tapia and Santiago García. The adults successfully reproduced at the Balsa de los Sapos, Centre of Amphibian Investigation and Conservation (CICA), School of Biological Sciences, Pontificia Universidad Católica del Ecuador
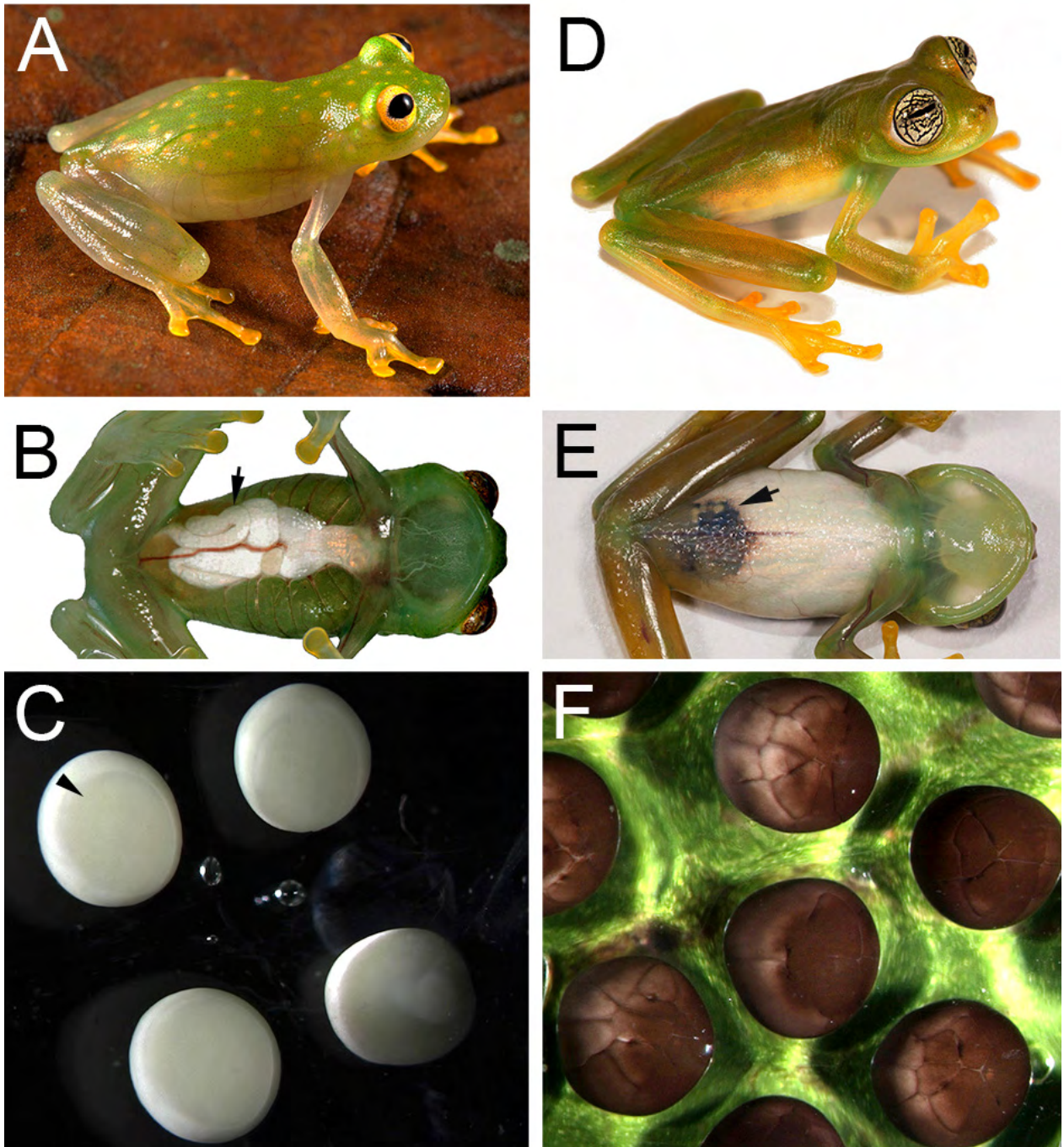
Fig. 1. The adults and egg clutches of glass frogs. **(A–C)** *Hyalinobatrachium fleischmanni*. **(A)** Lateral view of an adult male. **(B)** Ventral view of an adult male. The arrow indicates the border of the transparent body wall. The intestine and a blood vessel are visible. **(C)** Partial view of an egg clutch at the gastrula stage. The embryos are uniformly pale and the blastocoel roof is translucent (arrowhead). **(D–F)** *Espadarana callistomma*. **(D)** Lateral view of an adult female. **(E)** Ventral view of an adult female. The arrow signals the pigmented oocytes visible through the transparent body wall. The size of the transparent region is smaller than in *H. fleischmanni* shown in B. **(F)** Partial view of an egg clutch. The embryos were at stages 5–6 (Table 2). *Photographs of adult frogs by Santiago Ron (A–B, D–E).*

(PUCE). The permit 016-IC-FAU-DNBAP-MA from the Ministry of the Environment, Ecuador, allowed the collection and maintenance of these frogs at Balsa de Sapos. Egg clutches were donated to the Laboratory of Developmental Biology for analysis of embryonic development. This study was based on the analysis of embryos derived from seven egg clutches of *H. fleischmanni* and four egg clutches of *E. callistomma*.

The number of eggs of each egg clutch was recorded and the embryos were cultured in humid chambers at room temperature, as described for embryos of the dendrobatid frog, *E. machalilla* (del Pino et al. 2004). At various intervals, some embryos were moved to a Petri dish filled with 15% Steinberg's solution (del Pino et al. 2004) and the egg-jelly was manually removed to study embryogenesis. Embryos were staged according to the general table of frog development (Gosner 1960). Egg diameter was measured in fixed embryos with the measuring tool of the program, Axiovision (Carl Zeiss, Oberkochen, Germany).

Fixation, staining and analysis of embryonic development. Embryos were fixed in Smith's fixative (del Pino et al. 2004). The procedures for the bisection of embryos, vibratome sectioning, cell nuclei staining with the fluorescent dye Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA), and the staining of cell boundaries with silver nitrate were previously described (Moya et al. 2007; del Pino et al. 2004). Sections were mounted in glycerol, and were examined with a Stemi SV6 stereo microscope (Carl Zeiss, Oberkochen, Germany) or with fluorescent optics using a Z1 Axio Observer microscope (Carl Zeiss, Oberkochen, Germany). Embryos were photographed with Axiocam cameras, attached to microscopes, and the image capture program, Axiovision (Carl Zeiss, Oberkochen, Germany). The images were edited with Adobe Photoshop CS6.

Results and Discussion

Internal organs of adult glass frogs can be observed through their transparent belly; however the size of the transparent window varies in the different genera of centrolenid frogs as detected for *H. fleischmanni* and *E. callistomma* (Fig. 1A–B, D–E) (Cisneros-Heredia and Mcdiarmid 2007). In contrast with adults, the eggs of these frogs were opaque (Fig. 1C, F). We also detected significant pigmentation differences as the *H. fleischmanni* eggs and embryos were pale-green and those of *E. callistomma* were dark brown (Fig. 1C, F). Egg pigmentation is a distinctive character of the different genera of Centrolenidae; moreover some species deposit their eggs in the upperside and others in the underside of leaves. However, some species show no particular preference for the upper or underside of leaves for the deposition of their eggs (Cisneros-Heredia and Mcdiarmid 2007).

Clutch size, egg pigmentation and developmental time. The number of eggs ranged from 14–30 eggs, with a mean of 23 eggs per clutch in *H. fleischmanni*, and 32–39 eggs, with a mean of 35 eggs per clutch in *E. callistomma*. The eggs of both species measured about 2.1 mm in diameter (Table 1). The embryos of *H. fleischmanni* were uniformly pale-green (Figs. 1C; 2A–L; 3A–D). In contrast, the animal hemisphere of *E. callistomma* embryos was dark brown, and the vegetal hemisphere was pale-brown (Figs. 1F; 4A–L; 5A–F).

Dark pigmentation of the animal hemisphere of the egg may provide protection against solar UV radiation and may capture solar heat required to accelerate early development of frog embryos exposed to solar radiation in moist or aquatic environments. In contrast, there is lack of dark pigment in frog eggs and embryos that develop in secluded places (Duellman and Trueb 1986; Elinson and del Pino 2012). We propose that *H. fleischmanni* embryos do not require dark pigmentation because the underside of plant leaves may provide protection against solar radiation. In contrast, the presence of dark pigment in eggs and embryos of *E. callistomma* may be needed, as the egg clutches are directly exposed to UV solar radiation on the upper surface of plant leaves.

The differences in pigmentation were detectable in eggs and embryos until tadpole hatching (Figs. 1C, F; 2–5). At hatching, the tadpoles of *H. fleischmanni* were pale green with little dark pigmentation on the dorsum; whereas *E. callistomma* tadpoles had a brown color (Figs. 3C–D; 5F). The fossorial free-living tadpoles of *H. fleischmanni* remained nearly unpigmented had elongated bodies, and narrow tail fins to enable digging in the sandy stream bottoms. The eyes were reduced in size and were covered by skin characters likely associated with the fossorial habits of *H. fleischmanni* tadpoles (Delia et al. 2010; Duellman and Trueb 1986; Savage 2002). The characteristics of the *E. callistomma* free-living tadpoles are unknown. The differences in tadpole pigmentation at hatching suggest that the larval stages of these two centrolenids may occur in dissimilar aquatic environments.

The differences in egg pigmentation observed in *H. fleischmanni* and *E. callistomma* may depend on different expression levels of the gene Shroom2 during oogenesis. Shroom2, an actin-binding protein, controls pigment granule localization in the animal cortex of *X. laevis* oocytes (Lee et al. 2009). The oocytes of *Engystomops pustulosus* (Leptodactylidae) contain small amounts of Shroom2 protein and are white in color. However, *Engystomops* embryos have dark pigment granules around nuclei of blastomeres (Lee et al. 2009; Romero-Carvajal et al. 2009). Embryos of *H. fleischmanni* are pale and do not have dark pigment around the nuclei of blastomeres; whereas, in *E. callistomma* embryos dark pigment was observed on the cell surface of animal pole blastomeres, as well as around blastomere nuclei.

Embryos of *H. fleischmanni* and *E. callistomma* were maintained under identical laboratory conditions with a

Hyalinobatrachium fleischmanni
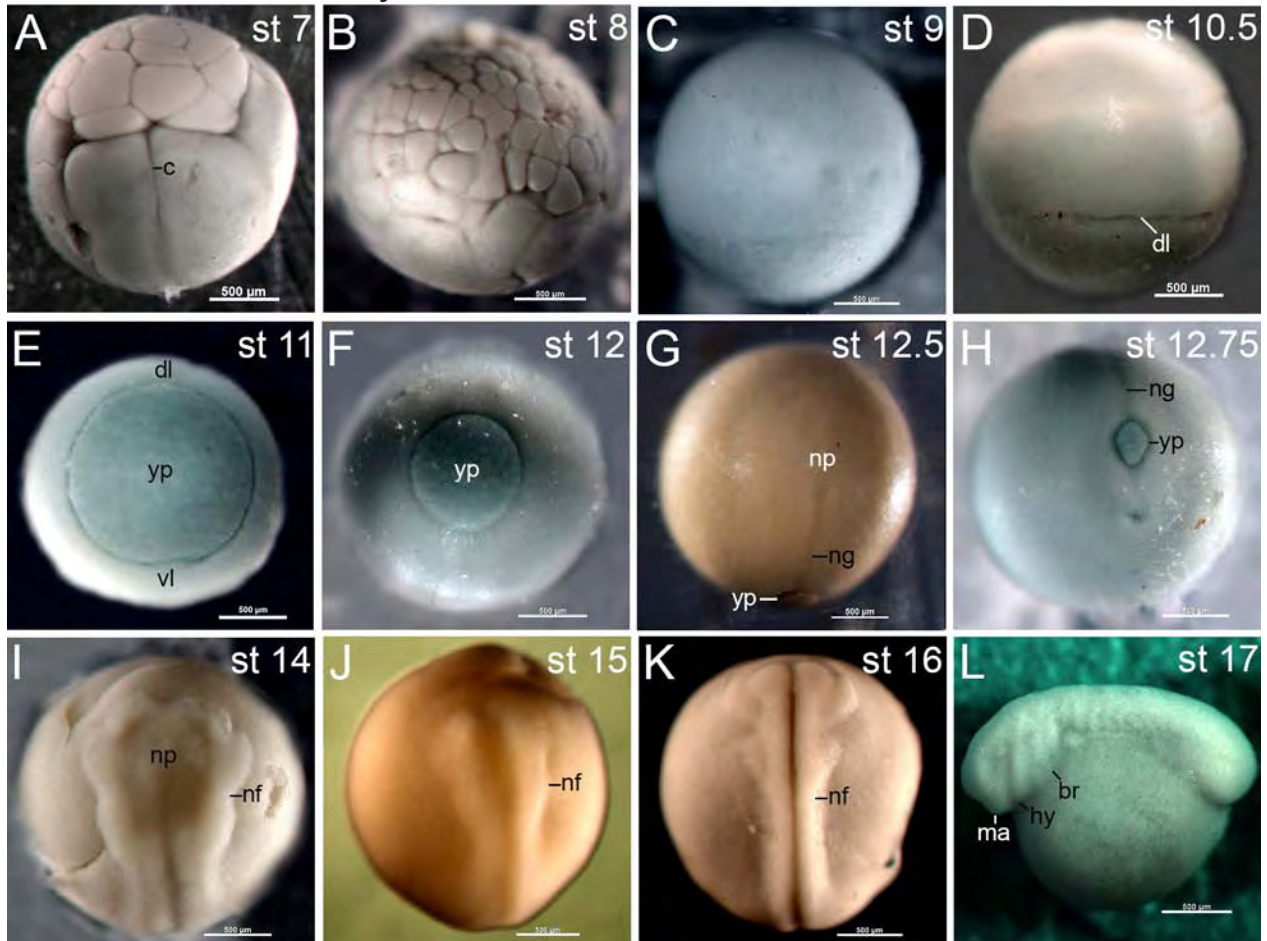
Fig. 2. External morphology of *Hyalinobatrachium fleischmanni* embryos from cleavage to the tail bud stage. **(A)** Stage 7: Thirty-two cell stage. Animal micromeres were much smaller than the vegetal macromeres. **(B)** Stage 8: Mid cleavage. **(C)** Stage 9: Blastula. **(D)** Stage 10.5: Early gastrula. The dorsal blastopore lip can be seen in the dorsal subequatorial region. **(E)** Stage 11: Mid gastrula. The yolk plug was large. **(F)** Stage 12: Late gastrula. **(G)** Stage 12.5: Late gastrula with a small yolk plug. Neural groove and neural plate were visible in embryos of this stage. **(H)** Stage 12.75: Late gastrula. The neural groove was visible. The yolk plug was small. **(I)** Stage 14: Early neural fold. **(J)** Stage 15: Mid-neural fold. The neural folds were more elevated. **(K)** Stage 16. Closure of the neural tube. The neural folds were near each other. **(L)** Stage 17. Tail bud stage. The branchial arches were visible. In this and the following figures, numbers in the top right-hand corner give the developmental stage. br, branchial arch; c, cleavage furrow; dl, dorsal blastopore lip; hy, hyoid arch; ma, mandibular arch; nf, neural fold; ng, neural groove; np, neural plate; vl, ventral blastopore lip; yp, yolk plug.

temperature fluctuation of 18–23 °C. However, developmental time diverged greatly between these frogs, as embryos of *H. fleischmanni* required six days and those of *E. callistomma* required 12 days from the 32–cell stage until tadpole hatching. However in nature, great variation in developmental time was observed in *H. fleischmanni*, as egg clutches required 8–21 days from oviposition to tadpole hatching (Greer and Wells 1980). In our laboratory, development of *H. fleischmanni* and *E. callistomma* was slower than in the floating foam-nests of *Engystomops* (Leptodactylidae), and faster than in the terrestrial nests of Dendrobatidae. In two species of *Engystomops*, development from egg deposition until hatching required only three days whereas 19–21 days were required for the same developmental processes by six species of den-

drobatid frogs (del Pino et al. 2004, 2007; Romero-Carvajal et al. 2009) (Table 1).

Reproductive strategies. We propose that rapid development may be favored in *H. fleischmanni* in comparison with *E. callistomma* because eggs deposited on the underside of plant leaves are at a greater risk of desiccation in comparison with eggs deposited on the upperside of leaves (Delia et al. 2010; Savage 2002). Moreover, rapid development may be required in all centrolenids, including frogs of the genus *Espadarana*, to overcome predation from a number of insect families and other arthropods (Cabanzo-Olarte et al. 2013; Duellman and Trueb 1986; Villa 1977; Vockenhuber et al. 2008). The deposition of eggs on the underside of plant leaves and

Table 1. Comparison of reproductive and developmental characteristics of glass frogs.

Family and Species	Reproduction	Clutch size and (egg diameter, mm)	Gastrulation time (hrs)[a]	Presence of the grp in the neurula	Onset of noto-chord elongation	Refs[d]
Rapid Development						1
Centrolenidae						
Hyalinobatrachium fleischmanni	Leaves underside	23 (2.1)	24	Yes	mid gastrula[b]	2
Espadarana callistomma	Leaves upperside	35 (2.1)	23	Yes	mid gastrula[b]	2
Leptodactylidae						
Engystomops randi	Floating foam-nest	110 (1.1)	12.5	Yes	mid gastrula[b]	3, 4
Engystomops coloradorum	Floating foam-nest	130 (1.3)	12.5	Unknown	mid gastrula[b]	3
Ceratophryidae						
Ceratophrys stolzmanni	Aquatic	664 (2.2)	5	Yes	mid gastrula[b]	4, 5
Pipidae						
Xenopus laevis	Aquatic	1000 (1.2)	6	Yes	mid gastrula[b]	6, 7
Slow Development						1
Dendrobatidae						
Epipedobates machalilla	Terrestrial nest	15 (1.6)	65	Yes	After gastrulation[c]	4, 8
Epipedobates tricolor	Terrestrial nest	13 (2.0)	36	Yes	After gastrulation[c]	8, 9
Hemiphractidae						
Gastrotheca riobambae	Egg brooding	128 (3.0)	168	Yes	After gastrulation[c]	1, 4

[a]Time from stages 10–13. Embryo culture temperatures for: *X. laevis* 23 °C, and 18–23 °C for other frogs.; [b]Stage 11; [c]Stage 13; [d]References: 1, (del Pino et al. 2007); 2, This work; 3, (Romero-Carvajal et al. 2009); 4, (Sáenz-Ponce et al. 2012b); 5, (Ortiz, 2013); 6, (Nieuwkoop and Faber 1994); 7, (Blum, et al. 2009); 8, (del Pino et al. 2004); 9, (Sáenz-Ponce et al. 2012a).

predation of eggs and embryos by wasps, ants, katydids and other arthropods are likely determining factors in favor of rapid development in *H. fleischmanni*.

Aquatic eggs and embryos characterize the basal mode of frog reproduction, as exemplified by *X. laevis* and *Ceratophrys stolzmanni* (Table 1). These frogs release a large number of small eggs in the water. However, frogs have invaded different environments for reproduction due to competition for water resources, predation, and the dangers of desiccation. Accordingly, clutch size, egg size and developmental time vary among species (Table 1) (Duellman and Trueb 1986). The dissimilar developmental times of *H. fleischmanni* and *E. callistomma* may relate to their egg deposition sites and to different predation pressure on eggs and embryos. Egg deposition in the upperside or underside of leaves associated with differences in egg pigmentation and developmental time, as observed in centrolenid frogs, are different reproductive modes that deserve further investigation.

Development of *H. fleischmanni* and *E. callistomma*. The characteristics of development are detailed in Table 2, and illustrated in Figs. 2–13. It was of interest to document the characteristics of development of these glass frogs, given the observed differences in embryonic pigmentation and developmental time. The development from early cleavage to tadpole hatching of *H. fleischmanni* and *E. callistomma* was characterized according to the generalized table of frog development (Gosner 1960)

(Table 2). Embryos of *H. fleischmanni* from fertilization to the sixteen cell stage were not available.

Micrographs of the external morphology of embryos illustrate the developmental stages of both species, and clearly demonstrate the pigmentation differences among species (Figs. 1C, F; 2A–L; 3A–D; 4A–L; 5A–F). The internal morphology of embryos from cleavage until the completion of neurulation follows the typical frog pattern, as outlined in the generalized table of development (Gosner 1960) (Figs. 6–13). The most notable differences are the overlap between gastrulation and the onset of neural development, and the lack of pigment in embryos of *H. fleischmanni* in comparison with embryos of *E. callistomma*. In both species cleavage was holoblastic (Figs. 6A–D; 7A–D), and the blastocoel roof was reduced to two-cells in thickness during gastrulation. At gastrulation, a conspicuous dorsal blastopore lip developed in the subequatorial dorsal region (Figs. 8A–F; 9A–E). The onset of neurulation began before completion of blastopore closure (Figs. 10A–D; 11A–F).

Developmental time, gastrulation and body elongation. Our comparative analysis includes frog species with rapid and slow development (Table 1). Embryonic development occurs rapidly in frog species with aquatic reproductive modes. The analyzed frogs with rapid development and embryos suspended on the vegetation included *H. fleischmanni*, *E. callistomma* (Centrolenidae). Frogs with aquatic eggs and embryos included *X. laevis*

Table 2. Characteristics of development of the glass frogs *Hyalinobatrachium fleischmanni* and *Espadarana callistomma*.

G	Morphology observed in Centrolenid frogs[1]
1	Fertilization (not available).
2	Gray crescent (not available).
3	Two cell stage (not available).
4	Four cell stage. The first two cleavage furrows passed from the animal to the vegetal pole. This stage was available only for *E. callistomma* (not shown).
5	Eight cell stage. The third cleavage furrow was latitudinal in some embryos and longitudinal in others. This stage was available only for *E. callistomma* (not shown).
6	Sixteen cell stage. Cleavage became asynchronous after the eight cell stage, and embryos with variable numbers of blastomeres were observed. This stage was available only for *E. callistomma* (not shown).
7	Thirty-two cell stage. Cleavage in both species was holoblastic, and the animal micromeres were much smaller than the vegetal macromeres, as observed for other frogs. (Figs. 2A; 4A; 6A–B; 7A–B).
8	Mid cleavage. Development of the blastocoel began during cleavage, as shown for *H. fleischmanni*. (Figs. 2B; 6C–D).
9	Blastula. The blastocoel roof was thick and consisted of several cell layers (Figs. 2C; 4B; 7C–D).
10	Early gastrula. A conspicuous blastopore groove was observed on the dorsal subequatorial region of the embryo, and there were bottle cells marking cell ingression at the blastopore groove as shown for both species (Figs. 4C; 8A; 9A–B). In slightly more advance embryos, the dorsal blastopore lip was detected in the dorsal subequatorial region, as shown for *H. fleischmanni* (Figs. 2D; 8B).
11	Mid gastrula. The blastopore lip surrounded a large yolk plug in embryos of both frogs (Figs. 2E; 4D; 8C). Internally, the archenteron was elongated, without inflation (Figs. 8D; 9C). The blastocoel roof was translucent (Fig. 1C) and consisted of two-cell layers (not shown).
12	Late gastrula and development of the neural plate (Figs. 2F; 4E; 8E). The neural groove and the neural plate were visible in gastrula stage embryos with a small yolk plug (stage 12.5) (Figs. 2G; 4F, 11 A). The archenteron was elongated in an anterior direction and it was inflated, and the blastocoel was reduced in size. The cleft of Brachet, that separates the ectoderm from the endomesoderm, was visible in the roof of the primitive gut (Figs. 8F; 9D–F; 10A; 11 B–C) The notochord was detected in stage 12.5 embryos, as shown for *H. fleischmanni* (Fig. 10B). In stage 12.75, the neural plate was visible in both species (Figs. 2H; 4G; 11 D). The yolk plug was small, the archenteron was fully inflated, and the germ layers were visible (Fig. 10C–D; 11 E–F). A triangular dorsal structure, considered to be the gastrocoel roof plate (grp), was located in the roof of the primitive gut, and was exposed to the cavity of the gastrocoel (Fig. 12C). The grp included the ventral surface of the notochord and paraxial mesoderm, and was bordered by the lateral endodermal crests (lec). The grp is illustrated for *E. callistomma* (Figs. 12D).
13	The closed blastopore and the neural plate. The yolk plug was totally retracted, the blastopore was at the slit blastopore stage, and the neural plate was visible (Figs. 4H; 12A). The grp was located in the roof of the primitive gut, and it was bordered by the lec, shown in whole mount for *H. fleischmanni* (Fig. 12B).
14	Early neural fold stage. The neural folds were slightly elevated (Figs. 2I; 4I; 13A). The grp included the ventral surface of the notochord, and somites, and it was bordered by the lec, shown for *E. callistomma* (Fig. 12 E–F). The neural ectoderm, paraxial mesoderm, notochord, and endoderm were visible (Fig. 13B).
15	Mid neural fold stage. The neural folds were elevated (Figs. 2J; 4J; 13C). In cross sections, the neural ectoderm, notochord, paraxial mesoderm and endoderm were visible, as shown for *H. fleischmanni* (Fig. 13 D).
16	Closure of the neural tube. The neural folds were closed (Figs. 2K; 4K; 13 E). In cross sections, the neural tube was visible dorsal to the notochord. The somites were visible on each side. The endoderm completely lined the archenteron, as shown for *E. callistomma* (Figs. 13 F).
17	Tail bud stage. The tail bud and the head region protruded beyond the yolky endoderm. The branchial arches were visible (Figs. 2L; 4L).
18	Muscular activity. The branchial arches protruded on the sides of the head. The tail became elongated. This stage is only shown for *E. callistomma* (Fig. 5A).
19	Heart beat. The heart beated, and the gill buds were visible. This stage is only shown for *E. callistomma* (Figs. 5B, C).
20	Circulation to the external gills. There were two gill pairs, each with two small branches. This stage is not shown.
21	The gills were larger, the first pair gill had two branches for both species and the second pair gill was unbranched in *H. fleischmanni* (Figs. 3A; 5D).
22	Tail fin circulation. Not observed.
23	The external gills reached their full size. There were five gill branches in the first pair and four branches in the second pair of external gills in embryos of *H. fleischmanni*. The opercular fold was developing. There were four gill branches in the first pair and three branches in the second pair of external gills of *E. callistomma* embryos (Figs. 3B–5E).
24	Larval stage. Not observed
25	Tadpole at hatching. Only a small portion of the external gills protruded from the opercular aperture in the hatching tadpoles. The eyes were very small. (Figs. 3C–D; 5F).

[1] The development of the Centrolenid frogs (C), *H. fleischmanni* and *E. callistomma*, was compared with the general staging table for frogs (G) (Gosner, 1960).

Hyalinobatrachium fleischmanni
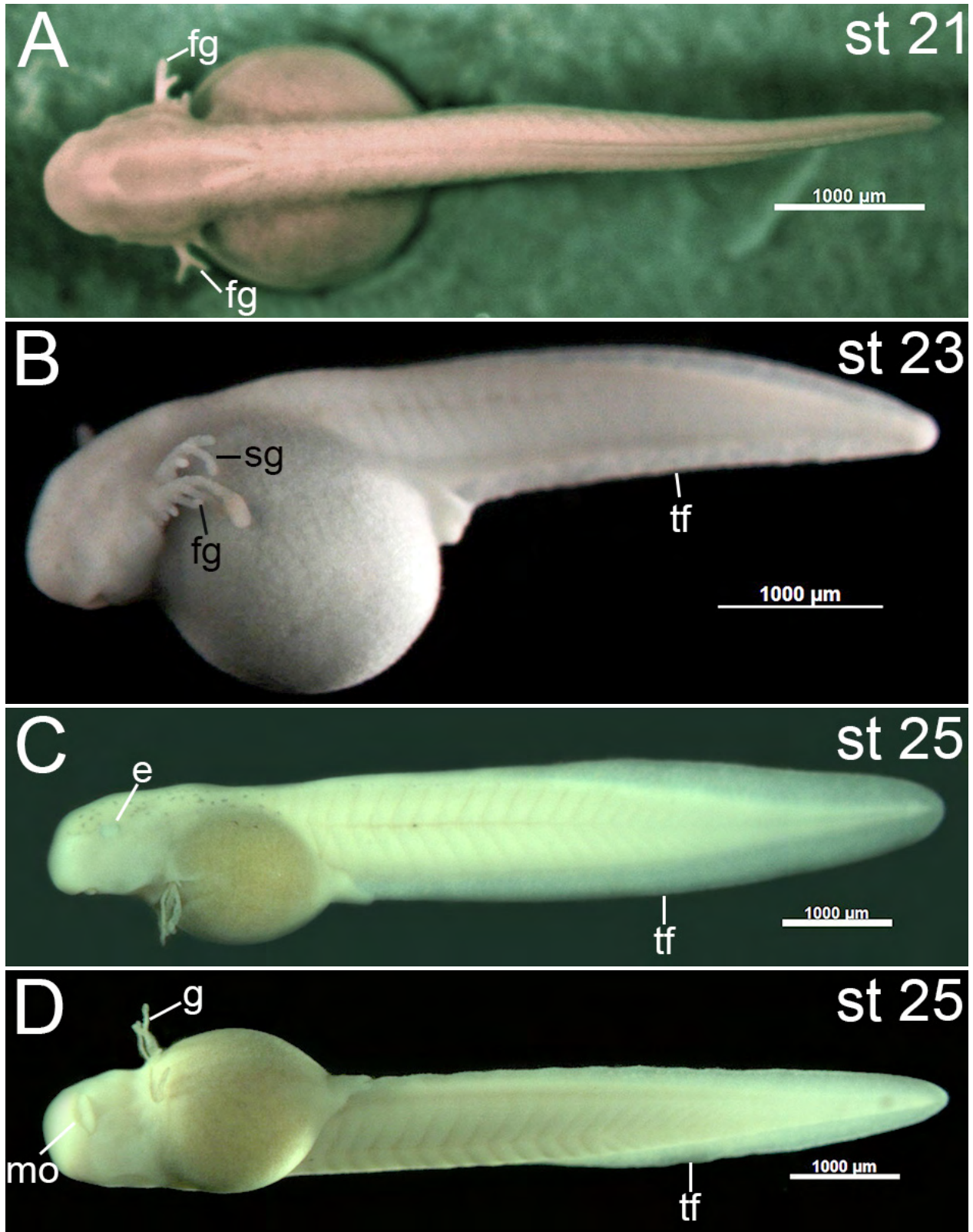
Fig. 3. External morphology of *Hyalinobatrachium fleischmanni* embryos from the development of the gills stage to hatching. **(A)** Stage 21: The gills were large, and each gill pair had two branches. **(B)** Stage 23: Full development of the external gills. There were five gill branches in the first pair and four branches in the second pair of gills. **(C)** Stage 25: Lateral view of a tadpole at hatching. The eyes were very small. **(D)** Stage 25: Ventral view of a tadpole at hatching. Only a small portion of the external gills protruded from the opercular aperture. The pink color of the embryo in A was an artifact of fixation. e, eye; fg, first pair gills; g, gills; mo, mouth; sg, second pair gills; tf, tail fin.
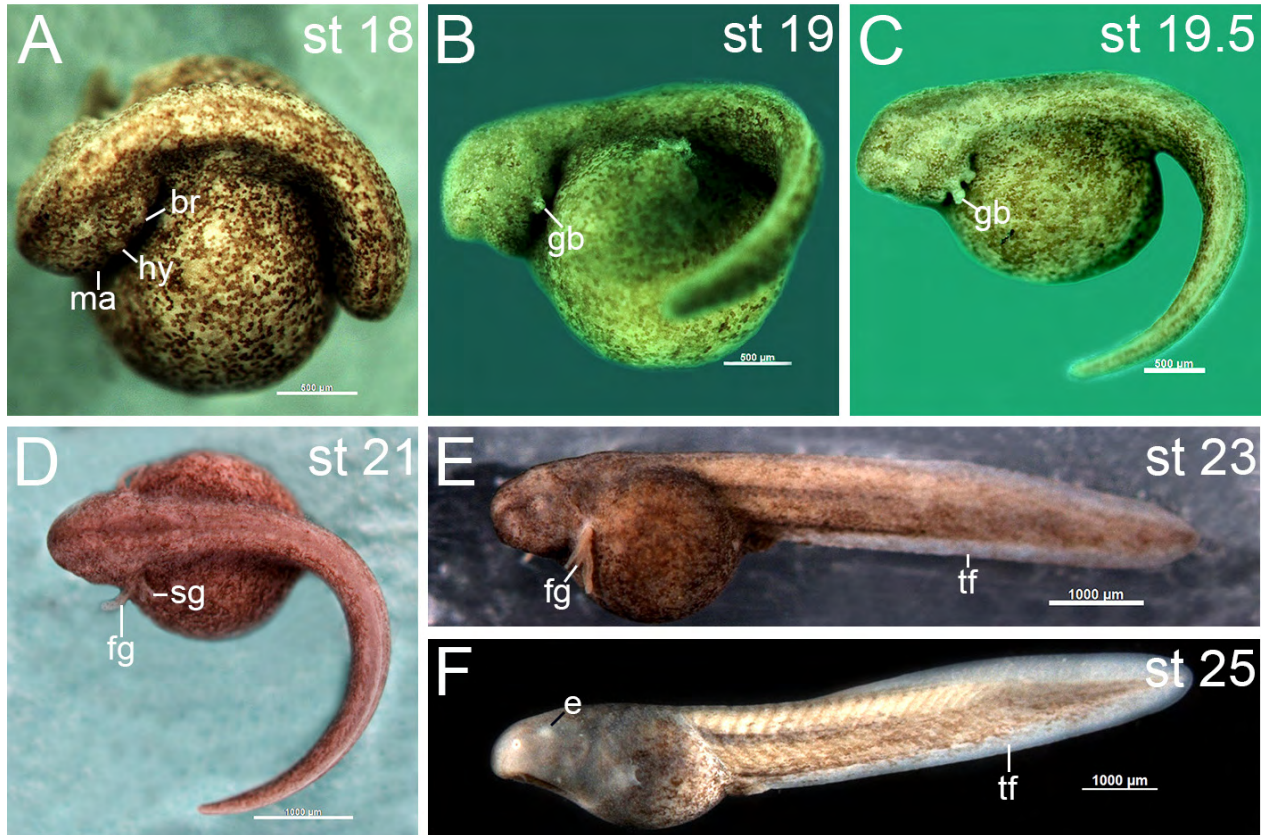
E. callistomma
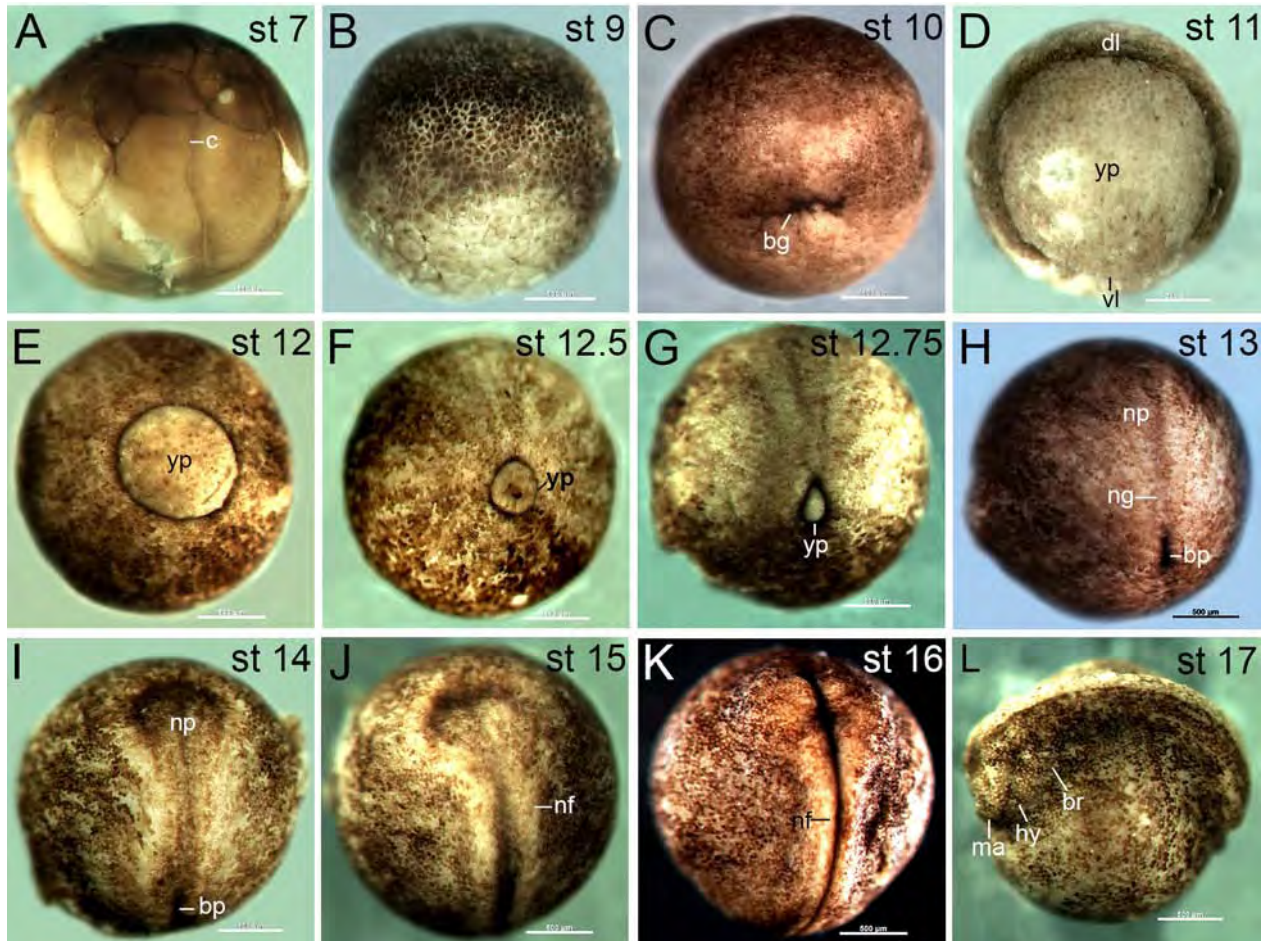
Fig. 4. External morphology of *Espadarana callistomma* embryos from cleavage to the tail bud stage. **(A)** Stage 7: Thirty-two cell stage. Animal micromeres were much smaller than the vegetal macromeres. **(B)** Stage 9: Blastula. **(C)** Stage 10: Early gastrula. A conspicuous blastopore groove was observed on the dorsal subequatorial region of the embryo. **(D)** Stage 11: Mid gastrula. The blastopore lip surrounded a large yolk plug. **(E)** Stage 12: Late gastrula. **(F)** Stage 12.5: Late gastrula with a small yolk plug. **(G)** Stage 12.75: Late gastrula with a very small yolk plug. The neural plate was visible. **(H)** Stage 13: The neural plate was visible. The yolk plug was totally retracted and the blastopore was at the slit blastopore stage. **(I)** Stage 14: Early neural fold. The neural folds were visible. **(J)** Stage 15: Mid neural fold. The neural folds were elevated. **(K)** Stage 16. Closure of the neural tube. The neural folds were closed. **(L)** Stage 17. Tail bud stage. The branchial arches were visible. bg, blastopore groove; br, branchial arch; bp, closed blastopore; c, cleavage furrow; dl, dorsal blastopore lip; hy, hyoid arch; ma, mandibular arch; nf, neural fold; ng, neural groove; np, neural plate; vl, ventral blastopore lip; yp, yolk plug.

(Pipidae), and *Ceratophrys stolzmanni* (Ceratophryidae), and frogs with embryos placed in floating foam-nests were *Engystomops randi* and *Engystomops coloradorum* (Leptodactylidae) (Table 1). In contrast, embryonic development was much slower in embryos of frogs with terrestrial adaptations. Frogs with slow development included the Marsupial frog *Gastrotheca riobambae* (Hemiphractidae) that broods its embryos in a dorsal pouch of the mother and the dendrobatid frogs *Epipedobates machalilla* and *Epipedobates tricolor* (Dendrobatidae) that deposit their eggs in terrestrial nests (Table 1) (del Pino et al. 2007; Elinson and del Pino 2012).

Gastrulation characteristics vary among frogs according to their developmental speed. Gastrulation and body elongation, as detected by the onset of notochord elongation, overlapped in embryos of *X. laevis*, *C. stolzmanni*,

E. randi, and *E. coloradorum*, frogs with rapid development (Table 1). Similarly, elongation of the notochord overlapped with gastrulation in the rapidly developing embryos of the centrolenid frogs *H. fleischmanni* and *E. callistomma* (Figs. 8D, F; 9C–F; 10A–D; 11B–C, E–F; 12D; Table 2). In contrast, gastrulation movements occurred before the onset of notochord elongation in the slowly developing dendrobatids *E. machalilla* and *E. tricolor*, and in the Marsupial frog, *G. riobambae*. Egg size is larger in these slowly developing frogs in comparison with the rapidly developing species (Table 1), (Elinson and del Pino 2012; del Pino et al. 2007).

The modular nature of gastrulation allows the separation of dorsal convergence and extension, the mechanism that triggers elongation of the notochord and the body, from gastrulation in the slowly developing frogs, and

E. callistomma

Fig. 5. External views of *Espadarana callistomma* embryos from the stage of muscular activity to hatching stages. **(A)** Stage 18: Muscular activity. The branchial arches protruded on the sides of the head. **(B)** Stage 19: Heartbeat. The gill buds were visible. **(C)** Stage 19.5: Two gill pairs were visible, each with two small branches. **(D)** Stage 21: The gills were larger, and each gill pair had two branches. **(E)** Stage 23: Full development of the external gills. There were four gill branches in the first pair and three branches in the second pair of gills. **(F)** Stage 25: Tadpole at hatching. The eyes were very small. br, branchial arch; e, eye; fg, first gill pair; gb, gill bud; hy, hyoid arch; ma, mandibular arch; sg, second gill pair; tf, tail fin.

the overlap of these two processes in rapidly developing frog species (Elinson and del Pino 2012). Overlap of gastrulation and body elongation is associated with rapid development in the unstable conditions of the reproductive modes that involve aquatic reproduction of *X. laevis* and *C. stolzmanni*, floating foam-nest development in *Engystomops*, and suspension of eggs on the vegetation, in the case of centrolenids frogs (Table 1), (Elinson and del Pino 2012). The distinct modes of gastrulation likely relate to the reproductive mode of frogs, rather than to phylogenetic relationships.

The gastrocoel roof plate (grp) and left-right asymmetry. It was of interest to determine whether frogs with different reproductive modes, and different onset of notochord elongation share the pattern of left-right asymmetry determination by cilia driven fluid flow towards the left side in the grp, described for *X. laevis* (Blum et al. 2014b; Sáenz-Ponce et al. 2012b). The question is particularly important because the mechanism of symmetry breakage by cilia driven fluid flow in the grp or equivalent structures is universal among vertebrates with

exception of the chick and the pig (Blum et al. 2014a,b). In all frogs analyzed, the gastrocoel roof plate (grp) had a triangular shape and was detected in the dorsal lining of the primitive gut of the late gastrula and neurula, as detected in *H. fleischmanni* and *E. callistomma* embryos (Fig. 12A–F; Table 1). As in *X. laevis* and other frogs, the grp of *H. fleischmanni* and *E. callistomma* embryos consisted of the ventral surface of the posterior notochord and paraxial mesoderm, and it was bordered by the lateral endodermal crests (lec), illustrated for *E. callistomma*, (Figs. 12D–E). However, in a more rostral region, only the notochord was exposed to the cavity of the primitive gut because the paraxial mesoderm was already covered by the closing lec (Fig. 12F). The major difference detected among frogs was the presence of the grp already in the late gastrula of the centrolenid frogs, as shown for *E. callistomma* (Fig. 12D), whereas the grp developed in the neurula of *X. laevis* (Blum et al. 2014b). The precocious onset of grp formation may relate to the overlap of neurulation and gastrulation in centrolenid frogs, another example of the modular nature frog gastrulation.
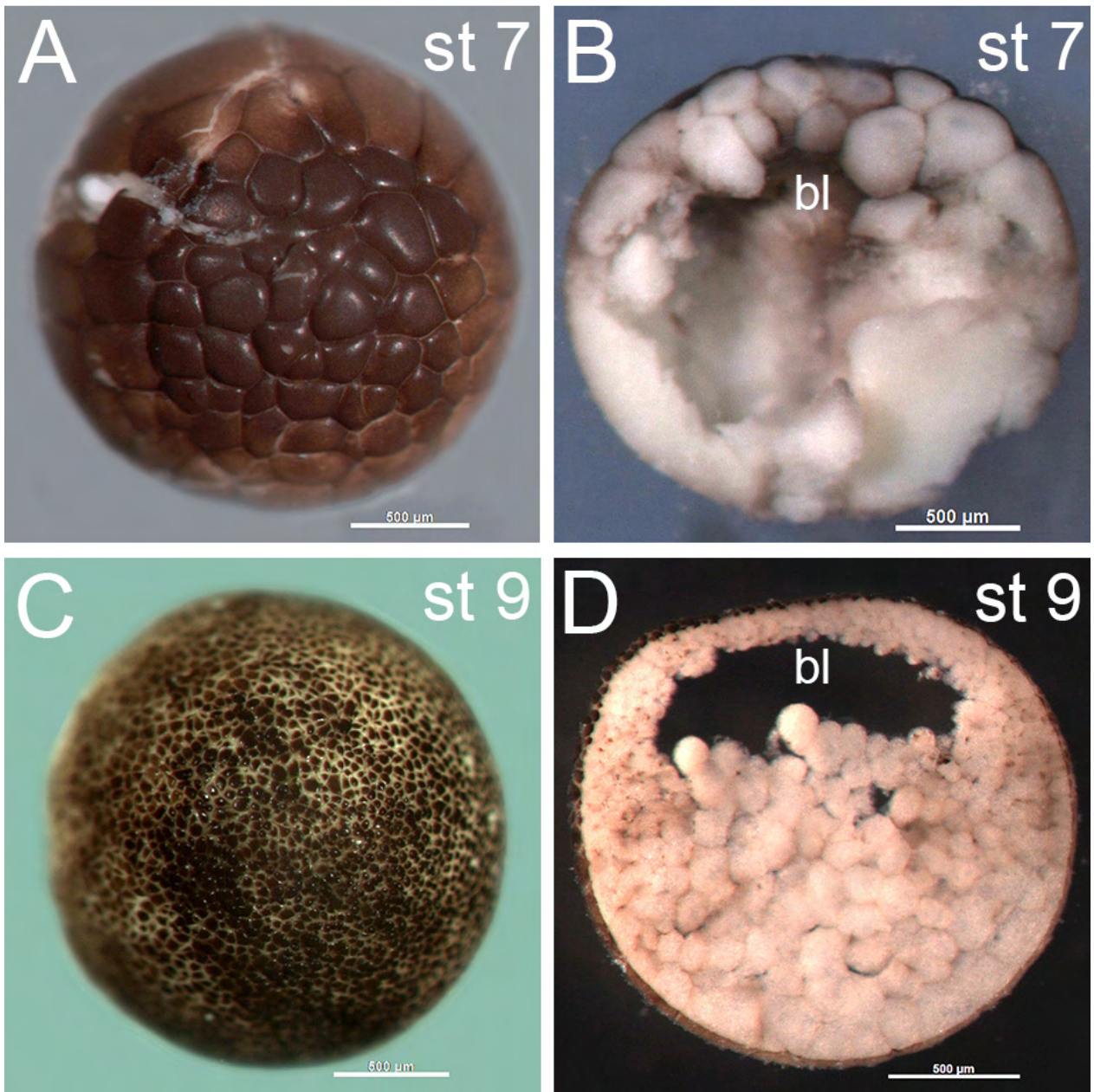
H. fleischmanni
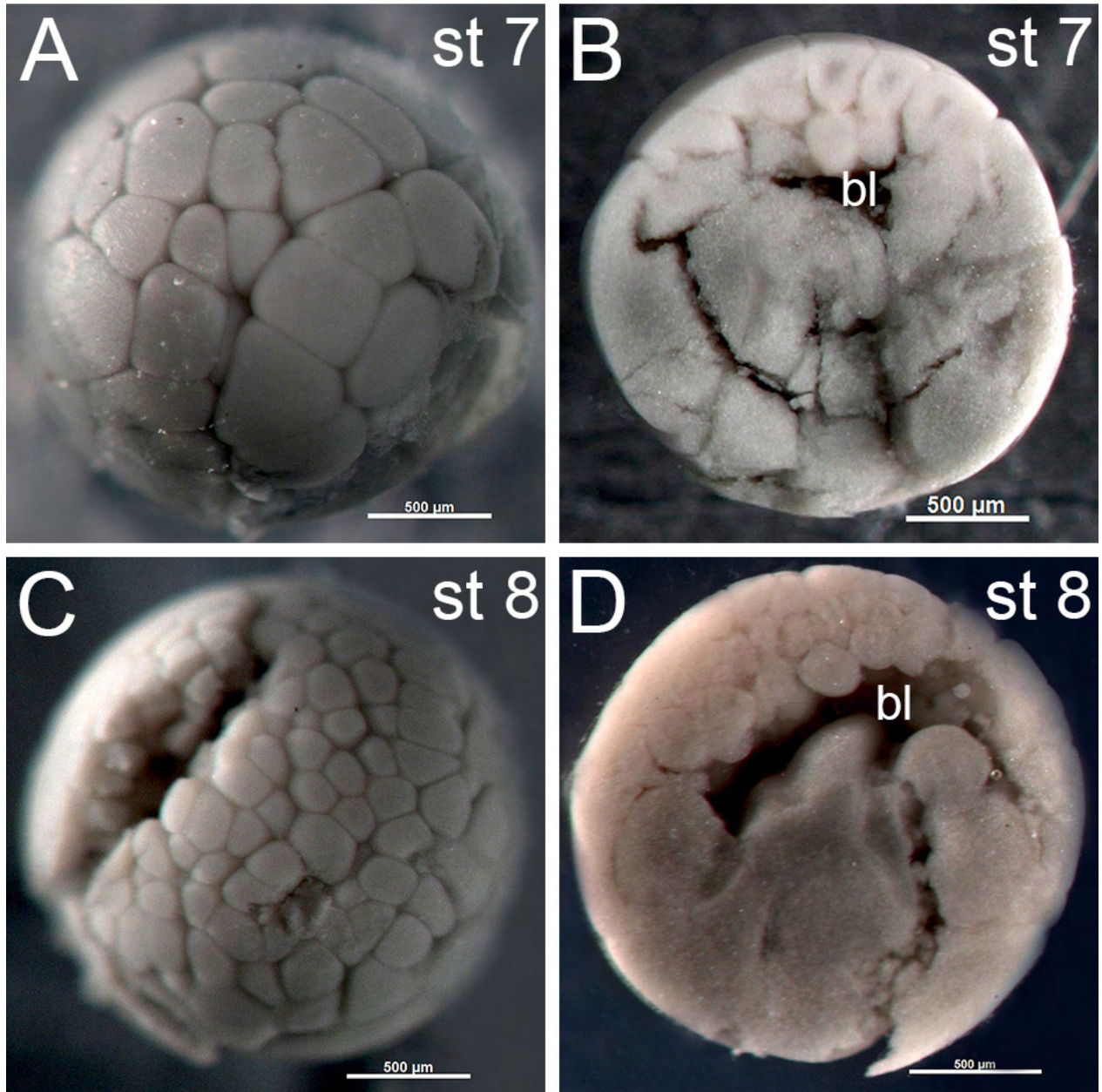
Fig. 6. Cleavage in *Hyalinobatrachium fleischmanni*. **(A)** Stage 7: Animal view of a 32–cell embryo. **(B)** Stage 7: The blastocoel of a 32–cell embryo, observed in a sagittal bisection. **(C)** Stage 8: Animal view of an embryo at mid–cleavage. **(D)** Stage 8: The blastocoel of a mid–cleavage embryo, observed in a sagittal bisection. bl, blastocoel.

The grp was detected in the neurula of eight frog species with a wide range of reproductive adaptations, and belonging to six different frog families, (Table 1) (Sáenz-Ponce et al. 2012a, b). The presence of the grp in this wide range of frogs suggests that determination of left-right asymmetry may follow mechanisms similar to those described for *X. laevis*. Moreover, cilia were detected in the grp epithelium that lines the dorsal roof of the primitive gut of these various frogs (Sáenz-Ponce et al. 2012a, b). The presence of cilia in the grp in centrolenid frogs was not analyzed.

Conclusions. The reproductive and developmental strategies of the two centrolenid frogs, analyzed in this work, differ from each other. The eggs of *E. callistomma*, deposited on the upper sides of plant leaves, contain dark pigment, and take twice as long to reach the hatching stage in comparison with *H. fleischmanni* embryos. In contrast, the *H. fleischmanni* development on the underside of plant leaves is accompanied by the lack of dark pigment in the egg and embryos and reduced developmental time. As in other frogs with rapid development, there was overlap between gastrulation and body elonga-
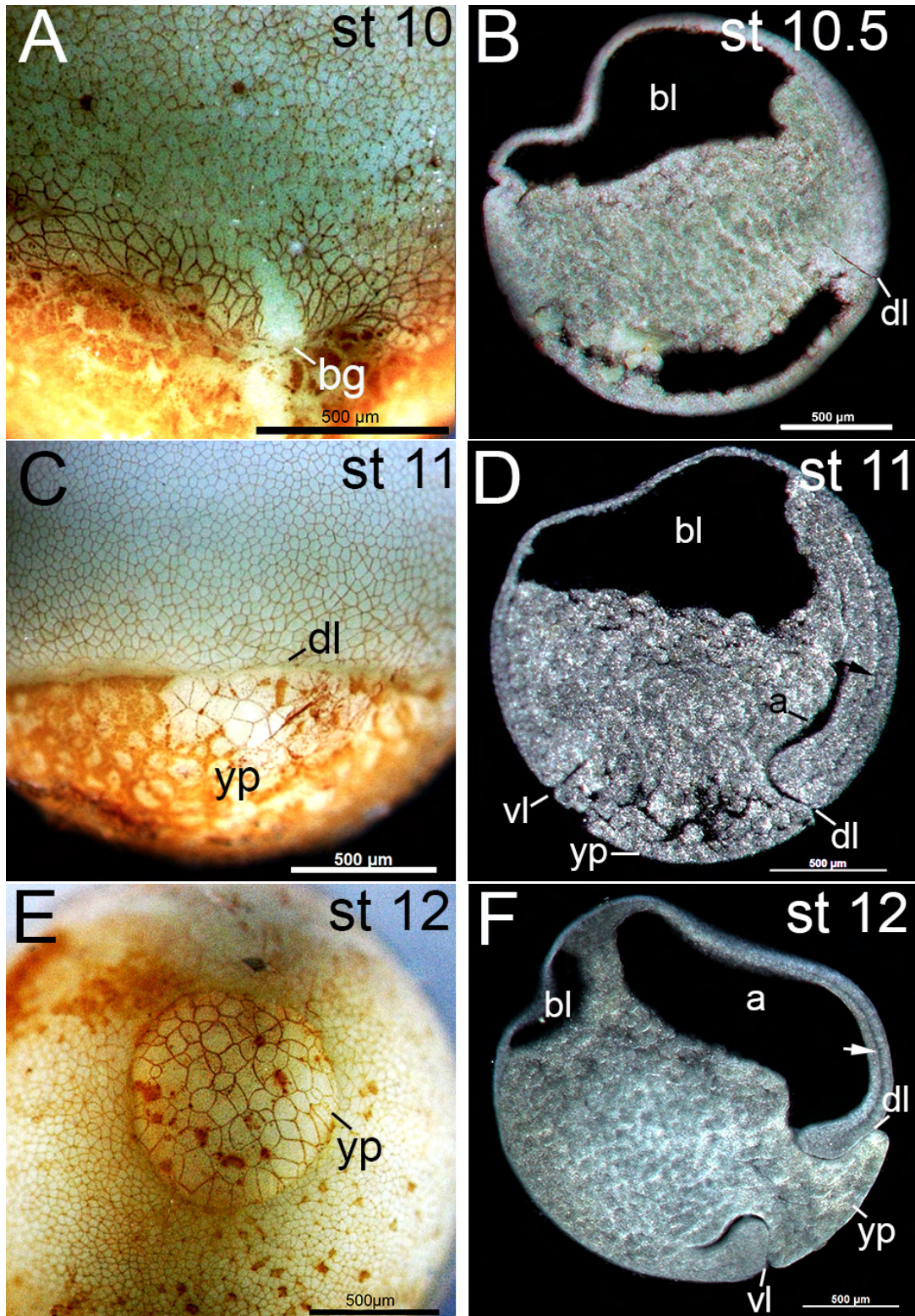
E. callistomma

Fig. 7. Cleavage in *Espadarana callistomma*. **(A)** Stage 7: Animal view of a 32–cell embryo. **(B)** Stage 7: The blastocoel of a 32–cell embryo, observed in a sagittal bisection. **(C)** Stage 9: Animal view of a blastula. **(D)** Stage 9: The blastocoel of a blastula, observed in a sagittal section. The blastocoel roof consisted of several cell layers. bl, blastocoel.

tion. Moreover, the process of neurulation already started during gastrulation, and the grp became visible in the late gastrula. Presence of the grp in embryos of these centrolenid frogs suggests that the mechanisms of left-right asymmetry is likely similar with the cilia-driven pattern of the *X. laevis* grp.
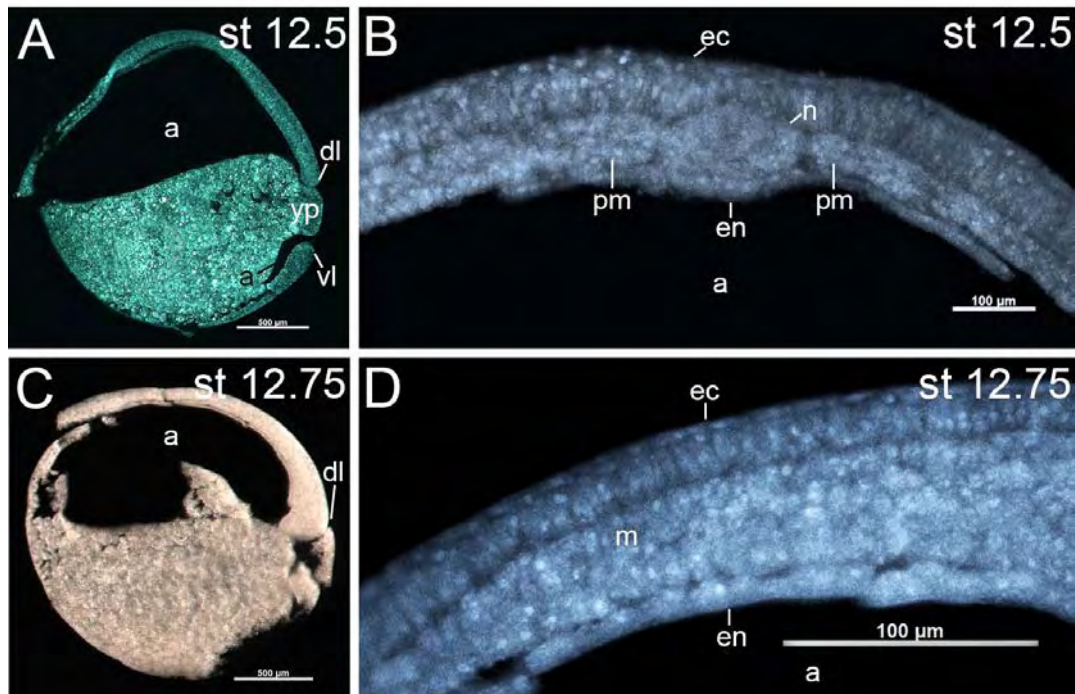
Hyalinobatrachium fleischmanni

Fig. 8. Gastrulation of *Hyalinobatrachium fleischmanni* (Stages 10–12). Embryos in A, C, E were stained for cell borders. **(A)** Stage 10: Early gastrula. Dorsal subequatorial region. The dorsal blastopore groove was visible between the small cells of the animal region with clearly delineated borders, and the vegetal cells, whose borders were not as clear. **(B)** Stage 10.5: Sagittal section of an early gastrula. The dorsal blastopore lip was visible. **(C)** Stage 11: Mid gastrula. Higher magnification of the dorsal blastopore lip region. There was difference in size of animal and vegetal cells. **(D)** Stage 11: Sagittal section of a mid gastrula. The archenteron was elongated, and the blastocoel roof was reduced to about two cell layers. **(E)** Stage 12: Late gastrula. Higher magnification of the yolk plug region. **(F)** Stage 12: Sagittal section of late gastrula. The arrow indicates the cleft of Brachet. a, archenteron; bg, blastopore groove; bl, blastocoel; dl, dorsal blastopore lip; vl, ventral blastopore lip; yp, yolk plug.
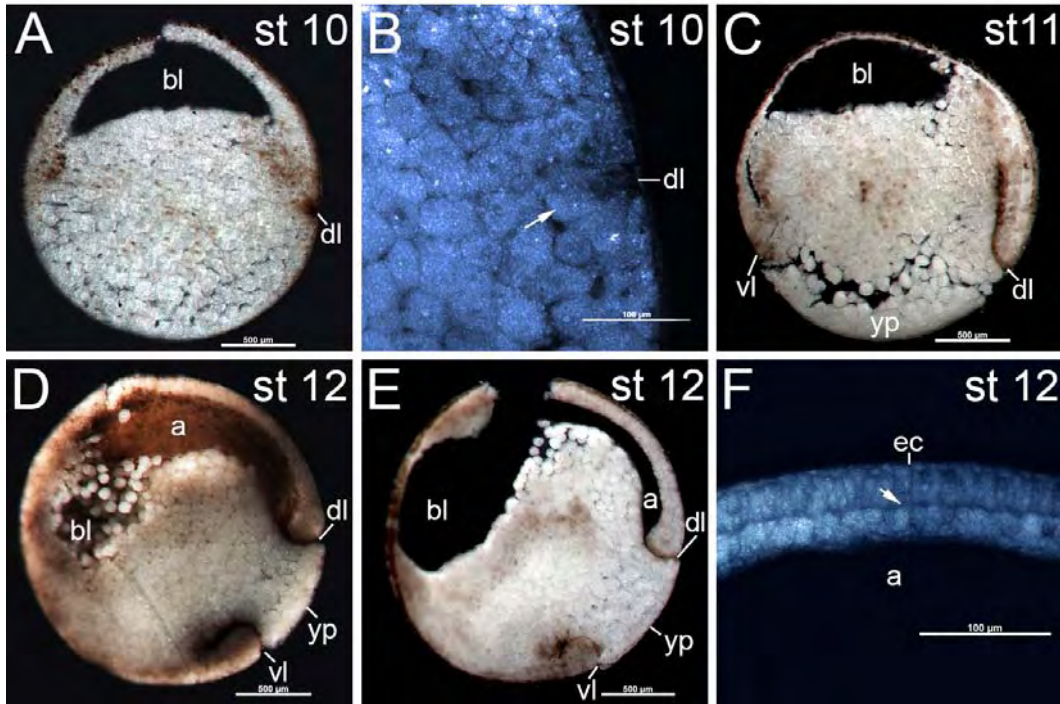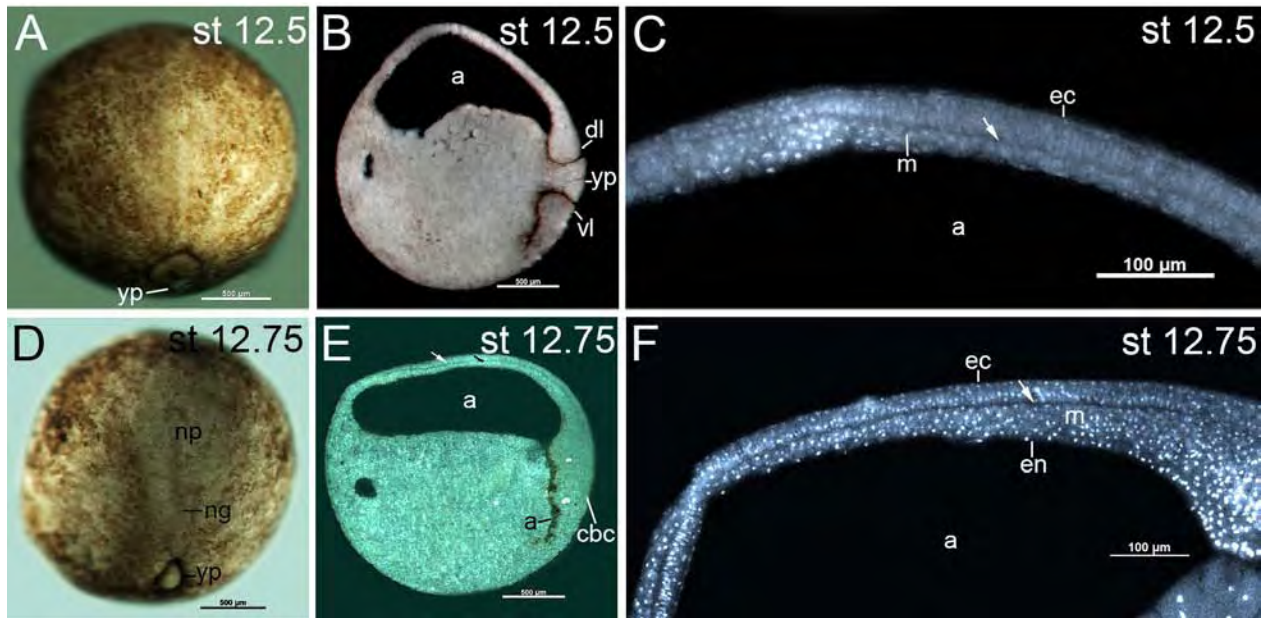
E. callistomma

Fig. 9. Gastrulation of *Espadarana callistomma* (Stages 10–12). **(A)** Stage 10: Sagittal section of an early gastrula. The dorsal blastopore groove was visible. **(B)** Stage 10. Higher magnification of the embryo in A, stained for cell nuclei. The arrow indicates a bottle cell of the blastopore groove area. **(C)** Stage 11: Sagittal section of mid gastrula. **(D)** Stage 12: Sagittal bisection of late gastrula. **(E)** Stage 12: Sagittal section of the late gastrula shown in D. The single cavity is an artifact of sectioning, it corresponds to the blastocoel and archenteron, as shown in D. **(F)** Higher magnification of the archenteron roof from the embryo in E, stained for cell nuclei. The arrow indicates the cleft of Brachet. a, archenteron; bl, blastocoel; dl, dorsal blastopore lip; ec, ectoderm; vl, ventral blastopore lip; yp, yolk plug.

H. fleischmanni

Fig. 10. Gastrulation of *Hyalinobatrachium fleischmanni* (Stages 12.5–12.75). **(A)** Stage 12.5: Sagittal section of a late gastrula. **(B)** Stage 12.5: Cross section through the rostral region of a late gastrula, stained for cell nuclei. The endoderm covered the notochord in this rostral section. **(C)** Stage 12.75: Sagittal section of a late gastrula. **(D)** Stage 12.75: Higher magnification of the archenteron roof from the embryo in E, stained for cell nuclei. The three germ layers were visible. a, archenteron; dl, dorsal blastopore lip; ec, ectoderm; en, endoderm; m, mesoderm; pm; paraxial mesoderm, vl, ventral blastopore lip; yp, yolk plug.

E. callistomma

Fig. 11. Gastrulation of *Espadarana callistomma* (Stages 12.5–12.75). **(A)** Stage 12.5: Late gastrula with a small yolk plug. **(B)** Stage 12.5: Sagittal section of a late gastrula. **(C)** Stage 12.5: Sagittal section of the archenteron roof through the rostral region of a late gastrula, stained for cell nuclei. **(D)** Stage 12.75: Late gastrula. The neural plate was visible. **(E)** Stage 12.75: Parasagittal section of a late gastrula. **(F)** Stage 12.75: Higher magnification of the archenteron roof from the embryo in E, stained for cell nuclei. The arrows in C, E and F indicate the cleft of Brachet. a, archenteron; cbc, circumblastoporal collar; dl, dorsal blastopore lip; ec, ectoderm; en, endoderm; m, mesoderm; ng, neural groove; np, neural plate; vl, ventral blastopore lip; yp, yolk plug.

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H. fleischmanni
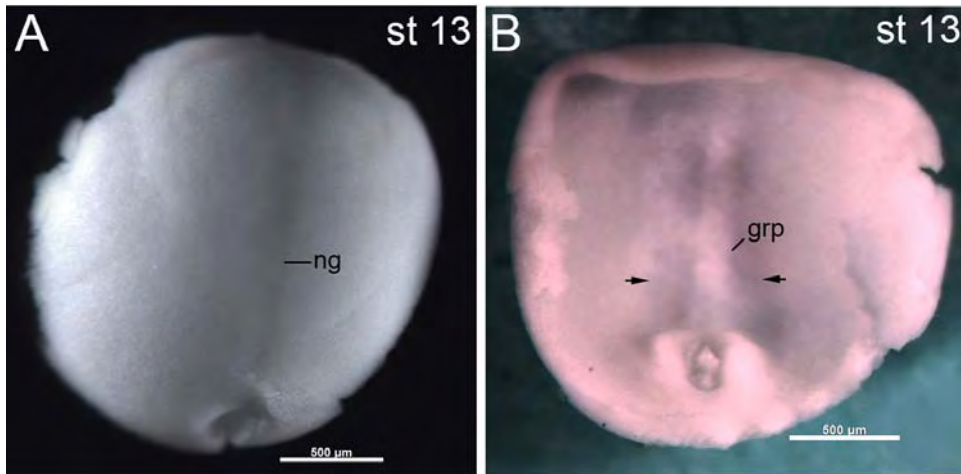
E. callistomma
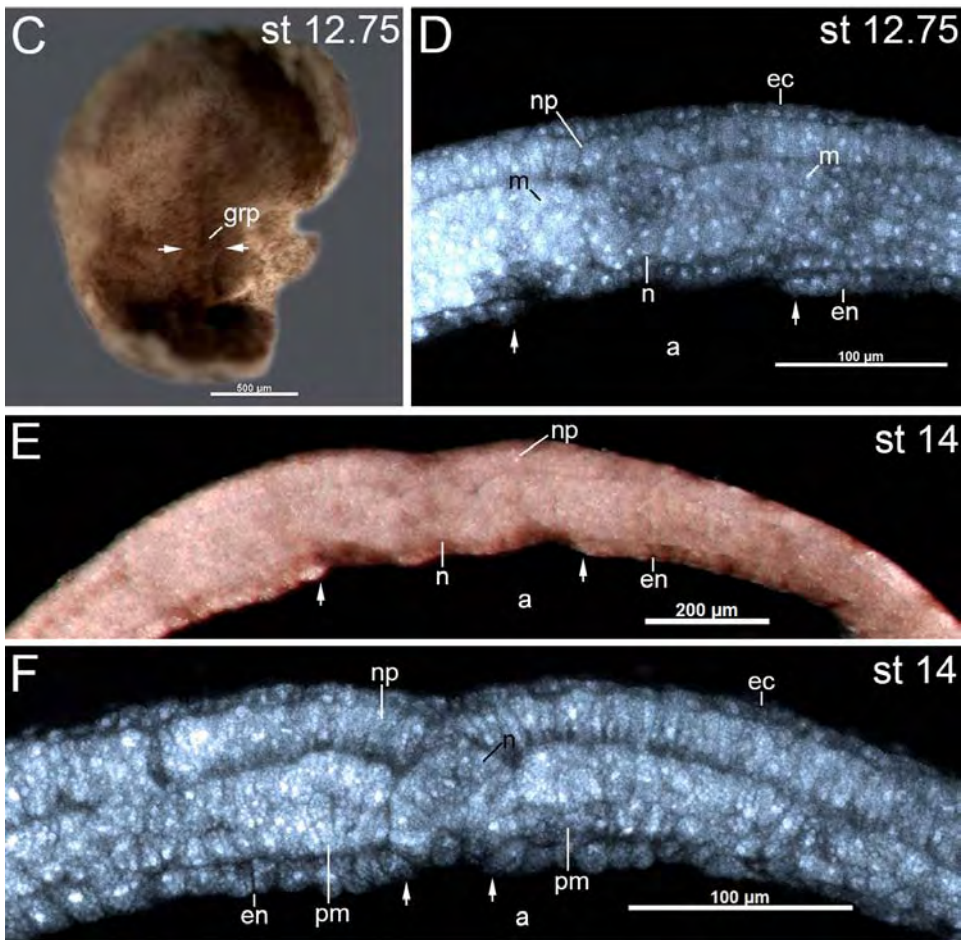
Fig. 12. The gastrocoel roof plate (grp) in embryos of *Hyalinobatrachium fleischmanni* and *Espadarana callistomma*. **(A)** Stage 13: External view of the neural plate of *H. fleischmanni*. **(B)** Stage 13: Internal view of the neural plate region of *H. fleischmanni*. The grp was visible in the midline. Arrows indicate the lateral endodermal crests (lec) at the border of the grp in B−F. **(C)** Stage 12.75: Internal view of the neural plate region of *E. callistomma*. The grp was visible in the midline. **(D)** Stage 12.75: Cross section through the caudal region of a late gastrula of *E. callistomma*, stained for cell nuclei. The grp was exposed in the midline and bordered by the lec. The grp consisted of the ventral surface of the notochord and paraxial mesoderm. **(E)** Stage 14: Early neural fold of *E. callistomma*. Cross section through the caudal region. The grp was exposed in the midline and bordered by the lec. The grp consisted of the ventral surface of the notochord and paraxial mesoderm, as in stage 12.75 embryos (shown in D). **(F)** Stage 14: Early neural fold of *E. callistomma*. Cross section through the rostral region, stained for cell nuclei. The grp included only the ventral surface of the notochord, due likely to the rostral closure of the lec. Only the notochord was exposed in the midline, and bordered by the lec. a, archenteron, ec, ectoderm; en; endoderm; grp, gastrocoel roof plate; m, mesoderm; n, notochord; np, neural plate; pm, paraxial mesoderm; ng, neural groove.

H. fleischmanni
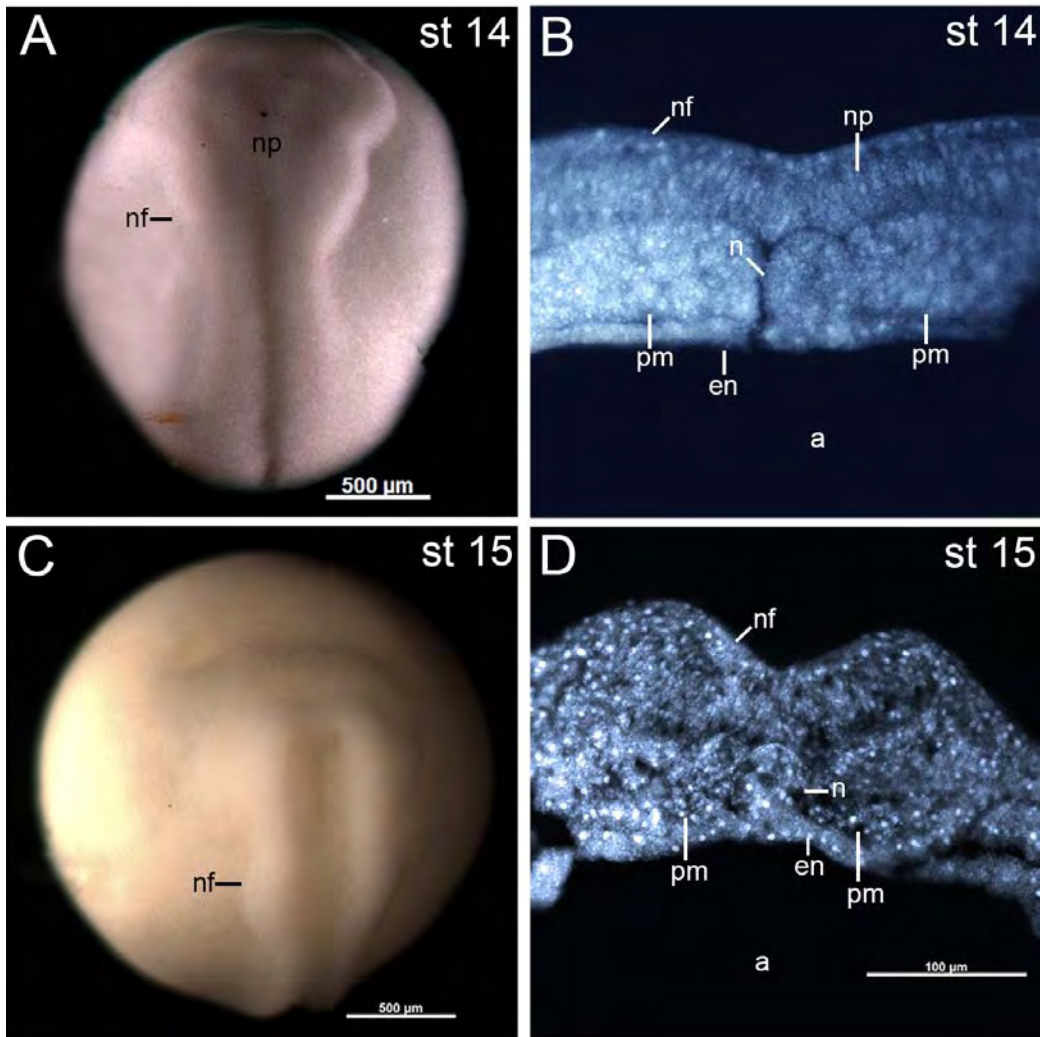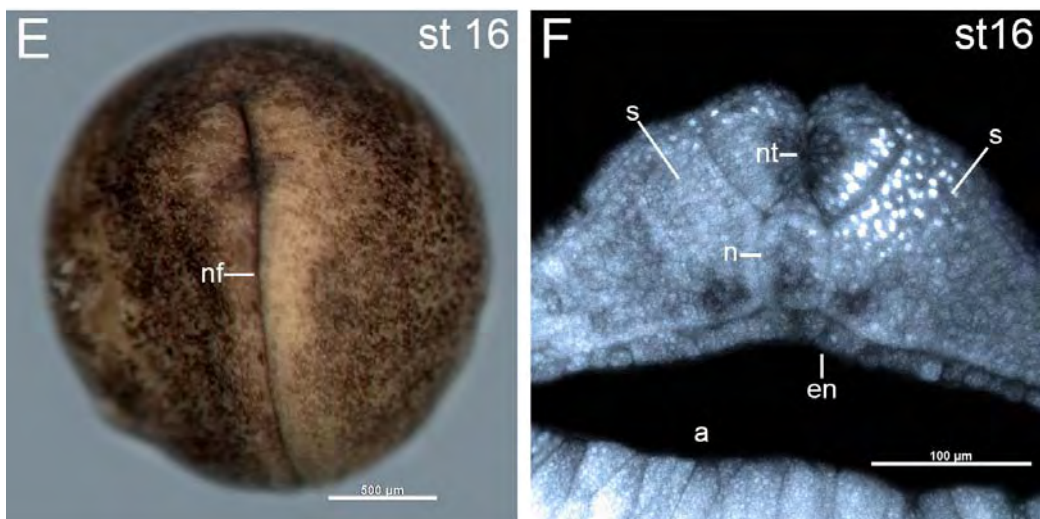
Fig. 13. Neurulation of *Hyalinobatrachium fleischmanni* and *Espadarana callistomma*. **(A)** Stage 14. Early neural fold stage of *H. fleischmanni*. **(B)** Stage 14: Cross section through the rostral region of the embryo in A, stained for cell nuclei. The notochord is totally covered by endoderm. **(C)** Stage 15: Mid neural fold of *H. fleischmanni*. **(D)** Stage 15: Cross section through the rostral region of the embryo in C, stained for cell nuclei. **(E)** Stage 16: Mid neural fold stage of *E. callistomma*. **(F)** Stage 16: Cross section through the rostral region of *E. callistomma*, stained for cell nuclei. The neural folds were closed. a, archenteron; en, endoderm; n, notochord; nf, neural fold; np, neural plate; nt, neural tube; pm, paraxial mesoderm; s, somite.

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A new species and country record of threadsnakes (Serpentes: Leptotyphlopidae: Epictinae) from northern Ecuador

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Abstract.—We describe a new species of *Trilepida* Hedges 2011 from cloud forests of the extreme northern Ecuadorian Andes, Carchi province. Among other characters, the new species is distinguished from all congeners by having a subhexagonal ocular with its anterior border barely rounded at eye level, rostral reaching the anterior border of ocular scales in dorsal view, three supralabials, four or five infralabials, thicker body width, 203–214 middorsal scales, 12 scales around middle of tail, uniform gray dorsum, and gray venter with interspaces between scales cream. Morphologically, the new species is most similar to *T. guayaquilensis* and *T. joshuai* from Ecuador and Colombia, respectively. We also report the first records of *T. macrolepis* for the country from the lowland and foothill evergreen forests of northwestern Ecuador.

Introduction

Fossorial snakes of the family Leptotyphlopidae are among the least known terrestrial vertebrates (Adalsteinsson et al. 2009). Even though some species in the family may be locally abundant and the group has a wide distribution from sea level to mountaintops in Africa, the Americas, and parts of Asia, their secretive habits make them rarely encountered in the field (Curcio et al. 2002; McDiarmid et al. 1999; Passos et al. 2005; Pinto et al. 2010). Most leptotyphlopids are small (150 to 250 mm snout-vent length), thin, and burrowing animals that feed on social insects (termites are probably the main food source for some species) (Vitt and Caldwell 2013). Recent phylogenetic analyses based on molecular data partitioned the 112 species now recognized in the family in 12 genera (Adalsteinsson et al. 2009; Wallach et al. 2014).

As a combination of limited morphological variation in fossorial squamates and paucity of specimens, morphological synapomorphies for these lineages have not been clearly established yielding differences in researchers' opinions as to which genus some species should be allocated. Especially problematic has been the classification of threadsnakes in the Neotropical genera *Rena*, *Siagonodon*, and *Trilepida* (Pinto and Curcio 2011; Pinto and Fernandes 2012).

In Ecuador, four species of threadsnakes have been confirmed on the basis of voucher specimens: *Epictia signata*, *E. subcrotilla*, *Trilepida anthracina*, and *T. guayaquilensis* (Cisneros-Heredia 2008; Pinto et al. 2010; Salazar-Valenzuela et al. 2010; Torres-Carvajal et al. 2014; Wallach et al. 2014). With the exception of *E. subcrotilla*, which seems to be a common taxon in natural history collections (Cisneros-Heredia 2008; Purtschert

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2007), most of the leptotyphlopid species reported for the country are known from a few specimens or exclusively from the holotype in the case of *T. guayaquilensis* (Cisneros-Heredia 2008).

Cryptozoic species of snakes are difficult to find and the usefulness of regularly including digging techniques during herpetological surveys has been proposed for fossorial reptiles (Measey 2006). While performing fieldwork on the extreme northern Andes of Ecuador, we found specimens of an undescribed species of *Trilepida* with the help of local people who usually dig for archaeological remains of Los Pastos pre-hispanic culture. Here we recognize this species based on morphological data, increasing the number of species of *Trilepida* to 14 (Uetz and Hosek 2014; Wallach et al. 2014). While reviewing material for this study we came across specimens assignable to *Trilepida macrolepis*, which constitute the first record of this species for Ecuador and are also reported herein.

Materials and Methods

We examined specimens housed in the Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ) and the Museo Ecuatoriano de Ciencias Naturales (DHMECN), Quito, Ecuador. In addition, we analyzed photographs of specimens deposited in the National Museum of Natural History, Smithsonian Institution (USNM), Washington, D.C., USA, and the Museum für Naturkunde (ZMB) Berlin, Germany. Characters used for description and comparisons were based on internal (skull of a MicroCT Scanned specimen, X-ray plates) and external morphology (meristic and morphometric data, shape of cephalic plates, and color pattern) of examined individuals, as well as published data provided by Rojas-Morales and González-Durán (2011) and those summarized in Pinto and Fernandes (2012). We consider the unique combination of morphological characters present in the new species as delimitation criteria, following the general species concept of de Queiroz (1998, 2007). Terminology for cephalic plates, scale features, cloacal sacs, and measurements follows Broadley and Wallach (2007), Kroll and Reno (1971), Passos et al. (2006), Pinto and Curcio (2011), and Pinto and Fernandes (2012). Color description in life is based on analysis of a series of photographs of the holotype and paratypes. Color terminology and codes follow Köhler (2012). Measurements were taken with a dial caliper to the nearest 0.1 mm, except for total length (TL) and tail length (TL), which were measured with a ruler to the nearest 1.0 mm. Measurements and descriptions of paired cephalic scales are provided for the right side. Sex was determined by the presence or absence of hemipenial muscles through a ventral incision at the base of the tail. Characters recorded are: (1) Total length (TL); (2) tail length (TAL); (3) TL/TAL ratio; (4) middorsal scales (rostral and terminal spine excluded);

(5) midventral scales (mental scale, cloacal shield, and subcaudals excluded); (6) subcaudal scales (terminal spine excluded); (7) dorsal scale rows around the middle of the tail (DSR); (8) midbody diameter (MB); (9) midtail diameter (MT); (10) head length (HL); (11) head width (HW); (12) relative eye diameter (ocular width at eye level/eye diameter); (13) presence of fused caudals; (14) relative rostral width (rostral width/head width) (Pinto and Curcio 2011; Pinto et al. 2010). The head of one preserved specimen (QCAZ 5778) was scanned on a Skyscan 1176 in-vivo high-resolution micro-CT scan at Universidade de São Paulo, Brazil. The specimen was scanned at 40 kV and 533 uA, and the dataset was rendered in three dimensions through the use of CTVox for Windows 64 bits version 2.6. Additionally, the skeleton of the holotype and paratypes were examined dorsally and ventrally through X-ray plates. Digital radiographies of QCAZ 5778 were made with a Faxitron X-Ray LLC MX 20 at Departamento de Vertebrados, Museu Nacional, Universidade Rio de Janeiro, Brazil and X-ray scans of QCAZ 8990 and QCAZ 5846 were made with a Thermo Kevex X-ray Imaging System at QCAZ. Terminology for the braincase, mandible, and vertebral column follows Rieppel et al. (2009), Kley (2006), and Holmann (2000), respectively.

Results

Trilepida pastusa sp. nov.

urn:lsid:zoobank.org:act:E7C8FF1C-07E8-4985-B673-80A52DACA8D1

Figs. 1–3.

Holotype.—Adult female, QCAZ 8690, collected on 23 February 2009 by O. Torres-Carvajal, S. Aldás-Alarcón, E. Tapia, A. Pozo and local people, surroundings of Chilmá Bajo on the way to Tres Marías waterfall (0°51'53.82" N, 78°2'59.23" W; 2071 m), Tulcán County, Carchi province, Ecuador.

Paratypes.—Two specimens with same locality data as holotype: one juvenile female (QCAZ 5778) collected on 21 February 2013 by D. Salazar-Valenzuela, H. Pozo, A. Chalapud, and D. Males, and one juvenile of undetermined sex (QCAZ 5846) collected on 20 March 2013 by D. Salazar-Valenzuela and A. Loaiza-Lange.

Diagnosis.—*Trilepida pastusa* is distinguished from all congeners by the following combination of characters: Snout truncate in dorsal and ventral view, rounded in lateral view; supraocular present; ocular subhexagonal with superior border straight and anterior border barely rounded at eye level; rostral subtriangular in dorsal view, reaching anterior border of ocular scales; frontal as long as supraocular and other middorsal cephalic shields, except for postfrontal which is smaller; temporal distinct; supralabials three (2+1); infralabials four or five; body
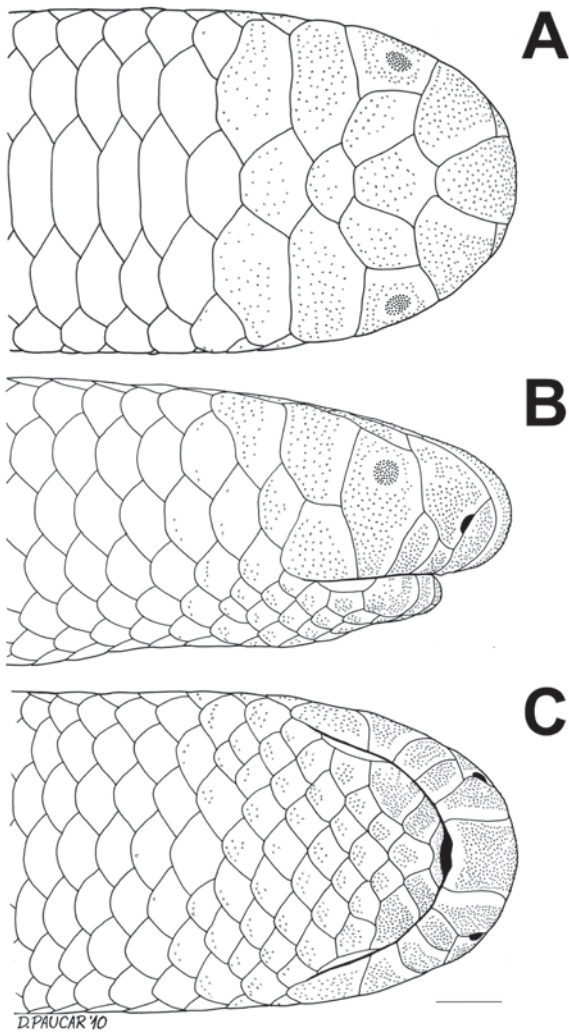
Fig. 1. Dorsal (A), lateral (B), and ventral (C) views of the head of the holotype of *Trilepida pastusa* sp. nov. (QCAZ 8690). Scale bar = 1.5 mm. *Drawings by D. Paucar.*

width relatively thick (TL/MB = 28.6–34.6); middorsal scales 203–214; midventral scales 182–193; subcaudal scales 18–19; fused caudals present; terminal spine conical, slightly longer than wide; scales around middle of tail 12; dorsum uniform gray; venter gray with interspaces between scales cream.

Comparisons (Table 1).—Among all congeners, *Trilepida pastusa* is more similar to *T. guayaquilensis* and *T. joshuai* in sharing 12 scales around midtail, three supralabials, and a uniform dark dorsum and pale venter (Pinto and Fernandes 2012). The new species differ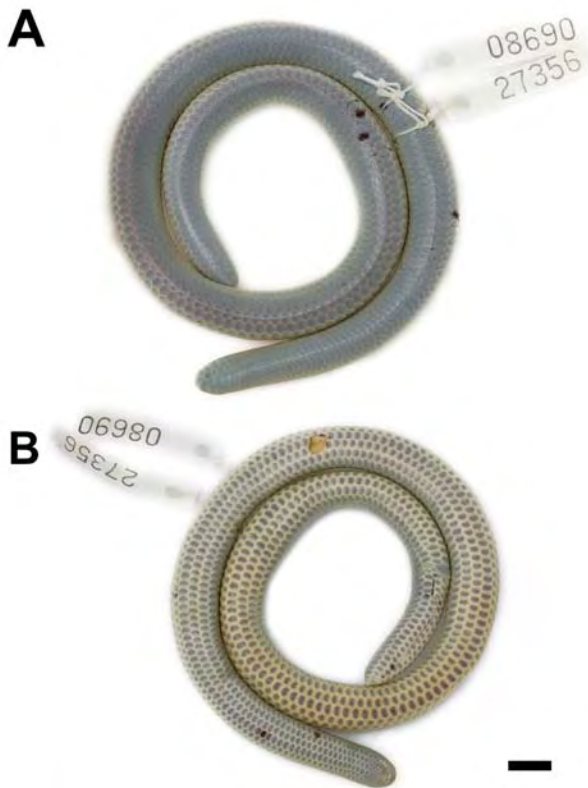s from both by having an ocular with an anterior border barely rounded at eye level, a thick body (TL/MB = 28.6–34.6), and a coloration pattern consisting of a uniform gray dorsum and a venter with gray on the center and cream on the outside of each scale (Figs. 3, 4A) (vs ocular with rounded anterior border, thin body [TL/MB = 48.6], and uniform brown color dorsally and pale brown ventrally in *T. guayaquilensis*; ocular with rounded anterior border, moderate to thin body width [TL/MB = 34.0–55.2], and uniform dark brown dorsally and cream ventral scales in *T. joshuai*) (Orejas-Miranda and Peters 1970; Pinto and Fernandes 2012; Pinto et al. 2010; Rojas-Morales and González-Durán 2011). Moreover, *T. pastusa* differs from *T. guayaquilensis* in having a lower number of middorsal (203–214 vs 253, respectively) and midventral (182–193 vs 233, respectively) scales (Orejas-Miranda and Peters 1970; Pinto and Fernandes 2012). The new species also differs from *T. joshuai* by having a higher number of middorsal (203–214 vs 174–199, respectively) and midventral (182–193 vs 165–187, respectively) scales, and a higher number of subcaudals (18–19 vs 13–18, respectively) (Pinto and Fernandes 2012; Pinto et al. 2010; Rojas-Morales and González-Durán 2011). Some specimens of *T. macrolepis* can have 12 scales around

Table 1.—Meristic and morphometric variation of the four species of *Trilepida* occurring in Ecuador. Data are from Pinto and Fernandes (2012) and references therein, as well as our own scale counts and measurements. Abbreviations: DO = middorsal scales; VE = midventral scales; SC = subcaudals; TL = total length; TAL = tail length; MB = midbody diameter; MT = midtail diameter; SL = supralabials; IL = infralabials; SO = supraocular. Color pattern adapted from Passos et al. (2006) and Pinto and Fernandes (2012): 1 = uniform violet black dorsally and ventrally; 2 = reticulate dark brown dorsally and reticulate pale brown ventrally; 3 = uniform brown dorsally and pale brown ventrally; 4 = uniform gray dorsally and reticulate gray ventrally.

| Character	*Trilepida pastusa* sp. nov.	*Trilepida macrolepis*	*Trilepida guayaquilensis*	*Trilepida anthracina*
DO	203–214	211–255	253	182–193
VE	182–193	201–237	233	167–176
SC	18–19	16–24	20	15–19
TL/TAL	10.86–13.67	8.2–15.9	13.1	12.2–16.6
TL/MB	28.63–34.55	32.2–68.3	48.6	31.7–43.7
TAL/MT	2.67–3.81	3.8–7.9	–	3.6–10.1
SL	2+1	2+1	2+1	2+1
IL	4–5	4	4	4
SO	present	present	present	present
Midtail scales	12	10	12	10
Color pattern	4	2	3	1

Fig. 2. Dorsal (A) and ventral (B) views of the holotype of *Trilepida pastusa* sp. nov. (QCAZ 8690) in preservative. Scale bar = 1 cm.

midtail (see Discussion), but *T. pastusa* differs from this species by having an ocular with barely rounded anterior border, thick body (TL/MB = 28.6–34.6), uniform gray dorsum and venter with gray on the center and cream on the outside of each scale, 203–214 middorsal scales, 182–193 midventral scales, 18–19 subcaudals, non-enlarged eyes, and rostral reaching ocular level in dorsal view (vs ocular with rounded anterior border, moderate to thin body width [TL/MB = 32.2–68.3], reticulate dark brown dorsally and reticulate pale brown ventrally, 211–255 middorsal scales, 201–237 midventral scales, 16–24 subcaudals, enlarged eyes, and rostral not reaching ocular level in *T. macrolepis*) (Fig. 4) (Pinto and Fernandes 2012; Pinto et al. 2010).

Description of the holotype (Figs. 1, 2).—Adult female, TL 315 mm, TAL 29 mm; MB 10.2 mm; MT 7.6 mm; TL/TAL 10.9; TL/MB 30.9; TAL/MT 3.8; HL 6.2 mm, HW 6.0 mm; relative eye diameter 3.1; relative rostral width 0.4. Body subcylindrical, robust, head slightly compressed compared to body and slightly tapered caudally near tail. Head subcylindrical, as long as wide and slightly distinguishable from neck. Snout slightly rounded in dorsal and ventral views, rounded in lateral view; rostral straight in frontal and ventral views, subtriangular in dorsal view but with rounded apex, reaching imaginary transverse line between anterior border of oculars; rostral contacting supranasal and infranasal laterally, and

frontal dorsally; nasal completely divided horizontally by oblique suture crossing nostril and descending posteriorly to contact first supralabial; nostril roughly elliptical, obliquely oriented and located in middle of nasal suture; supranasal higher than wider, bordering rostral anteriorly, infranasal inferiorly, first and second supralabials, and ocular posteriorly, and frontal and supraocular dorsally; supranasal ventral margin half the length of upper border of infranasal scale; infranasal about twice as high as wide, longer than any of the supralabials; upper lip border formed by rostral, infranasal, two anterior supralabials, ocular, and posterior supralabial; temporal distinct in size from dorsal scales of lateral rows; three supralabials, first two anterior to ocular and one posterior (2+1); first supralabial almost twice as high as wide, not reaching nostril and eye levels, second supralabial almost twice as high as wide, higher than first supralabial, reaching nostril level; third supralabial trapezoidal, as high as wide, reaching nostril level, its posterior margin in broad contact with temporal; ocular enlarged, subhexagonal, anterior border barely rounded at eye level, higher than wide, contacting posterior margins of supranasal and second supralabial anteriorly, parietal and third supralabial posteriorly, and supraocular dorsally; eye distinct (diameter = 0.7 mm), located in central area of upper part of ocular, displaced above nostril level; supraocular longer than wide, as long as frontal, between ocular and frontal, contacting supranasal anteriorly, frontal and ocular laterally, and parietal and postfrontal posteriorly; frontal, interparietal, and interoccipital subequal in size, hexagonal and imbricate, postfrontal smaller; frontal longer than wide, contacting rostral, supranasal, supraocular, and postfrontal; postfrontal as long as wide, contacting frontal, supraocular, parietals, and interparietal; interparietal as long as wide, contacting postfrontal, parietals, occipitals, and interoccipital; interoccipital wider than long, contacting interparietal, occipitals, and first dorsal scale of vertebral row; parietal and occipital subequal, irregularly heptagonal; parietal longer than occipital, twice as high as wide, lower margin contacting upper border of third supralabial, posterior margin contacting temporal, occipital, and interparietal, anterior border in contact with ocular, supraocular, and postfrontal; occipital twice as high as wide, its lower limit attaining upper margin of third supralabial, separated from the latter by temporal; symphysial trapezoidal, anterior border slightly concave and posterior border convex except in the middle, five times wider than high; four infralabials; first infralabial twice as high as wide; second infralabial as high as wide; third infralabial twice as wide as high and not pigmented; fourth infralabial as high as wide. Cephalic shields with uniformly scattered sensory pits. Middorsal scales 203; midventral scales 182; scales rows around middle of body 14, reducing to 12 rows in middle of tail; cloacal shield triangular, as wide as long; subcaudals 19; fused caudals present; terminal spine conical, slightly longer than wide; elongated cloacal sacs present. Dorsal scales
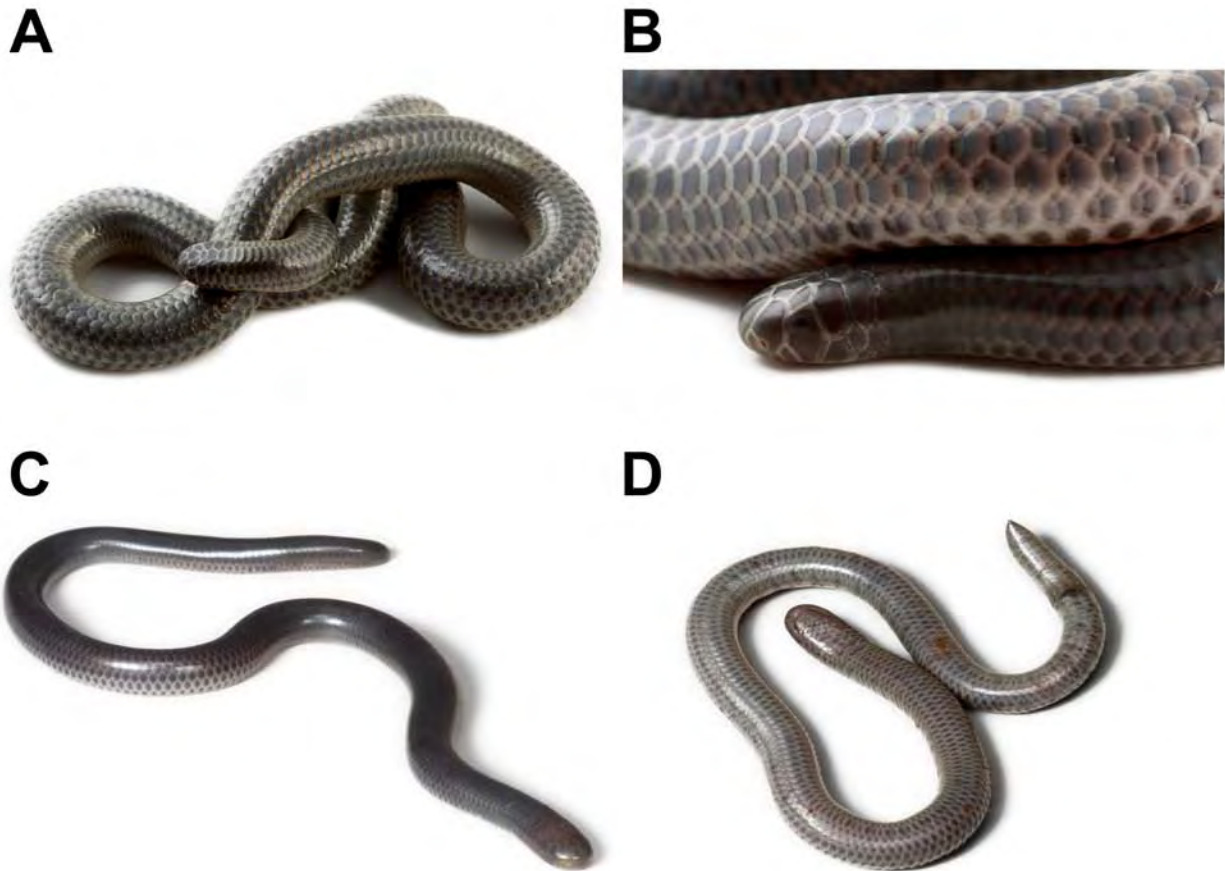
Fig. 3. *Trilepida pastusa* in life. Lateral view of body (A) and head (B) of holotype (QCAZ 8690) and lateral (C) and ventral (D) views of body of juvenile paratype (QCAZ 5846). *Photographs by O. Torres-Carvajal and S.R. Ron.*

homogeneous, cycloid, smooth, imbricate, and wider than long.

Coloration in preservative of the holotype (Fig. 2).— Middorsal scales (i.e., seven longitudinal rows) bluish gray. The remaining seven scale rows forming the ventral and lateral sides of the body are occupied on the center by the same bluish gray color, but the margins of each scale are cream white; the latter pattern is less apparent on the anterior fourth of the body. Border of mouth, mental scale, nostrils and eyes are cream. Cloacal shield bluish gray except on its posterior margin, which is cream with bluish-gray dots.

Color variation.—Dorsal ground color of body similar to that of the holotype in one of the juveniles (QCAZ 5846), the other juvenile (QCAZ 5778) is dark gray; ventral coloration is similar in all specimens.

Color in life.—Dorsum uniform Dark Blue Gray (Color 194) (Figs. 3A, 3C, 4A), with upper part of head Brick Red (Color 36) on both juveniles (Figs. 3C, 4A). Venter of body and tail Dark Blue Gray, with interspaces between scales Cream White (Color 52) (Figs. 3B, 3D, 4A). Anal plate entirely Dark Blue Gray. After a few minutes of handling, the borders of each scale became apparent

and they turned Pale Neutral Gray (Color 296) (Fig. 3D). Tongue Smoky White (Color 261).

Quantitative variation.—Scale counts in *Trilepida pastusa* vary as follows: middorsal scales 203–214 ($\bar{x}$ = 206.67 ± 6.35, n = 3); midventral scales 182–193 ($\bar{x}$ = 186 ± 6.08, n = 3); subcaudals 18–19 ($\bar{x}$ = 18.67 ± 0.58, n = 3); TL 315 mm (n = 1) in adult and 123–136 mm ($\bar{x}$ = 129.5 mm ± 9.19, n = 2) in juveniles; TAL 29 mm (n = 1) in adult and 9–12 mm ($\bar{x}$ = 10.5 mm ± 2.12, n = 2) in juveniles; TL/MB ratio 30.94 (n = 1) in adult and 28.63–34.55 ($\bar{x}$ = 31.59 ± 4.19, n = 2) in juveniles; TAL/MT ratio 3.81 (n = 1) in adult and 2.67–3.41 ($\bar{x}$ = 3.04 ± 0.52, n = 2) in juveniles; infralabials 4 (n = 1) in adult and 5 (n = 2) in juveniles; relative eye diameter 3.07 (n = 1) in adult and 1.70–1.73 ($\bar{x}$ = 1.71 ± 0.02, n = 2) in juveniles; relative rostral width 0.37 (n = 1) in adult and 0.31–0.38 ($\bar{x}$ = 0.35 ± 0.05, n = 2) in juveniles.

Skull (Fig. 5).—Premaxilla roughly rectangular in frontal and ventral views, edentulous, pierced by six foramina; transverse process of premaxilla absent and vomerian process double; nasals paired, approximately quadrangular dorsally, and pierced by a pair of foramina in lateral border of contact with prefrontals; nasal septum descending as medial vertical flanges; prefrontals paired,
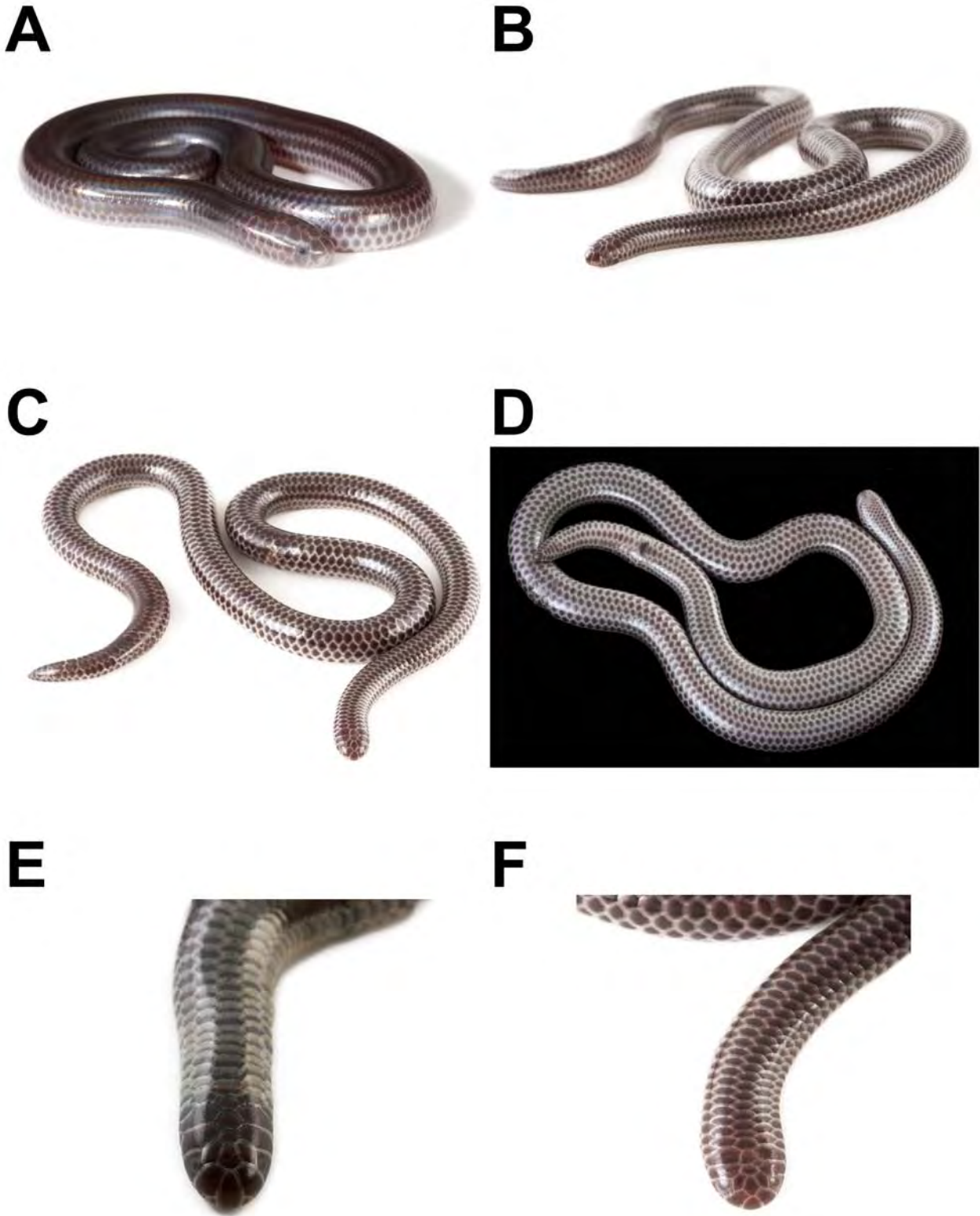
Fig. 4. Lateral (A) view of body of juvenile paratype of *Trilepida pastusa* (QCAZ 5778). Lateral (B), dorsal (C), and ventral (D) views of body of *T. macrolepis* (DHMECN 11400). Dorsal view of head of the holotype of *T. pastusa* (QCAZ 8690) (E) and *T. macrolepis* (DHMECN 11400) (F). *Photographs by L.A. Coloma, O. Torres-Carvajal, and S.R. Ron.*

subtriangular in dorsal view; septomaxillae paired, complex in shape, expanding dorsally into the naris; conchal invagination absent; ascending process of premaxilla pierced by single large foramen; vomers paired, located midventral to vomeronasal cupola, bearing transversal arms, and with short posterior arms; frontals paired, nearly rectangular dorsally, without anterolateral projections to attach to prefrontals; frontal pillars absent; optic nerve restricted to lateral descending surface of frontals; maxilla edentulous, irregular in shape, pierced by three large juxtaposed foramina; posterior orbital element absent; parietal single, wide, representing largest bone of braincase; parabasisphenoid arrow-like, with tapered anterior tip lying bellow palatine, and fitting in medial line of vomeronasal cupola; parabasisphenoid with shallow pituitary fossa; basioccipital single and pentagonal; supraoccipitals fused into single unit, subpentagonal; prootics paired and triangular; large statolythic mass present in *cavum vestibuli*; *crista tubelaris* not enclosing juxtastapedial recess; stapedial footplate apparently not co-ossified with prootic; otoccipitals paired and rectangular; palatines paired and triraadiate; anterior margin of maxillar process slightly flexing ventrally; pterygoids slender and rod-like, not contacting quadrate posteriorly, and not extending beyond the anterior margin of basioccipital; ectopterygoid indistinct; quadrate long and slender, about 50% of skull length; dentary supports a series of five teeth ankylosed to the inner surface of the anterolateral margin of dental concha; mental foramen nearly under the 3rd–4th teeth; splenial conical, representing smallest bone in lower jaw; anterior mylohyoid foramen absent on splenial; posterior milohyoid foramen on the ventral surface of angular; angular conical; compound bone pierced by two foramina in the surangular lamina, posterior surangular foramen large and anterior to the articular portion of compound bone, anterior surangular foramen located below the coronoid; coronoid rests on the compound bone.

Postcranial data.—Precloacal vertebrae 176–184 ($\bar{x}$ = 179 ± 4.16, n = 3); cervical vertebrae 6 + trunk vertebrae 170 (n = 1); caudal vertebrae 23–24 ($\bar{x}$ = 23.3 ± 0.6, n = 3), the last vertebrae representing the fusion of three vertebrae (n = 2). Correlation (n = 3) between middorsal scales and precloacal + subcaudal vertebrae (1:0.99), between midventral scales and precloacal vertebrae (1:1.02), and between subcaudal scales and caudal vertebrae (0.8:1). Pelvic girdle located at the level of the 171st and 176th precloacal vertebrae (QCAZ 5778), or 176th precloacal and 2nd subcaudal (QCAZ 8690). Pelvic girdle represented by four non-fused bones: ilium, ischium, femur, and pubis. Ilium, ischium, and femur rod-like; ilium represents the longest bone of pelvic girdle; femur stout.

Etymology.—The specific epithet is used as a noun in apposition. As explained in Coloma et al. (2010), *pastuso* or *pastusa* is a Spanish word used to refer to the inhabitants of the Pasto region in northern Ecuador and southern Colombia. Here, we also use it to recognize the presence of Los Pastos pre-hispanic culture (500–1500 CE) (Delgado-Troya 2004), whose vestiges remain in the type locality and allowed the discovery of *Trilepida pastusa* and specimens of another cryptozoic snake species: *Atractus savagei* (Salazar-Valenzuela et al. 2014).

Proposed standard English and Spanish names.—Pastuso threadsnakes; Serpientes hebra pastusas.

Distribution and natural history (Figs. 6, 7).—Western versant of the Cordillera Occidental of extreme northern Ecuadorian Andes in Tulcán County, Carchi province. *Trilepida pastusa* is known only from the type locality, which belongs to Montane Cloud Forest (Valencia et al. 1999) at 2,071 m. The holotype was found below mounds of dirt, stones, pasture and moss in conjunction with eggs of *Liophis vitti* (nomenclature following recommendations expressed in Curcio et al. 2009) and adult specimens of *Atractus savagei* (Salazar-Valenzuela et al. 2014). Juvenile paratypes were found below rocks (ca. 40 cm in diameter) in areas of pasture.

First records of Trilepida macrolepis for Ecuador.—The big-scaled threadsnake, *T. macrolepis*, is a relatively large (126–322 mm) leptotyphlopid snake with the widest geographical distribution of all species in the genus (Pinto et al. 2010). Localities for the species in northern South America include Panama, Colombia, Venezuela, Guyana, Suriname, French Guiana, Brazil, and Peru (Wallach et al. 2014). It is distinguished from congeners by having three supralabial and four infralabial scales, 10 rows in the middle of the tail, more than 210 middorsal scales, and more conspicuously because of a dorsal and ventral coloration pattern consisting of dark brown to black scales with white borders (i.e., reticulate) (Passos et al. 2005; Pinto and Fernandes 2012; Pinto et al. 2010). Two specimens from Esmeraldas province in northern Ecuador agree with most of these characters, their scale counts vary as follows (QCAZ 10247, juvenile of undetermined sex and DHMECN 11400, adult male, respectively): middorsal scales 239, 250; midventral scales 220, 228; subcaudals 21, 20; TL 158 mm, 333 mm; TAL 11 mm, 23 mm; SL 2+1, 2+1; IL 4, 6; SO 1, 1; midtail scales 12, 10. Coloration pattern on both is reticulate dark brown dorsally and reticulate pale brown ventrally (Figs. 4B–4D). Specimen QCAZ 10247 was collected on 13 February 2010 in Otokiki Reserve, Alto Tambo (0°54'21.6" N, 78°36'21.6" W, 620 m), San Lorenzo County, Esmeraldas province; the snake was found in primary forest 30 cm below ground among fern roots. Specimen DHMECN 11400 was collected on 01 April 2012 near Durango (1°02'30.7" N, 78°37'26.6" W, 243 m), San Lorenzo County, Esmeraldas province; the snake was found in secondary forest one m above ground among leaf litter accumulated on the junction of
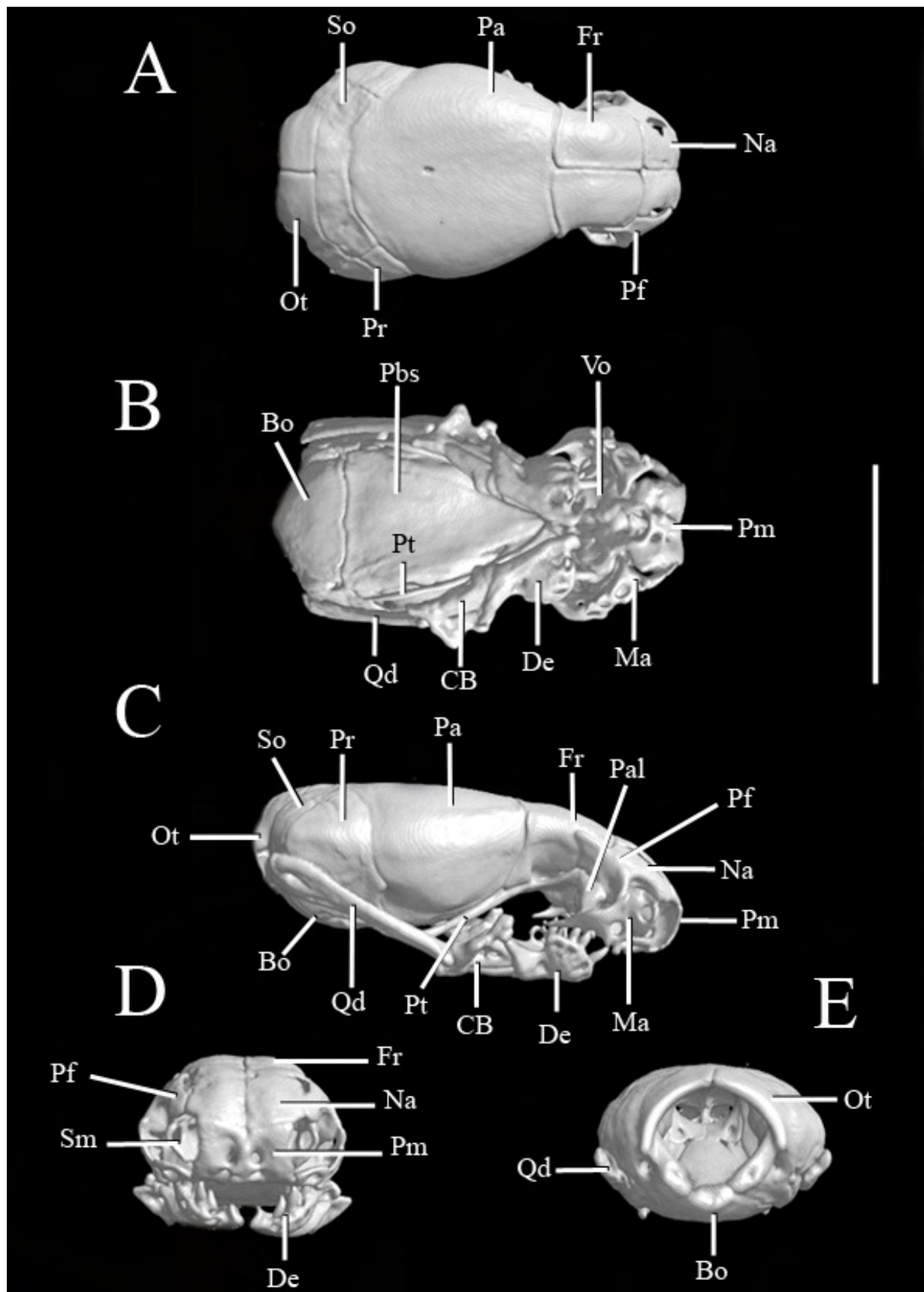
Fig. 5. Three-dimensional reconstruction of the skull of *Trilepida pastusa* based on HRXCT data. Dorsal (A), ventral (B), lateral (C), anterior (D), and posterior (E) views of juvenile paratype (QCAZ 5778). Scale bar = 3.5 mm. Bo, basioccipital; CB, compound bone; De, dentary; Fr, frontal; Ma, maxilla; Na, nasal; Ot, otico-occipital; Pa, parietal; Pal, palatine; Pbs, parabasisphenoid; Pf, prefrontal; Pm, premaxilla; Pr, prootic; Pt, pterygoid; Qd, quadrate; Sm, septomaxilla; So, supraoccipital; Vo, vomer.

lianas. Both localities belong to Lowland and Foothill Evergreen Forests of northwestern Ecuador (Cerón et al. 1999) (Fig. 6).

Discussion

The conservation assessment of reptile species belonging to families that are completely or primarily fossorial (e.g., Amphisbaenidae, Anomalepididae, Leptotyphlopidae, Typhlopidae, Uropeltidae) is incomplete (Santos 2013). Due to their secretive habits and non-inclusion during routine herpetological surveys, knowledge about their distribution and population dynamics is scarce (Measey 2006; Pyron and Wallach 2014). Böhm et al. (2013) estimated that 10.5% (range: 5.6–57%) of species of fossorial reptiles are Threatened; however, the authors recognized that this low estimate and wide confidence intervals reflect the fact that a large number (47% from a subsample of 1,500 reptile species) of the included taxa had been classified as Data Deficient. Therefore, this study re-emphasized the need to target these groups in future research and surveys.

Records for *Trilepida macrolepis* and *T. pastusa* provided here come from a region where several new species of snakes have been discovered in the last 15 years (e.g., Dixon 2000; Passos et al. 2009; Salazar-Valenzuela
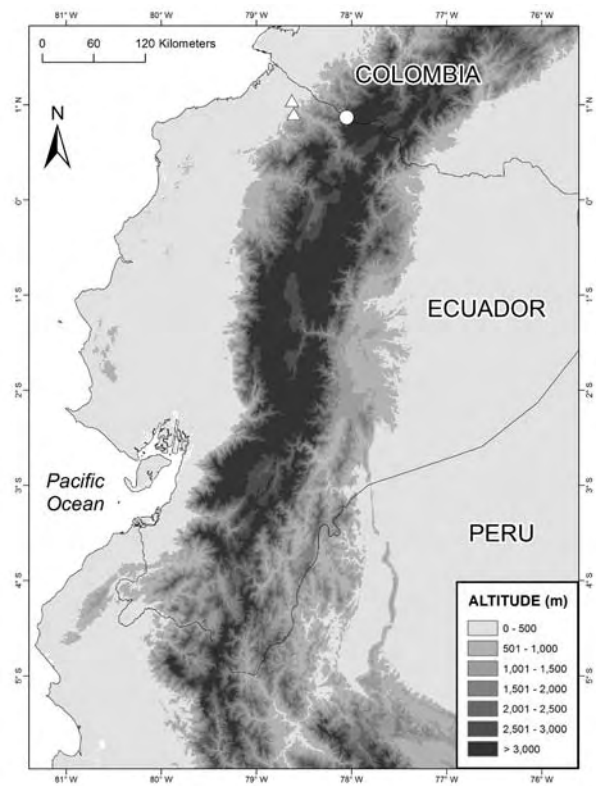
Fig. 6. Geographic distribution of *Trilepida pastusa* (circle) and *T. macrolepis* (triangles) in Ecuador.
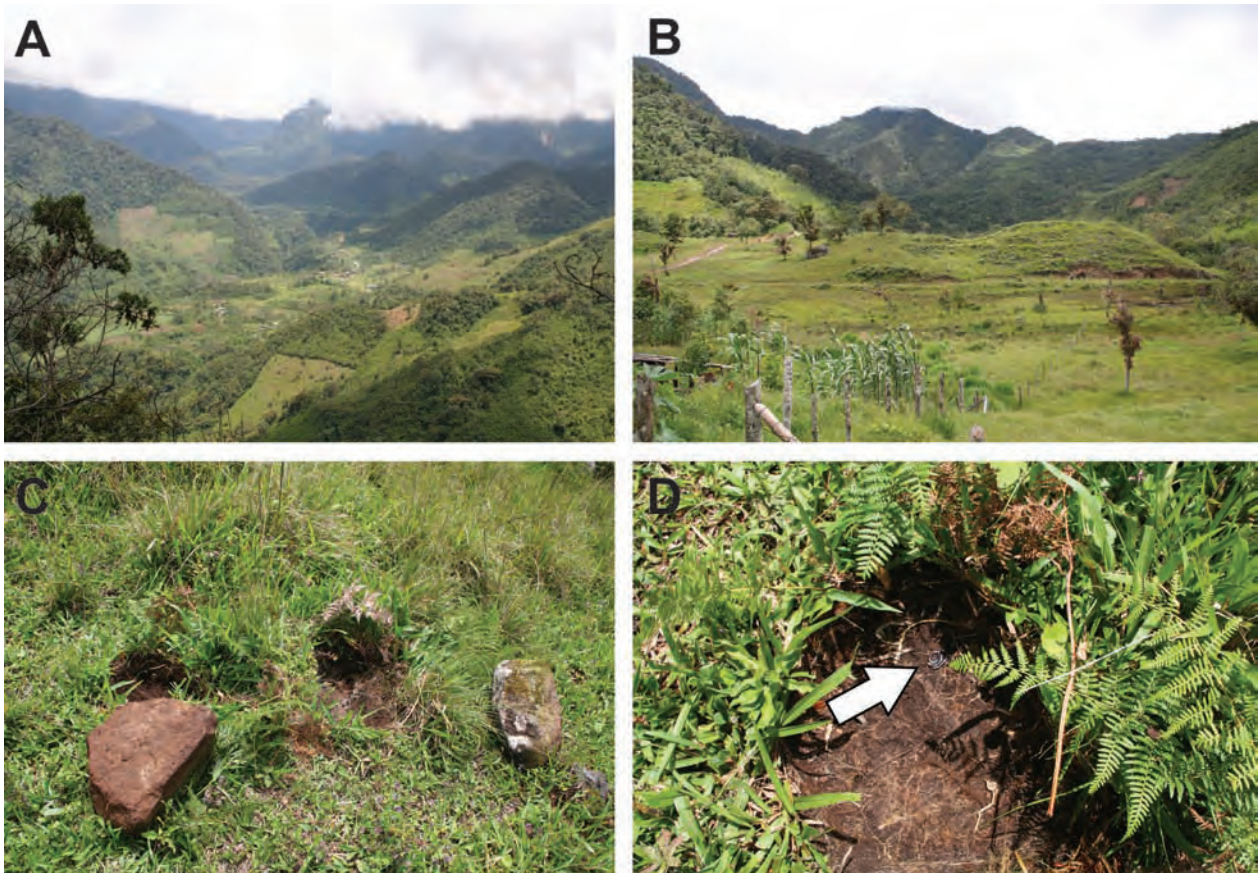
Fig. 7. Habitat of *Trilepida pastusa* surrounding the cloud forests near the town of Chilmá Bajo, Tulcán County, Carchi province (A); pasture areas where individuals were collected (B); microhabitat of *T. pastusa* (C); and a juvenile individual of the new species *in situ* (arrow) (D). *Photographs by D. Salazar-Valenzuela.*

et al. 2014; Torres-Carvajal et al. 2012). The singularity of this region may be attributable to the proposed existence of a habitat transition between northern and central parts of the Chocó bioregion (western Colombia and northwestern Ecuador) and communities found further south along the Pacific coast and adjacent Andean slopes of Ecuador (Anderson and Jarrín-V 2002; Anderson and Martínez-Meyer 2004; Cisneros-Heredia 2006; Salazar-Valenzuela et al. 2014).

Trilepida macrolepis has been suggested to represent a complex of species due to its wide distribution, presence on both sides of the Andes, and deep molecular divergence between individuals from two localities in northern Brazil and Guyana (Adalsteinsson et al. 2009; Orejas-Miranda 1967). Our data from Ecuador agree with the diagnosis provided for this species in Pinto et al. (2010), except for the presence of 12 midtail scales in specimen QCAZ 10247. This character has been used in the taxonomy of members of the genus (Orejas-Miranda and Peters 1970; Pinto et al. 2010) and will need to be evaluated in future studies. We tentatively assign QCAZ 10247 to *T. macrolepis* based mainly on the presence of a high number of middorsal and midventral scales (239 and 220, respectively), an ocular with rounded anterior border, and a reticulate dorsal and ventral coloration pattern, but acknowledge that a detailed revision of this taxon is needed to confirm the taxonomic identity of this specimen. Pérez-Santos and Moreno (1991) showed two color photographs of leptotyphlopid snakes of Ecuador without species identification. One of them (picture 148) seems to agree with the dorsal reticulate pattern present in *T. macrolepis*, suggesting that this taxon was already collected in Ecuador. However, the same image (picture 85) was also included in Pérez-Santos and Moreno (1988)'s book on snakes of Colombia rendering questionable the origin of that specimen. The discovery of individual DH-MECN 11400 among leaf litter one m above the forest floor is in agreement with the report of individuals of this species complex from the Amazonian lowlands wrapped two m above ground around small tree trunks and moving their heads back and forth (Vitt and Caldwell 2013). These authors suggested that the snakes were probably trying to detect airborne chemical cues associated with termite nests.

Putative synapomorphies for the genus *Trilepida* include a hemipenis body with a narrow base and a robust terminal portion, middorsal cephalic scales of moderate size (i.e., supraocular scales smaller or equal to frontal and postfrontal scales), and an enlarged terminal spine (Passos et al. 2006; Pinto and Curcio 2011; Pinto and Fernandes 2012). Although we could not examine hemipenis for *T. pastusa* since adult males were not available in our sample, we assign this species to the genus *Trilepida* based on the presence of middorsal cephalic scales of moderate size and a slightly enlarged terminal spine (see Pinto and Curcio, 2011). Also, the paired or unpaired condition of the nasal bone is variable in differ-

ent members along the Leptotyphlopidae family (Rieppel et al. 2009). Although osteological characters have not been employed in less inclusive phylogenetic analysis on the Renina subtribe (represented by the genus *Rena* and *Trilepida*), previous morphological studies on the skull of members pertaining to this subtribe (e.g., Brock 1932; List 1966; McDowell and Bogert 1954; Rieppel et al. 2009) indicate that the paired condition of the nasal bones may be a feature that could distinguish the genus *Trilepida* (paired condition) from the genus *Rena* (fused condition). As many other species currently allocated in the genus *Trilepida*, *T. pastusa* also has paired nasal bones. Intrageneric phylogenetic relationships have not been established for members of the genus *Trilepida*; out of the 14 species of the genus, three consistently show the presence of 12 midtail scales: *T. guayaquilensis*, *T. joshuai*, and *T. pastusa*. These species are restricted to Colombia and Ecuador and these shared features may indicate close phylogenetic relationships between them. *Trilepida guayaquilensis* is still only known from the holotype even though it was described 45 years ago from a specimen collected in Guayaquil, Guayas province, Ecuador. We analyzed photographs from specimens collected in this locality and misidentified as *T. guayaquilensis*. These specimens actually represent *Epictia subcrotilla*, which is a leptotyphlopid snake distributed in the lowlands of Ecuador and Peru, relatively common in natural history collections (Cisneros-Heredia 2008; Purtschert 2007). Indeed, specimen QCAZ 12769 collected by us in Guayaquil confirms the presence of this species in urban areas of this city. *Trilepida guayaquilensis* therefore remains to be confirmed as a species with a distribution that includes Guayaquil but no further records exist from this area because it is either extremely rare in abundance or searching efforts have not been enough to locate this fossorial animal. Alternatively, *T. guayaquilensis* could be a species whose only known specimen did not originate from Guayaquil but may have been brought there from a nearby locality in the Pacific lowlands or the western versant of the Andes, as has been suggested for a couple of other species of Ecuadorian snakes (Cadle 2005; Curcio et al. 2012).

As is common in tropical parts of the world where these groups have significantly diversified (Pyron and Burbrink 2012; Vitt and Caldwell 2013), knowledge about the diversity of fossorial snake fauna in Ecuador is still fragmented. Cisneros-Heredia (2008) clarified much of the confusion present at the time regarding the existence of voucher specimens for species registered in the country and concluded that only three species of the family Leptotyphlopidae were present in the country. The addition of two species of threadsnakes to the snake fauna of Ecuador reported in this study should bring us closer to the true diversity present in the country, even though it is clear that there are more species that remain to be described in this group (Cisneros-Heredia 2008, *pers. observ.*).

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APPENDIX

Specimens examined

Epictia subcrotilla (*n* = 4).—ECUADOR: Provincia Guayas: Guayaquil, 7 m, QCAZ 12769; USNM 232401–03 (photographs examined).

Trilepida anthracina (*n* = 1).—ECUADOR: Provincia Zamora Chinchipe: 6.5 km SE of Tundayme, 1,300–1,500 m, QCAZ 7396.

Trilepida guayaquilensis (*n* = 1).—ECUADOR: Provincia Guayas: Guayaquil, ZMB 4508 (holotype) (photographs examined).

Trilepida macrolepis (*n* = 2).—ECUADOR: Provincia Esmeraldas: Alto Tambo, Otokiki Reserve, 620 m, QCAZ 10247; Durango, 243 m, DHMECN 11400.

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Development and gastrulation in *Hyloxalus vertebralis* and *Dendrobates auratus* (Anura: Dendrobatidae)

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Abstract.—We document the embryonic development of *Hyloxalus vertebralis*, a frog species of the Ecuadorian highlands, declared as Critically Endangered by the International Union for the Conservation of Nature (IUCN) due to significant declines in its populations. Our work may be of value for conservation and management of this endangered frog, especially as it is being bred in captivity to ensure against extinction. We were able to analyze and compare the development of *H. vertebralis* with *Dendrobates auratus* (Dendrobatidae), and other frogs, because of the successful reproduction in captivity of Ecuadorian frogs at the Balsa de los Sapos, Centre of Amphibian Investigation and Conservation (CICA), of the Pontificia Universidad Católica del Ecuador, in Quito. Embryos were fixed, and the external and internal morphology was described from whole mounts, and serial sections. Cellular morphology was analyzed by staining nuclei. Embryos of *H. vertebralis* and *D. auratus* developed from eggs that were 2.6 and 3.5 mm in diameter, respectively. In spite of the large size of their eggs, the morphology of *H. vertebralis* embryos from cleavage to hatching was similar to the morphology of *Epipedobates machalilla* (Dendrobatidae) embryos. The comparison of gastrulation morphology was extended to six additional species of Dendrobatidae (*E. machalilla*, *Epipedobates anthonyi*, *Epipedobates tricolor*, *H. vertebralis*, *Ameerega bilinguis*, *D. auratus*), and to *Xenopus laevis* (Pipidae), and *Gastrotheca riobambae* (Hemiphractidae). We found that elongation of the notochord occurs after blastopore closure in the six species of dendrobatid frogs, as in *G. riobambae*; whereas gastrulation and notochord elongation overlap during *X. laevis* development. We propose that the separation of gastrulation from notochord elongation may relate to slower development patterns, probably associated with the terrestrial reproductive strategies of dendrobatid frogs and marsupial frogs. This analysis contributes to the knowledge of frog embryology and gastrulation, and provides developmental information that may be useful for the conservation and management of *H. vertebralis*.

Introduction

We analyzed the embryonic development of *Hyloxalus vertebralis* and *Dendrobates auratus* from cleavage to tadpole hatching and compared these patterns with the development of *Epipedobates machalilla* (Dendrobatidae) and *Xenopus laevis* (Pipidae), frogs with well-studied development. These comparisons were then extended to embryos of other Ecuadorian, neotropical frogs (del

Pino et al. 2004, 2007; Moya et al. 2007; Nieuwkoop and Faber 1994). Our aim was to extend the analysis of frog embryonic development to additional species and to provide information that may be useful for the conservation and management of *H. vertebralis*, an endangered frog. Most of the *H. vertebralis* population has disappeared, possibly due to chytridiomycosis infection and habitat destruction. For these reasons, the International Union for Conservation of Nature (IUCN) declared *H. verte-*

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H. vertebralis
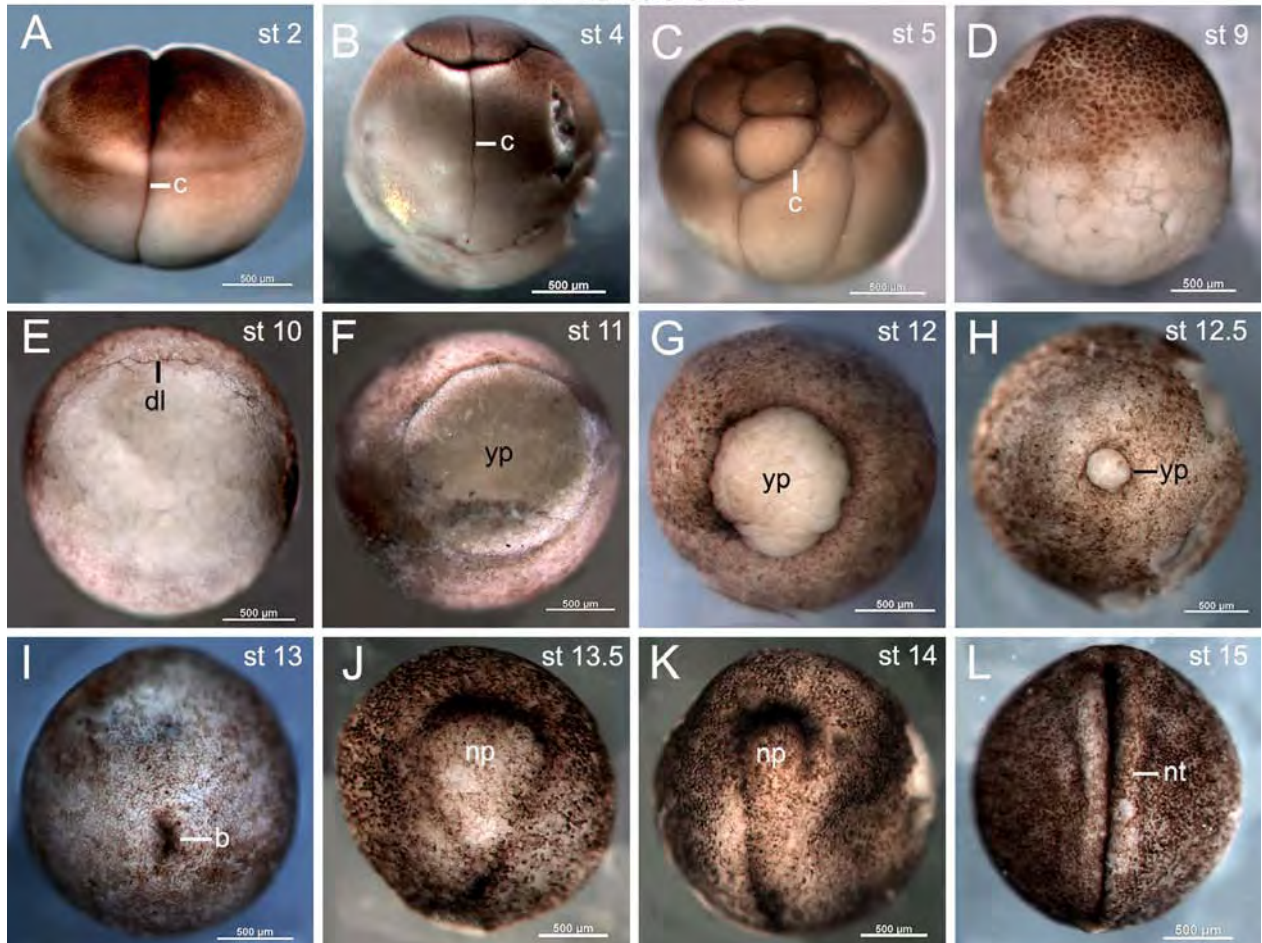
Fig. 1. External views of *H. vertebralis* embryos from cleavage to the neurula stage. (**A**) Stage 2: Two-cell stage. (**B**) Stage 4: Eight-cell stage. (**C**) Stage 5: Sixteen-cell stage. (**D**) Stage 9: Advanced blastula. (**E**) Stage 10: Early gastrula. (**F**) Stage 11: Mid-gastrula. (**G**) Stage 12: Late-gastrula. (**H**) Stage 12.5: Late-gastrula with a small yolk plug. (**I**) Stage 13: Slit-blastopore stage. (**J**) Stage 13.5: Advanced slit-blastopore stage. The neural plate was visible. (**K**) Stage 14: Early neural fold stage. (**L**) Stage 15: Mid-neural fold stage. In this and the following figures, the developmental stage (st) is given in top right-hand corner of each image; b, blastopore; c, cleavage furrow; dl, dorsal blastopore lip; np, neural plate; nt, neural tube; yp, yolk plug.

bralis as Critically Endangered (Coloma et al. 2004). It is currently being bred in captivity to guard against extinction.

Hyloxalus vertebralis occurs at elevations of 1,770–3,500 m above sea level in the inter-Andean valleys of Ecuador. In addition, it occurs on the eastern and western slopes of the Andes in central and southern Ecuador, respectively (Coloma 1995). Its habitat is the cloud forest and it has also been found in ponds, open areas, and streams. The nests consist of 5–12 eggs that are placed on the ground (Coloma 1995). After the tadpoles hatch, the males transport them to streams for further development (Coloma 1995).

Dendrobates auratus is distributed from southeastern Nicaragua to northwestern Colombia (Solís et al. 2004). This species does not occur in Ecuador. These frogs deposit their eggs in terrestrial nests, and embryonic development occurs inside the egg capsules until tadpole hatches in the leaf litter. Brood care is performed by the male. After hatching, tadpoles are transported individu-

ally by the male to small seasonal pools (Solís et al. 2004). Eggs of *D. auratus* are the largest among the dendrobatids and measure 3.5 mm in diameter (del Pino et al. 2007; Hervas and del Pino 2013).

Dendrobatid frogs are of great developmental interest because of their great variation in egg size (Table 1), and their modified pattern of gastrulation. Notochord elongation occurs after gastrulation in *E. machalilla*, and *Epipedobates anthonyi*, as in the Marsupial frog, *Gastrotheca riobambae* (Hemiphractidae); whereas, the onset of notochord elongation is a feature of the *Xenopus laevis* mid-gastrula (Benítez and del Pino 2002; Keller and Shook 2004; del Pino et al. 2007; Moya et al. 2007; Montenegro-Larrea and del Pino 2011; Elinson and del Pino 2012). For this reason, we compared the gastrulation characteristics of *Epipedobates anthonyi*, *Epipedobates tricolor*, *H. vertebralis*, *Ameerega bilinguis*, and *D. auratus* with *E. machalilla* (Dendrobatidae). In a previous study, *Ameerega bilinguis* was identified as *Epipedobates ingeri* (del Pino et al. 2007). This analysis
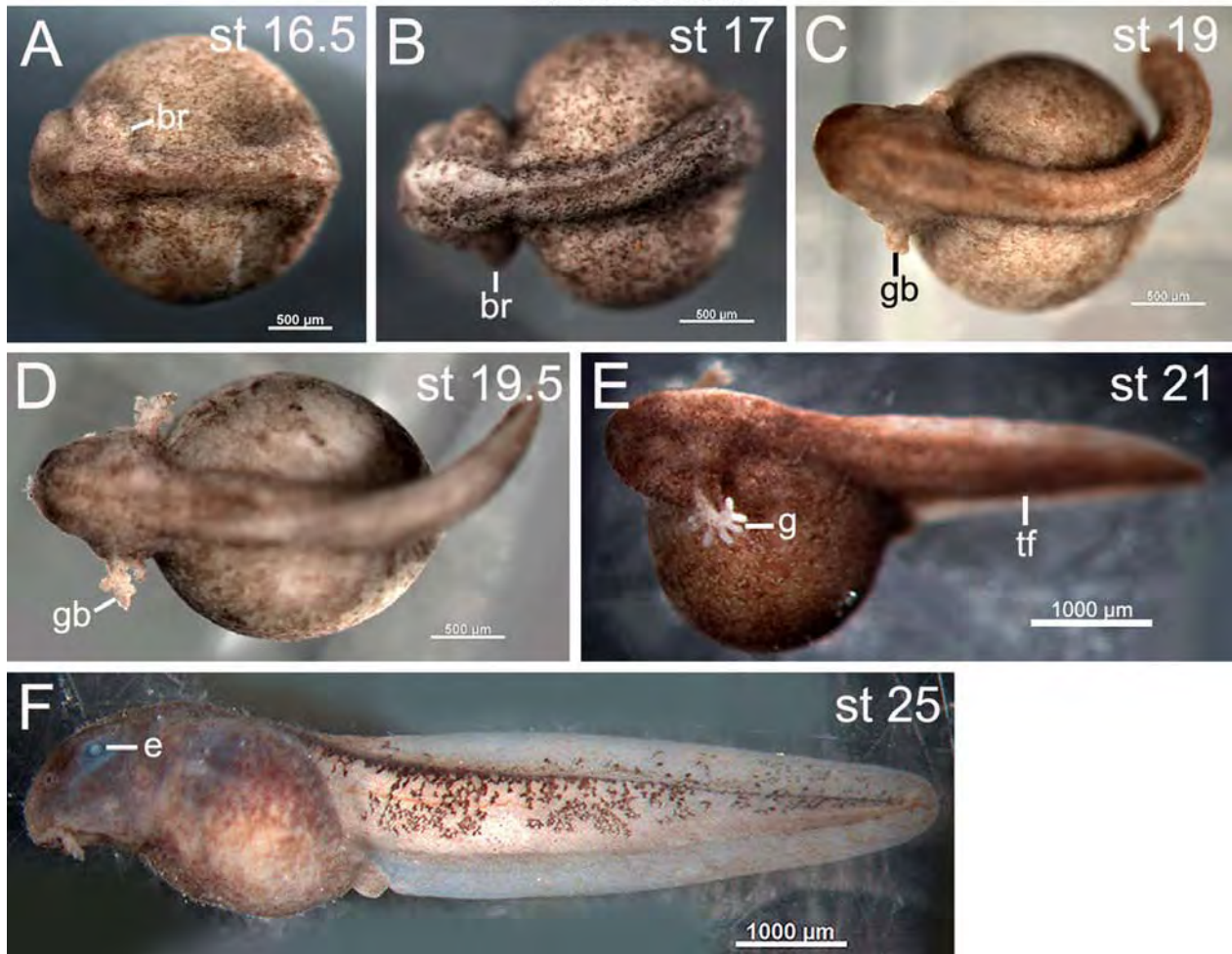
Fig. 2. External views of *H. vertebralis* embryos from closure of the neural tube to hatching. **(A)** Stage 16.5: Closure of the neural tube. **(B)** Stage 17: Tail bud stage. The brachial arches protruded on the sides of the head. **(C)** Stage 19: Embryo at the muscular response stage. **(D)** Stage 19.5: Gill buds of the two external gill pairs were visible. **(E)** Stage 21: Development of the external gills. There were seven branches in the first gill pair and the second gill pair was unbranched. **(F)** Stage 25: Embryo at hatching. br, branchial arch; e, eye; g, gills; gb, gill bud; tf, tail fin.

Table 1. Gastrulation in dendrobatid frogs in comparison with *X. laevis* (Pipidae) and *G. riobambae* (Hemiphractidae).

Family and Species	Eggs per clutch (No. of clutches)	Egg diameter (mm)	Gastrulation time (hrs)[a]	Archenteron elongation	Onset of notochord elongation	References[g]
Pipidae						
Xenopus laevis	--	1.3	5	Early gastrula[b]	Mid gastrula[f]	1
Dendrobatidae						
Epipedobates machalilla	15 (72)	1.6	65	Late gastrula[c]	After gastrulation[e]	2
Epipedobates anthonyi	18 (30)	2.0	36	Late gastrula[c]	After gastrulation[e]	3
Epipedobates tricolor	13 (34)	2.0	36	Late gastrula[c]	After gastrulation[e]	2
Hyloxalus vertebralis	13 (39)	2.6	39	Late gastrula[c]	After gastrulation[e]	4
Ameerega bilinguis	10 (04)	3.0	55	Late gastrula[d]	After gastrulation[e]	4
Dendrobates auratus	05 (42)	3.5	72	Late gastrula[d]	After gastrulation[e]	1
Hemiphractidae						
Gastrotheca riobambae	87	3.0	168	After gastrulation[e]	After gastrulation[e]	5

[a]Time from stages 10–13. Embryo culture temperatures for: *X. laevis* 23 °C, and 18–21 °C for other frogs; [b]Stage10; [c]Stage 12.5; [d]Stage 12; [e]Stage 13; [f]Stage 11; [g]References: 1, (del Pino et al. 2007); 2, (del Pino et al. 2004); 3, (Montenegro-Larrea and del Pino 2011); 4, This work; 5, (del Pino 1996; Moya et al. 2007).
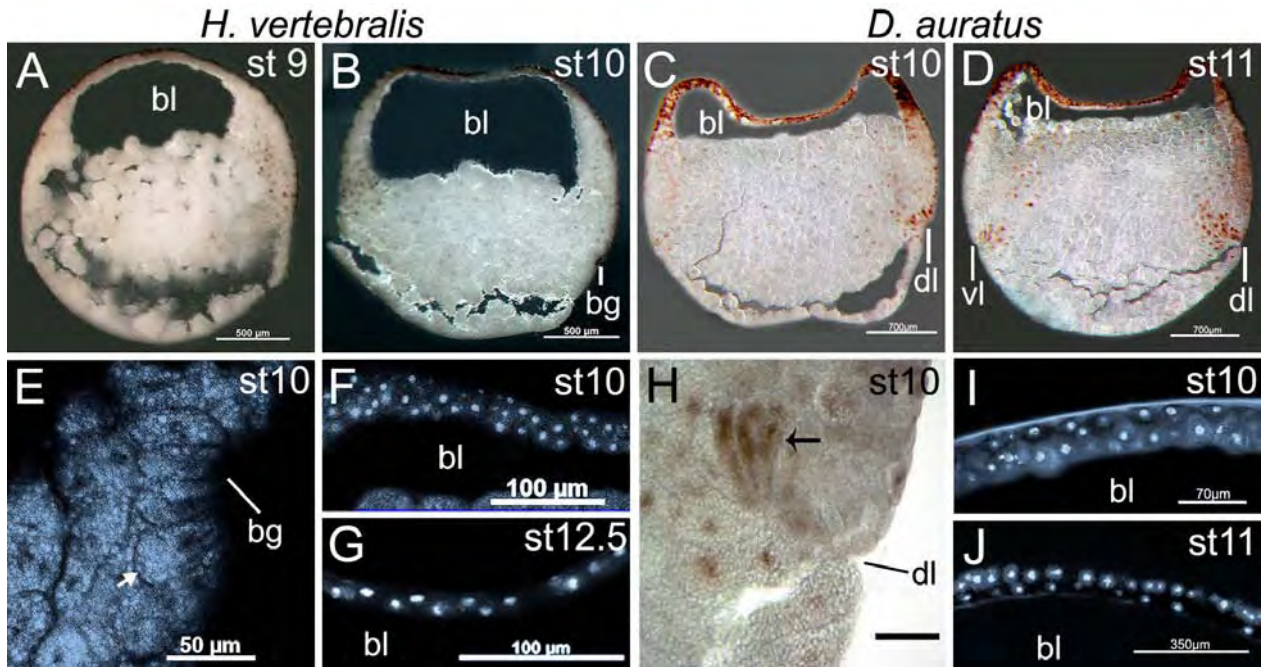
Fig. 3. Internal morphology of the *H. vertebralis* and *D. auratus* early gastrula. Micrographs of *H. vertebralis* embryos are shown in A, B, E–G, and micrographs of *D. auratus* embryos are shown in C, D, H–J. Sections shown in E–G, I–J were stained for cell nuclei. **(A)** Stage 9: Sagittal section of an advanced blastula. **(B)** Stage 10: Sagittal section of an early gastrula. **(C)** Stage 10: Sagittal section of an early gastrula. **(D)** Stage 11: Sagittal section of the mid-gastrula. **(E)** Stage 10: Higher magnification of the dorsal blastopore groove from the embryo in B. The arrow signals a bottle cell. **(F)** Stage 10: The blastocoel roof of an early gastrula. It was two-three cells in thickness. **(G)** Stage 12.5: One cell layer in the blastocoel roof of a late-gastrula. **(H)** Stage 10: Higher magnification from the embryo in C. The arrow signals a bottle cell. **(I)** Stage 10: The blastocoel roof of an early gastrula of two cells in thickness. **(J)** Stage 11: The blastocoel roof of mid-gastrula with one-two cells in thickness. bl, blastocoel; bg, blastopore groove; dl, dorsal blastopore lip; vl, ventral blastopore lip.

of gastrulation in several dendrobatids expands previous studies (del Pino et al. 2007; Montenegro-Larrea and del Pino 2011). The gastrulation pattern of these dendrobatids is similar to the pattern of *E. machalilla*, with the notochord elongation after completion of gastrulation (del Pino et al. 2004, 2007; Moya et al. 2007).

We report the features of development from cleavage to tadpole hatching of *H. vertebralis* and from gastrula to tadpole hatching of *D. auratus*. This study expands the report on the mode of myogenesis, neurulation, and internal features of embryos of these two dendrobatids (Hervas and del Pino 2013). In spite of the large size of their eggs, the external and internal morphology from cleavage until tadpole hatching of *H. vertebralis*, and *D. auratus* is similar to that of *E. machalilla* (del Pino et al. 2004 2007; Hervas and del Pino 2013). Moreover, myogenesis occurs by cell interdigitation, as in embryos of other dendrobatid frogs (del Pino et al. 2007; Hervas and del Pino 2013).

Materials and Methods

Collection sites

Adults of *Hyloxalus vertebralis* were collected by Fernando Dueñas and Ítalo Tapia on 10 September 2008. The locality of collection was Azuay Province, Sevilla de Oro, in southern Ecuador at an altitude 2,418 m above

sea level. The geographic coordinates of this site are W 78.60097, S 2.63605. The permit 016-IC-FAU-DNBAP-MA from the Ministry of the Environment, Ecuador, allowed the collection and maintenance of frogs at Pontificia Universidad Católica del Ecuador (PUCE). The Atlanta Zoo donated adults of *Dendrobates auratus* to the PUCE. Adults of both species reproduced successfully at the Balsa de los Sapos, Centre of Amphibian Investigation and Conservation (CICA) of PUCE. Egg clutches were donated to the laboratory of developmental biology for embryonic analysis.

Analysis of embryonic development

The number of eggs per egg clutch was recorded. Embryos were analyzed from cleavage until tadpole hatching, and were staged according to the *E. machalilla* table of stages (del Pino et al. 2004). Embryos were cultured in humid chambers at room temperature (18–23 °C). Procedures for fixation of embryos in Smith's fixative, vibratome sectioning, and the staining of sections for cell nuclei with the fluorescent dye Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA) were previously described (del Pino et al. 2004; Moya et al. 2007). Sections were mounted in glycerol, and microscopically examined with normal light using a Stemi SV6 stereomicroscope (Carl Zeiss, Oberkochen, Germany) or with fluorescent optics using a Z1 Axio Observer microscope (Carl Zeiss,
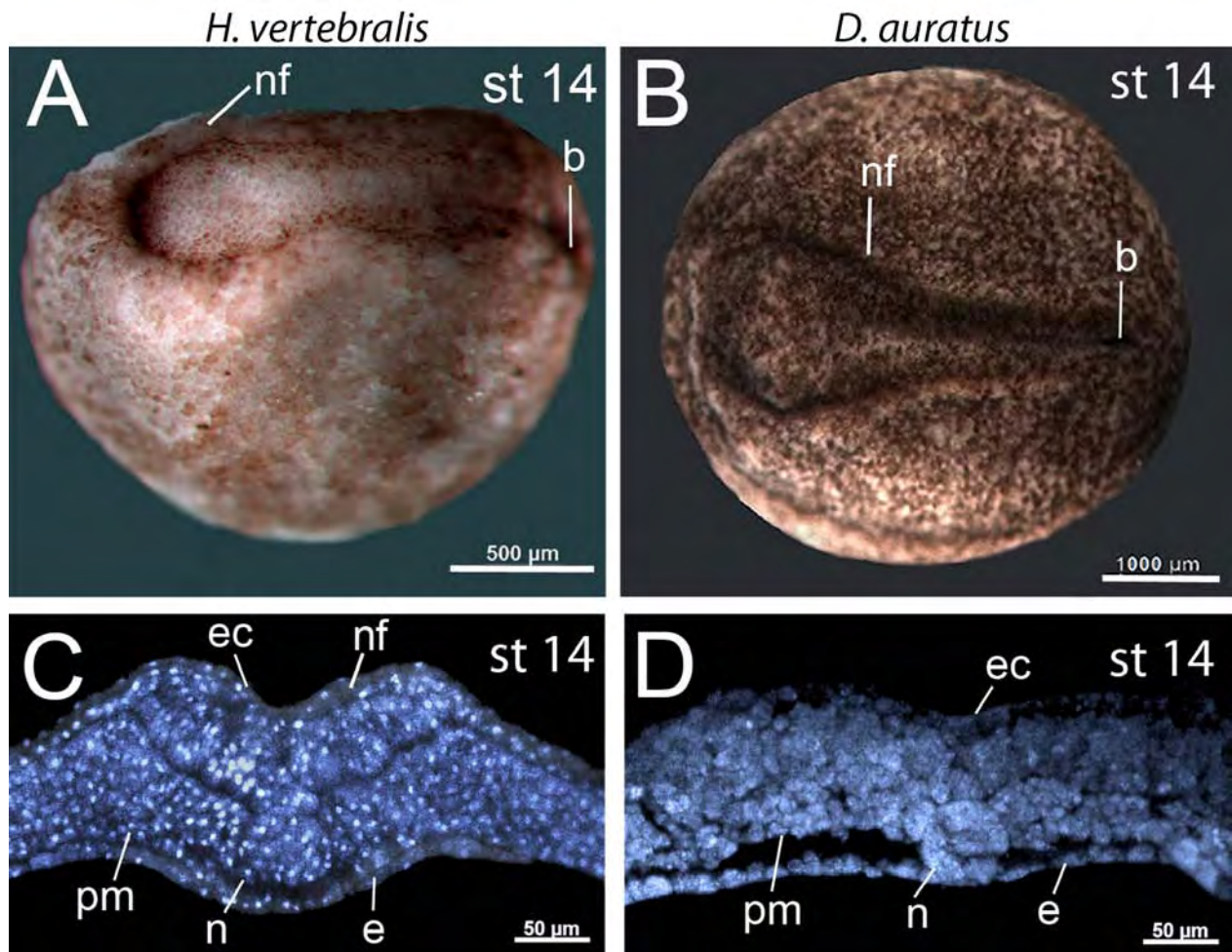
Fig. 4. Stage 14: Early neural fold stage of *H. vertebralis* and *D. auratus* embryos. Micrographs of *H. vertebralis* embryos are shown in A, C, and micrographs of *D. auratus* embryos are shown in B, D. Sections shown in C–D were stained for cell nuclei. **(A)** Lateral view of a neural fold stage embryo. **(B)** Dorsal view of a neural fold stage embryo. The neural plate was visible in embryos of the two species. **(C)** Cross section through the region of the trunk (Reproduced from Hervas and del Pino, 2013). **(D)** Cross section through the caudal region of an embryo. The notochord was visible in C and D. b, blastopore; e, endoderm; ec, ectoderm; n, notochord; nf, neural fold; pm, paraxial mesoderm.

Oberkochen, Germany). Embryos were photographed with Axiocam cameras and the image capture program Axiovision (Carl Zeiss, Oberkochen, Germany). The images were edited with Adobe Photoshop CS6. Egg diameter was measured in fixed embryos with the measuring tool of the program Axiovision (Carl Zeiss, Oberkochen, Germany).

Results and Discussion

Clutch size and developmental time

The number of eggs ranged from 2–25 eggs, with a mean of 13 eggs per clutch in *H. vertebralis*, and 2–8 eggs, with a mean of five eggs per clutch in *D. auratus* (Table 1). The eggs of *H. vertebralis* and *D. auratus* measured about 2.6 and 3.5 mm in diameter, respectively (Table 1). The diameter of *H. vertebralis* eggs was previously reported to be of about three mm (Coloma 1995). The animal hemisphere of embryos was dark brown and the

vegetal hemisphere was pale-yellow in embryos of both frogs (Fig. 1A–D; not shown for *D. auratus*). Egg clutches of *H. vertebralis* required 18 days from the two-cell stage to tadpole hatching under laboratory conditions; whereas 19–21 days were required from fertilization to tadpole hatching by the six species of dendrobatid frogs (del Pino et al. 2004, 2007; Hervas and del Pino 2013). The similarity of developmental times suggests that parental care allows slow development in all of the species of dendrobatid frogs examined in comparison with *X. laevis*.

Embryonic development of *H. vertebralis* and *D. auratus*

The development from early cleavage until tadpole hatching of *H. vertebralis* and *D. auratus* was divided into 25 stages, according to the staging criteria for *E. machalilla* (del Pino et al. 2004) given in Table 2. Micrographs of the external and internal morphology of *H. vertebralis*
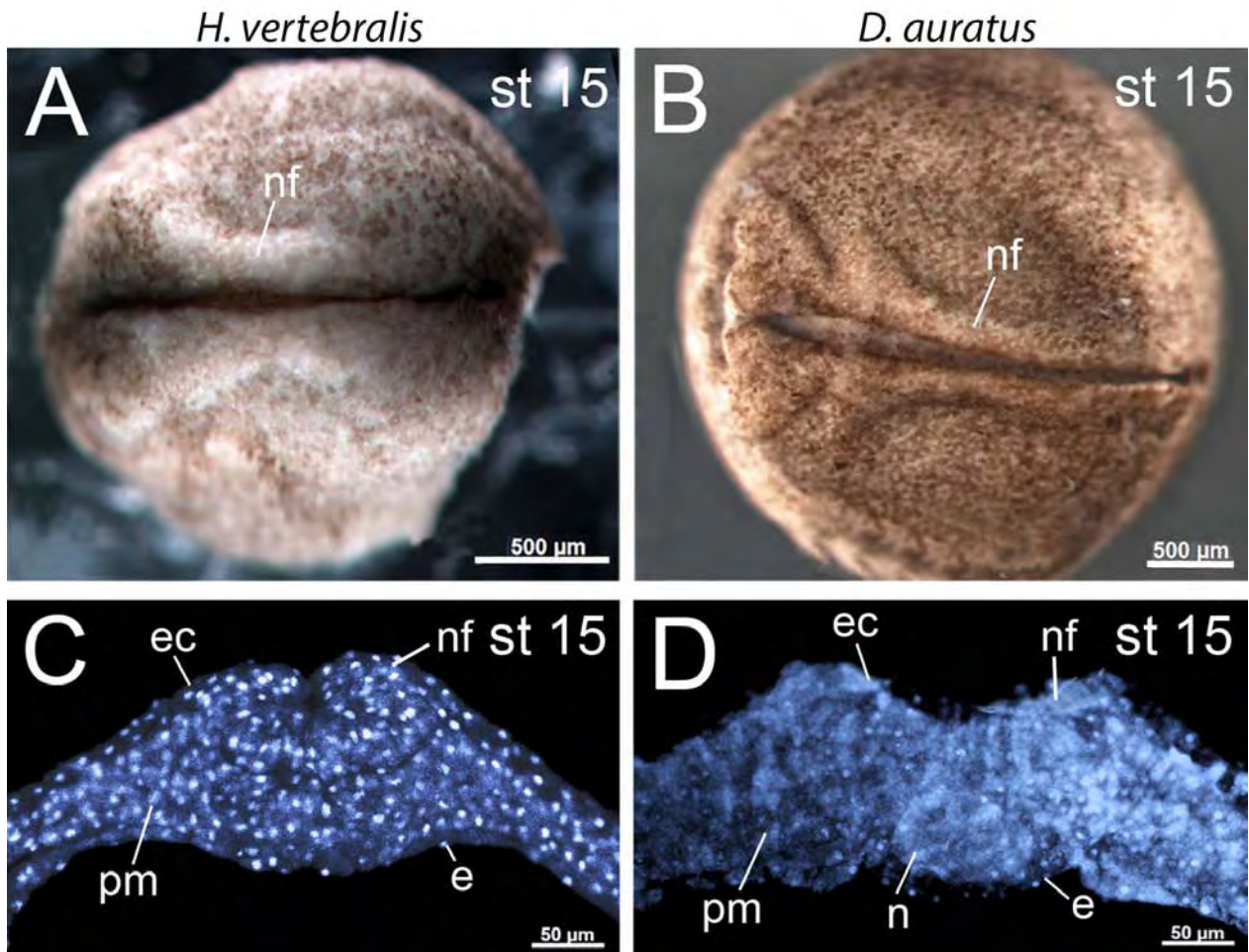
Fig. 5. Stage 15: Mid-neural fold stage of embryos of *H. vertebralis* and *D. auratus*. Micrographs of *H. vertebralis* embryos are shown in A, C, and micrographs of *D. auratus* embryos are shown in B, D. Sections shown in C–D were stained for cell nuclei. **(A–B)** Dorsal views of embryos. The neural tube was open in embryos of both species. **(C)** Cross section through the caudal region. The notochord was not detected in this caudal region (Reproduced from Hervas and del Pino, 2013). **(D)** Cross section through the trunk region. The notochord was visible. e, endoderm; ec, ectoderm; n, notochord; nf neural fold; pm, paraxial mesoderm.

and *D. auratus* embryos illustrate these developmental stages (Figs. 1–12).Cleavage of *H. vertebralis* was holoblastic as in *E. machalilla* (del Pino et al. 2004) (Fig. 1A–C). Cleavage of *D. auratus* was not observed. The *H. vertebralis* blastula consisted of small, pigmented cells in the animal hemisphere; whereas, cells of the vegetal hemisphere were larger. The blastocoel developed during cleavage, and was large in blastula and gastrula stage embryos (Fig. 3A–D). The blastocoel roof, of the two species, was several cell diameters in thickness at stage 10 (Fig. 3F and I), and it was reduced to one cell thickness in the late-gastrula stage of *H. vertebralis* (stage 12.5; Fig. 3G). Similarly the thickness of the blastocoel roof was reduced to one or two cells in thickness in the early gastrula of *D. auratus* (stage 11; Fig. 3J). Thickness of the blastocoel roof in the late-gastrula was not documented for this frog species. In *E. machalilla*, expansion of the blastocoel was accompanied by reduction in its thickness until it was a monolayer of cells in the late-gastrula (del Pino et al. 2004).

The onset of gastrulation in *H. vertebralis* and *D. auratus* was marked by the presence of the dorsal blastopore lip in a sub-equatorial location (Fig. 1E). A field of bottle cells was observed at the blastopore groove (Figs. 3E, H), as in *X. laevis* and *E. machalilla* (Hardin and Keller 1988; Moya et al. 2007). The gastrula developed a conspicuous yolk plug that became smaller during gastrulation, until it was totally retracted by the end of gastrulation (Fig. 1E–I). The closed blastopore looked like a small slit in stage 13 embryos (Fig. 1I), as in *E. machalilla* and other frogs (del Pino et al. 2004). Internally, a small dorsal archenteron developed, which did not elongate until stage 13 in *H. vertebralis* (Fig. 12J–L), as in *E. machalilla* (del Pino et al. 2004); whereas in *D. auratus*, the archenteron was already large and inflated at stage 12 (Fig. 12P) (del Pino et al. 2007).

The neural plate developed in late stage 13 (Fig. 1J). In stage 14, the neural folds were elevated (Figs. 1K; 4A–B). The notochord was observed underneath the neural plate of stage 14 embryos (Fig. 4C–D). The neu-

Table 2. Stages of development of *H. vertebralis* and *D. auratus* in comparison with the *E. machalilla* table of development.

| D	X	G	Characteristics of embryos
1	1	1	Fertilization (not shown).
1	-	2	Gray crescent (not shown).
2	2	3	Two-cell stage (Fig. 1A). This stage was observed only for *H. vertebralis*.
3	3	4	Four-cell stage (not shown).
4	4	5	Eight-cell stage (Fig. 1B). This stage was observed only for *H. vertebralis*.
5	5	6	Sixteen-cell stage (Fig. 1C). This stage was observed only for *H. vertebralis*.
6	6	7	Thirty-two cell stage (not shown).
7	7	8	Large-cell blastula (not shown).
8	8	-	Medium-cell blastula (not shown).
9	9	9	Advanced blastula (Fig. 1D; 3A).
10	10	10	Early gastrula. The dorsal blastopore lip was formed (Fig. 1E), had a subequatorial location (Figs. 3B, C), and there were bottle cells making the onset of cell ingression at the blastopore, as shown for both species (Figs. 3E, H).The blastocoel was a large cavity, and its roof was several cells in thickness. The thickness of the blastocoel roof was reduced to a single cell in the late gastrula (Figs. 3F, G, I, J).
11	11	11	Mid-gastrula with a yolk plug that measured about 1/2 of the embryo's diameter (Fig. 1F). Internally, the ventral blastopore lip was formed as shown for *D. auratus* (Fig. 3D).
12	12	12	Late gastrula with a yolk plug that was 1/3 of the embryo's diameter or smaller (Figs. 1G). The archenteron of *H. vertebralis* was smaller than *D. auratus* (Figs. 12J, K, P, Q).
13	13	13	Slit blastopore stage (Fig. 1I). Internally, the archenteron was elongated. A large circumblastoporal collar was visible (Figs. 12L; R). The neural plate became visible in the late stage 13 (Fig. 1J).
14	14	14	Early neural fold stage. Images of *H. vertebralis* (Fig. 1K; 4A, C), and of *D. auratus* (Figs. 4B, D). In the trunk region of both species, the neural plate, notochord, and mesoderm were visible (Figs. 4C, D).
15	16	15	Mid-neural fold stage. The neural folds approached each other. Images of *H. vertebralis* (Fig. 1L; 5A, C), and of *D. auratus* (Figs. 5B, D). The neural folds were elevated and touched each other in the trunk region (*H. vertebralis*, Fig. 5C); but were open in the cephalic region (*D. auratus*, Fig. 5D).
16	20	16	Closure of the neural tube in *H. vertebralis* (Fig. 2A; 6A, C), and *D. auratus* (Figs. 6B, D). Closure of the neural tube was complete in both species.
17	24	17	Tail bud stage. The tail bud and the head region protruded beyond the yolky endoderm in *H. vertebralis* (Fig. 2B; 7); not shown for *D. auratus*. The epidermis, neural tube, notochord, somites, and endoderm were visible in the trunk region (Fig. 7B, C).
18	26	18	Muscular activity. The branchial arches protruded on the sides of the head. The eye vesicles were small (not shown). Images of *H. vertebralis* (Figs. 8A, C, E), and of *D. auratus* (Figs. 8B, D, F). The epidermis, neural tube, notochord, rows of somites, and endoderm were visible in the trunk region of both species (Figs. 8C–F).
19	33	19	Heart beat and external gill buds. The gill buds of the two pairs of external gills were visible. Images of *H. vertebralis* (Figs. 2C, D; 9A, C, E), and of *D. auratus* (Figs. 9B, D, F). The dorsal fin was visible (Fig. 9C), the notochord was vacuolated (Fig. 9C), and the pronephros was detectable (Fig. 9D). The number of somites increased (Figs. 9E, F).
20	40	20	Circulation to the external gills. The first gill pair had four or more branches. Images of *H. vertebralis* (Fig. 10A). Internally, the otocysts, brain, notochord, and somites were observed, as shown for *H. vertebralis* (Fig. 10C).
21	41	21	Development of the external gills. The first pair of external gills had seven branches in *H. vertebralis*. The second pair of external gills was small and unbranched (Fig. 2E). In *D. auratus*, the first gill pair had six branches and the second gill pair developed two branches. The tail became elongated (not shown).
22	41	22	The external gills enlarged and eye pigment. The eyes contained pigment. Images of *H. vertebralis* (Fig. 10B). Internally, the otocysts, eye, internal gills, notochord, and somites were observed, as shown for *H. vertebralis* (Fig. 10D).
23	43	23	The external gills reached their full size (Fig. 11). The first pair of external gills had eight and nine branches in *H. vertebralis* and *D. auratus*, respectively. The second gill pair was unbranched in *H. vertebralis* and had two branches in *D. auratus* (Figs. 11A, B). The opercular fold was visible. The eyes and the body were pigmented. Internally the epidermis, eye, otocysts, and somites were detected. Images of *H. vertebralis* (Figs. 11A, C) and of *D. auratus* (Figs. 11B, D).
24	44	24	The external gills were visible only on the left side. The operculum was closed on the right side (not shown).
25	45	25	The spiracle was formed. The embryos hatched and had the appearance of a tadpole. Internally, the brain, otocysts, somites, and yolky endoderm were observed. Images of *H. vertebralis* (Fig. 2F, 11E–G).

[1]D, stages of the dendrobatid frogs, *H. vertebralis* and *D. auratus*, according to the *E. machalilla* standard stages of development (del Pino et al. 2004); X, normal stages of *X. laevis* development (Nieuwkoop and Faber 1994); G, the generalized table of frog development (Gosner 1960).
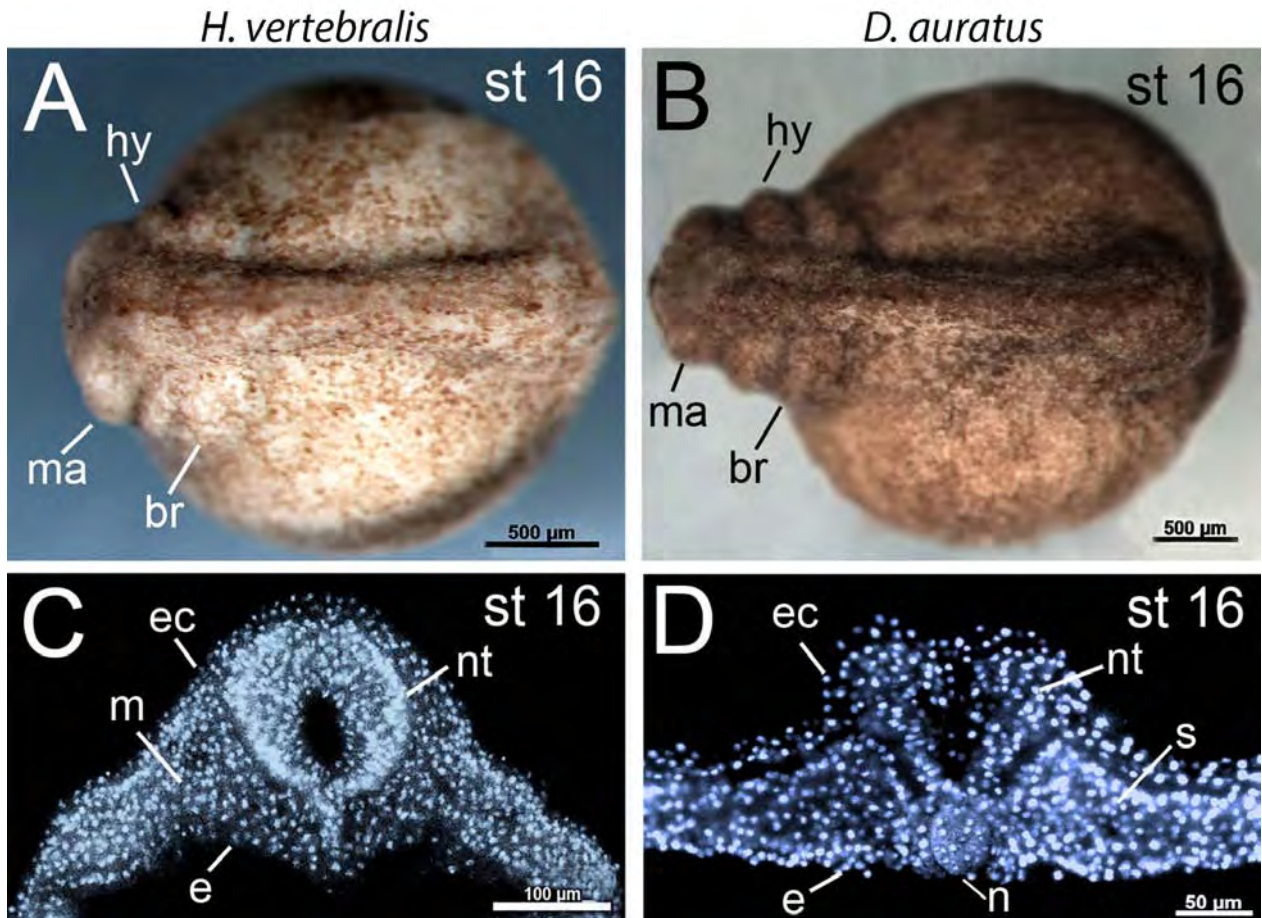
Fig. 6. Stage 16: Closure of the neural tube in embryos of *H. vertebralis* and *D. auratus*. Micrographs of *H. vertebralis* embryos are shown in A, C, and micrographs of *D. auratus* embryos are shown in B, D. Sections shown in C–D were stained for cell nuclei. **(A–B)** Dorsal views of embryos. The neural tube was closed. The branchial arches were visible in the head region. **(C)** Cross section through the rostral region, anterior to the notochord. The neural tube was completely closed. (Reproduced from Hervas and del Pino, 2013). **(D)** Cross sections through the trunk region of an embryo. The somites were visible. br, branchial arch; e, endoderm; ec, ectoderm; hy, hyoid arch; ma, mandibular arch; m, mesoderm; n notochord; nt, neural tube; s, somite.

ral folds became closed along the midline during stages 15–16 (Figs.1L; 2A; 5; 6). The external and internal characteristics of the neurula from stages 14–16 of *H. vertebralis* and *D. auratus* were compared (Figs. 4–6) and were found to be similar to *E. machalilla* embryos (del Pino et al. 2004).

The tail bud embryos (stage 17) of *H. vertebralis* were examined in their external and internal morphology (Figs. 2B; 7). The body became elongated and the head and tail regions protruded over the large yolky endoderm. The branchial arches were visible (Fig. 7A), and the brain and neural tube were detected. Embryos of this stage contained numerous somites (Fig. 7B, C). Embryos of stage 18 were characterized by muscular activity, and the embryos were longer. Buds of the external gills were detected in the head region (Fig. 8A, B). Somites, the notochord and neural tube were detected in the trunk region (Fig. 8C, D). A row of somites was detected on each side of the notochord (Fig. 8E, F). Myogenesis in both species occurred by cell interdigitation, as in other dendrobatid frogs and in the Marsupial frog, *G. riobambae*; whereas, cell rotation is the pattern for *X. laevis*

myogenesis (Gatherer and del Pino 1992; Hervas and del Pino 2013). Gill buds were larger in stage 19 embryos (Figs. 2C–D; 9), and the external gills were fully developed in embryos of stage 22–23. The first gill pair of *H. vertebralis* developed eight branches, and the second pair was unbranched; whereas, embryos of *D. auratus* developed nine and two branches in the first and second gill pairs, respectively (Figs. 10; 11A, B). The number of gill branches in the first and second pair of external gills varies among species of Dendrobatidae (del Pino et al. 2004). The tail became longer in embryos of stages 18–25, the brain, spinal cord, somites, and internal organs developed and the embryos gradually acquired the tadpole shape in both species (Figs. 2C–F, 8–11). The processes of neurulation, somitogenesis, and internal embryo morphology of *H. vertebralis* and *D. auratus* were similar to the patterns described for other species of dendrobatid frogs (del Pino et al. 2004, 2007). Embryos of *H. vertebralis* hatched at stage 25 (Figs. 2F, 11E–G). The mouth had darkly pigmented teeth (Fig. 11F), the body had dark pigment, and the embryo had the appearance of a tadpole (Figs. 2F, 11E, G).

H. vertebralis
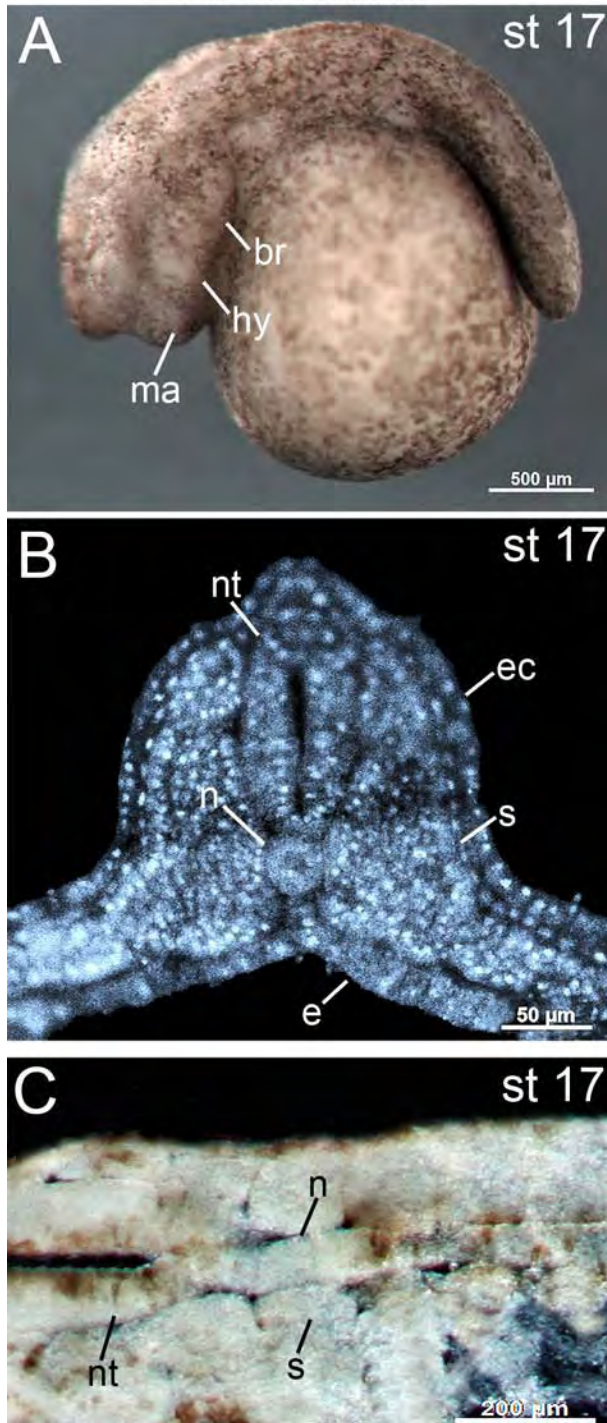
Fig. 7. Stage 17: Tail bud stage of *H. vertebralis* embryos. (**A**) Lateral view of an embryo. (**B**) Cross section through the trunk region of the embryo in A. (**C**) Horizontal section at the level of the notochord and somites with the rostral region towards the left. A row of somites was detected on each side of the notochord. A portion of the neural tube was detected in the rostral region of the section. br, branchial arch; e, endoderm; ec, ectoderm; hy, hyoid arch; ma, mandibular arch; n notochord; nt, neural tube; s, somite.

Comparative analysis of gastrulation

Gastrulation is characterized by common morphogenetic events that occur in all of the analyzed frog species. Formation of the dorsal blastopore lip, its development to enclose a yolk plug, and the process of internalization of cells at the blastopore lip by the movements of involution are among these common morphogenetic processes (Elinson and del Pino 2012). Other developmental events, however, may be dissociated from gastrulation in some frog species. In particular, dorsal convergence and extension and the onset of notochord elongation are separated from gastrulation in the Marsupial frog, *G. riobambae*, and in dendrobatid frogs; whereas, these events occur simultaneously with gastrulation in *X. laevis* and in *Engystomops* (Leptodactylidae) (Table 1) (del Pino 1996; Benítez and del Pino 2002; Keller and Shook 2004; Moya et al. 2007; Elinson and del Pino 2012).

The simultaneous occurrence of gastrulation and onset of notochord elongation may be related to the reproductive adaptation of frogs for rapid development under unstable environmental conditions such as the aquatic environment in which embryos of *X. laevis* develop, or the development in floating foam nests in species of *Engystomops* (Elinson and del Pino 2012). Embryos of these frogs require from 5 hours to 12.5 hours from the onset of gastrulation to blastopore closure (Stage 10–13) (Nieuwkoop and Faber 1994; Romero-Carvajal et al. 2009). Elongation of the notochord and gastrulation occur simultaneously in embryos of *X. laevis*, *Engystomops coloradorum*, and *Engystomops randi* (Leptodactylidae) (Keller and Shook 2004; Romero-Carvajal et al. 2009; Venegas-Ferrín et al. 2010). Early elongation of the notochord may be required for embryos to rapidly acquire the elongated tadpole shape in the unstable conditions of their reproductive environments.

The most divergent mode of gastrulation was detected in embryos of the Marsupial frog, *G. riobambae*. Gastrulation results in the formation of an embryonic disk from which the body of the embryo develops (del Pino and Elinson 1983). Cells that involute during gastrulation accumulate in the blastopore lip, and after blastopore closure give rise to an embryonic disk of small cells, visible on the surface. Internally, the small cells that involuted during gastrulation accumulated in the embryonic disk and in its internal circumblastoporal collar (Moya et al. 2007). Formation of the embryonic disk of *G. riobambae* is associated with delayed onset of notochord elongation that only starts once the blastopore is closed (del Pino 1996). Embryos of the Marsupial frog, *G. riobambae* develop slowly, and take a total of 168 hours from the onset of gastrulation to its completion (Table 1).

As in *G. riobambae*, cells that involuted during gastrulation became accumulated in a large circumblastoporal collar in embryos of dendrobatid frogs, with sepa-
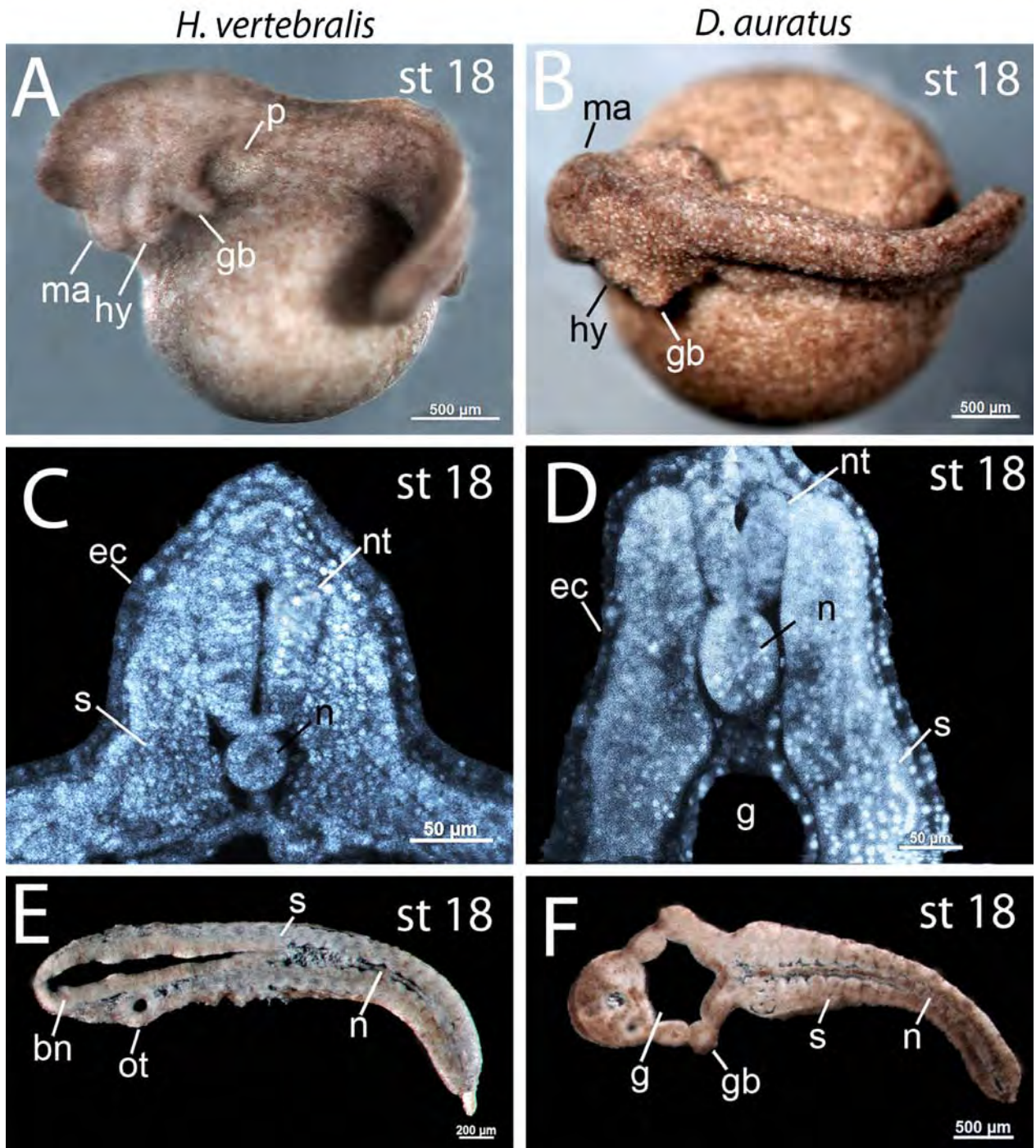
Fig. 8. Stage 18: Muscular activity stage of *H. vertebralis* and *D. auratus* embryos. Micrographs of *H. vertebralis* embryos are shown in A, C, E, and micrographs of *D. auratus* embryos are shown in B, D, F. Sections shown in C–D were stained for cell nuclei. **(A)** Lateral view of an embryo. **(B)** Dorsal view of an embryo. The gill buds were visible on each side of the head in embryos of both species. **(C–D)** Cross sections through the trunk. The cavity in D corresponds to the gut. **(E–F)** Horizontal sections. A row of numerous somites was detected on each side of the notochord. The brain and the otocysts were visible in E, and the gut was visible in F. bn, brain; ec, ectoderm; g, gut; gb, gill bud; hy, hyoid arch; ma, mandibular arch; n notochord; nt, neural tube; ot, otocyst; p, pronephros; s, somite.

ration of the morphogenetic events of gastrulation and the onset of notochord elongation. However, dendrobatid frogs do not develop an embryonic disk (Elinson and del Pino 2012). Egg size varied from 1.6 to 3.5 mm in diameter among dendrobatid frogs (Table 1), and their development was slow. Embryos of dendrobatid frogs require

36–72 hours from the onset of gastrulation to its completion (Stage 10–13; Table 1). We analyzed the characteristics of the gastrula in dendrobatid embryos derived from eggs of different diameters (Table 1; Fig. 12). Protection of embryos in the terrestrial nests of dendrobatids or inside a pouch of the mother in *G. riobambae* may al-
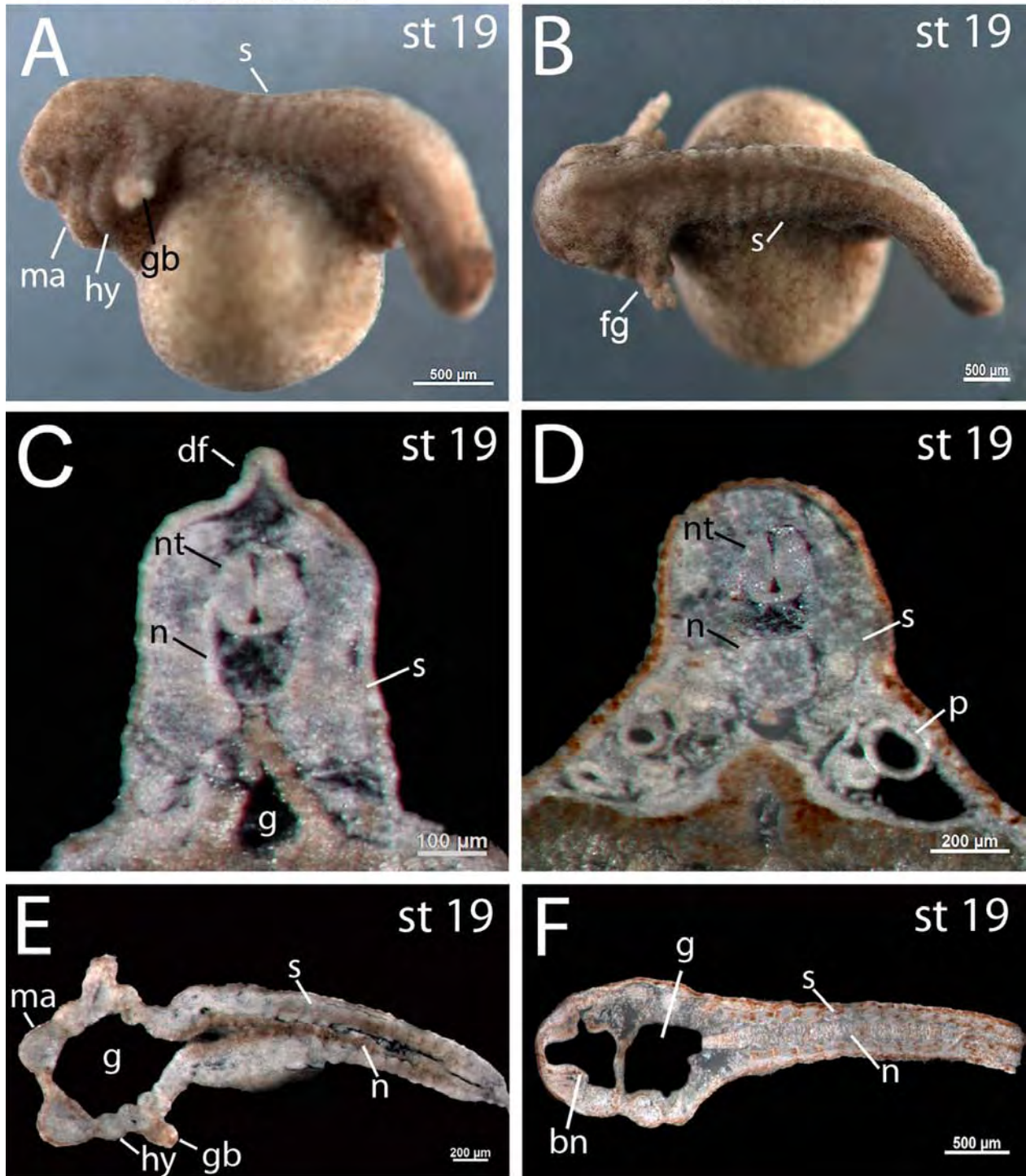
Fig. 9. Stage 19: Muscular response stage of *H. vertebralis* and *D. auratus* embryos. Micrographs of *H. vertebralis* embryos are shown in A, C, E, and micrographs of *D. auratus* embryos are shown in B, D, F. **(A)** Lateral view of an embryo. **(B)** Dorsal view of an embryo. The developing gills were visible. **(C–D)** Cross sections through the trunk. The dorsal fin was visible in C, and the pronephros in D. **(E)** Horizontal section at the level of the gut. **(F)** Horizontal section at the level of the brain and the gut. bn, brain; df, dorsal fin; fg, first gill pair; g, gut; gb, gill bud; hy, hyoid arch; ma, mandibular arch; n notochord; nt, neural tube; p, pronephros; s, somite.

low slow development and the separation of gastrulation from notochord elongation (Elinson and del Pino 2012).

Details of the morphology of the *H. vertebralis* and *D. auratus* gastrula are illustrated in Fig. 1E–I, Fig. 3B–J, and Fig. 12J–L, P–R. The archenteron roof remained

relatively thin during gastrulation in *H. vertebralis* and *D. auratus* in comparison with stage 13 embryos of *X. laevis* (Fig. 12C, J–L, P–R). Elongation and inflation of the archenteron varied greatly among dendrobatids. The archenteron remained small during gastrulation and be-

H. vertebralis
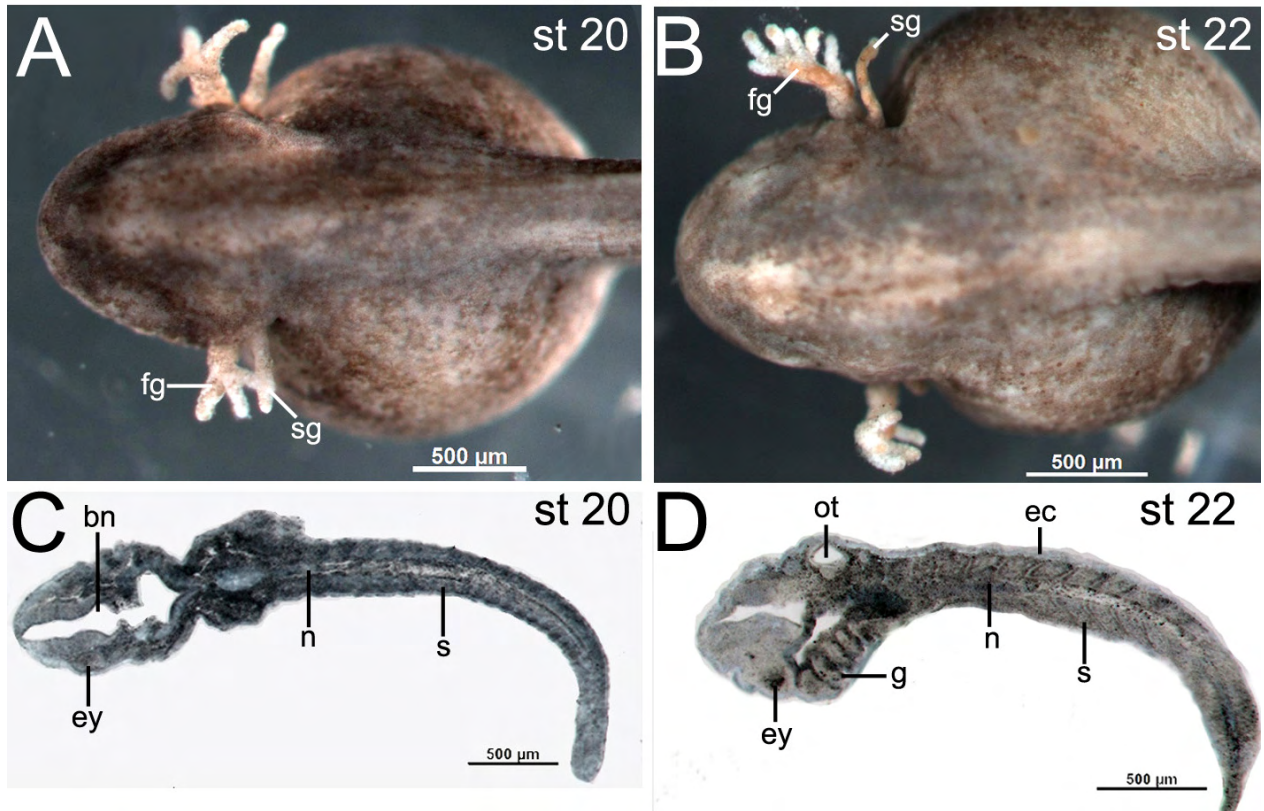
Fig. 10. Stages 20–22: External gill development in *H. vertebralis*. **(A)** Stage 20: Circulation to the external gills. Three branches were visible in the first gill pair. The second gill pair was unbranched. **(B)** Stage 22: The external gills enlarged. Seven branches were visible in the first gill pair. The second gill pair was unbranched. **(C)** Stage 20: Horizontal section at the level of the brain. **(D)** Stage 22: Horizontal section at the level of the internal gills. bn, brain; ey, eye; ec, ectoderm; fg, first gill pair; g, internal gill; nt, notochord; ot, otocyst; s, somite; sg, second gill pair.

came elongated and inflated after gastrulation in *H. vertebralis* (Fig. 12J–L); whereas, at stage 12, the archenteron was already elongated in the very large embryos of *A. bilinguis* and *D. auratus* (Fig. 12 M, P). Moreover inflation of the archenteron was already detected in stage 12 embryos of *D. auratus* (Fig. 12 P). In other species of dendrobatids, we detected variation in the level of archenteron elongation and inflation (Fig. 12D–R). We concluded that in *A. bilinguis*, and *D. auratus*, dendrobatids with very large eggs, the elongation of the archenteron begins earlier in comparison with embryos of dendrobatid frogs with smaller eggs such *E. machalilla* (Table 1) (del Pino et al. 2007).

In spite of the differences detected in the onset of archenteron elongation, the cells that involuted during gastrulation became accumulated in a large circumblastoporal collar at stage 13 in all of the dendrobatid frogs analyzed, as previously reported for *E. machalilla*, and shown for *E. anthonyi*, *E. tricolor*, *H. vertebralis*, *A. bilinguis*, and *D. auratus*, (Fig. 12F, I, L, O, R) (Moya et al. 2007). Notochord elongation is dissociated from gastrulation in these frogs (Benítez and del Pino 2002; del Pino et al. 2007; Moya et al. 2007; Venegas-Ferrín et al. 2010; Montenegro-Larrea and del Pino 2011).

The comparative analysis of gastrulation indicates that in spite of the great variation in egg size and onset of

archenteron elongation and inflation, the Dendrobatidae species examined develop a large circumblastoporal collar as a result of gastrulation (Fig. 12D–R; Table 1) (del Pino et al. 2007; Moya et al. 2007; Montenegro-Larrea and del Pino 2011). Moreover, notochord elongation is delayed until after blastopore closure as in *G. riobambae*. In spite of their large circumblastoporal collar, dendrobatid frog embryos did not develop an embryonic disk.

Conclusions

Development of the dendrobatid frogs, *H. vertebralis* and *D. auratus*, shared the developmental characteristics described for *E. machalilla* (del Pino et al. 2004). Gastrulation and notochord elongation occurred as separate morphogenetic events in these frogs in comparison with additional species of Dendrobatidae. Development in a somewhat stable terrestrial environment may be associated with the separation of these developmental events and with comparatively slow development. The developmental analysis of *H. vertebralis* and other frogs contributes to a better knowledge of their biology and may contribute to the conservation and reproductive management of endangered frogs.

H. vertebralis   ## *D. auratus*
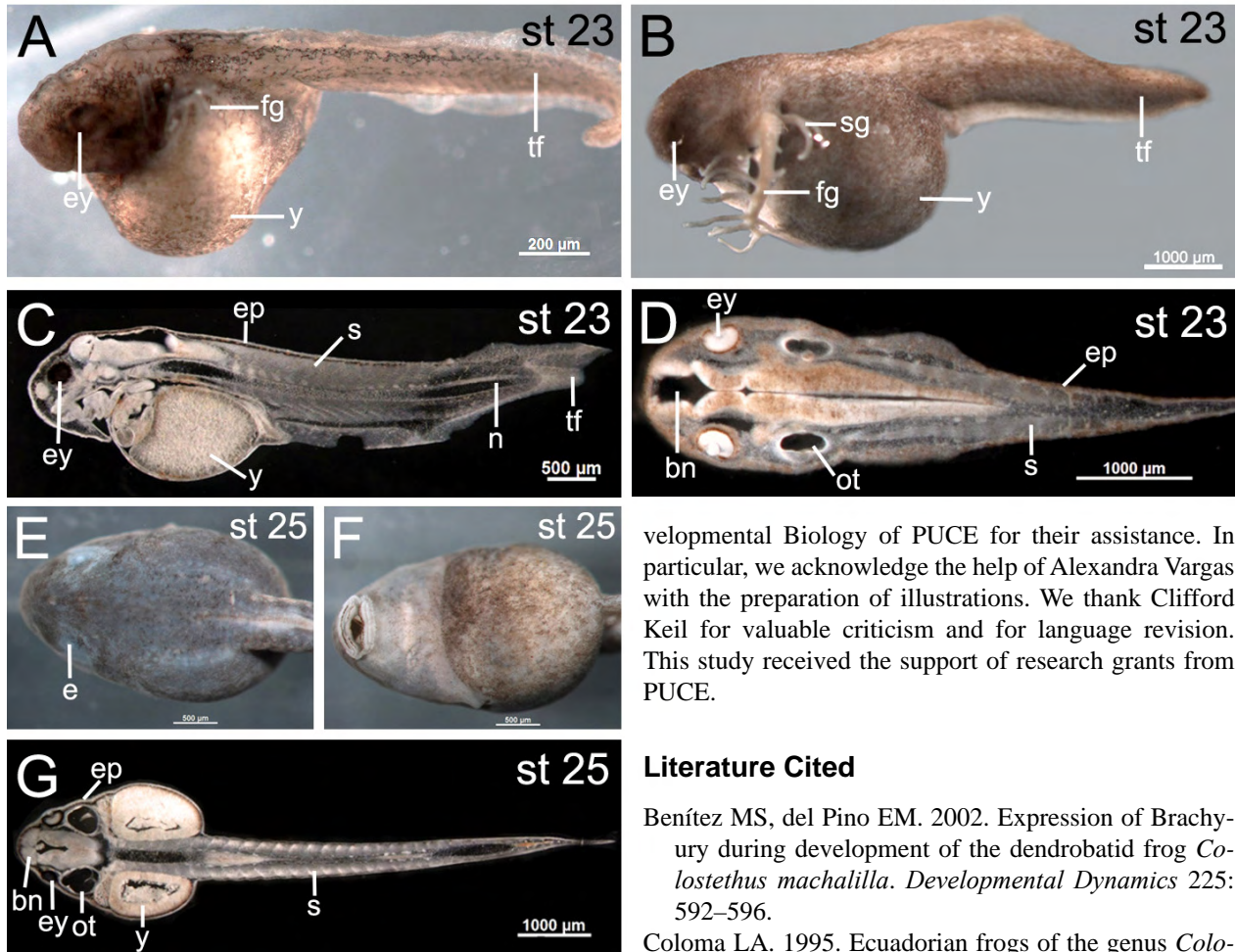
Fig. 11. Stages 23–25: Complete development of the external gills to tadpole hatching in embryos of *H. vertebralis* and *D. auratus*. Micrographs of *H. vertebralis* embryos are shown in A, C, E, F, G, and micrographs of *D. auratus* embryos are shown in B, D. **(A)** Stage 23 of *H. vertebralis*: Full development of external gills. The first gill pair of the external gills had eight branches, which at this stage were fully extended. The second gill pair of external gills was unbranched. **(B)** Stage 23 of *D. auratus*: The first gill pair of the external gills had nine branches, which at this stage were fully extended. The second pair of external gills was smaller and had two branches. In embryos of both species the eyes and the body were pigmented. The tail was elongated. **(C)** Stage 23: Sagittal section. The section was done through the embrionary brain and somites. The eyes, notochord, and tail fin were observed. **(D)** Stage 23: Horizontal section at the level of somites. The eyes and otocysts were visible. **(E)** Stage 25: Head of a tadpole at hatching in dorsal view. The eyes were visible. **(F)** Stage 25: Ventral view of the head of the tadpole shown in A. The spiracle was visible. **(G)** Stage 25: Horizontal section of a tadpole at hatching at the level of the otocysts. The eyes, otocysts, and somites were visible. bn, brain; ey, eye; ep, epidermis, fg, first gill pair; n, notochord; ot, otocyst; sg, second gill pair; s, somite; tf, tail fin; y, yolky endoderm.

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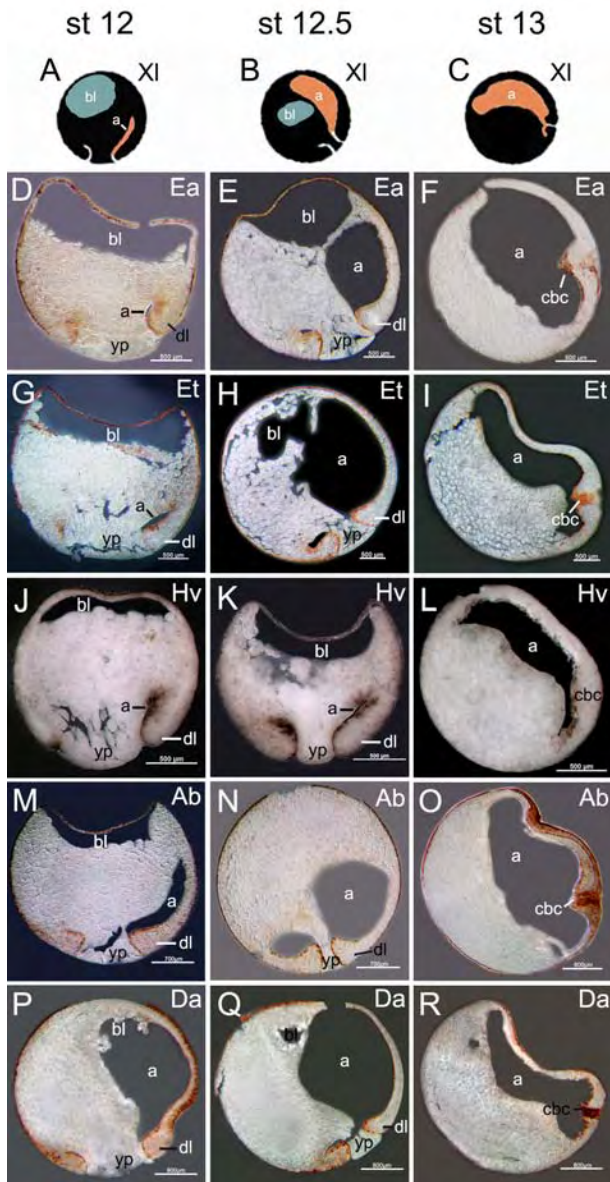
Fig. 12. Gastrulation of dendrobatid frogs in comparison with *X. laevis*. To facilitate the comparison, the stages (st) are given as column headings: Stage 12, late-gastrula; Stage 12.5, advanced late-gastrula; Stage 13, slit blastopore stage. The letters in the upper right-hand corner indicate the species: Ab, *Ameerega bilinguis*; Da, *Dendrobates auratus*; Ea, *Epipedobates anthonyi*; Et, *Epipedobates tricolor*; Hv, *Hyloxalus vertebralis*; Xl, *Xenopus laevis*. **(A–C)** Diagrams of *X. laevis* embryos. **(D–R)** Sagittal sections of gastrulae. **(D–F)** Embryos of *E. anthonyi*. (The section in E was reproduced from Montenegro–Larrea and del Pino, 2011). **(G–I)** Embryos of *E. tricolor*. **(J–L)** Embryos of *H. vertebralis*. **(M–O)** Embryos of *A. bilinguis*. **(P–R)** Embryos of *D. auratus*. a, archenteron; bl, blastocoel; cbc; circumblastoporal collar; dl, dorsal blastopore lip; yp, yolk plug.

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Short Communication

First records of *Anolis ventrimaculatus* Boulenger, 1911 (Squamata: Iguanidae) in Ecuador

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Abstract.—We report the first records of *Anolis ventrimaculatus* for Ecuador based on twelve specimens from three localities: Chical (Provincia Carchi), El Cristal (Provincia Esmeraldas), and Lita (Provincia Imbabura). The locality in the Provincia Carchi lies approximately 16 km S from the nearest record (Ñambí, Department Nariño, Colombia). We also present information on scalation and coloration.

Key words. Anole lizards, color, distribution, Ecuador, scalation

Thirty-seven species of *Anolis* have been reported formally from Ecuador (Ayala-Varela et al. 2014). *Anolis ventrimaculatus* Boulenger 1911 was described from two syntypes, an adult female and a juvenile from Río San Juan, Department Risaralda, Colombia. Williams and Duellman (1984) designated the adult female syntype (BMNH 1946.8.13.5) as the lectotype.

Anolis ventrimaculatus is assigned to the *aequatorialis* series Castañeda and de Queiroz (2013) by having a moderate to large body size, narrow toe lamellae, small head scales, smooth ventral scales, and uniform dorsal scalation. It is assigned to the eulaemus-subgroup based on a typical *Anolis* digit, in which the distal lamellae of phalanx III distinctly overlap the first proximal subdigital scale of phalanx II (Williams 1976; Williams and Duellman 1984; Castañeda and de Queiroz 2013).

Eleven species of the eulaemus-subgroup occur on both sides of the Andes (*Anolis anoriensis* Velasco et al. 2010, *A. antioquiae* Williams 1985, *A. eulaemus* Bou-

lenger 1908, *A. fitchi* Williams and Duellman 1984, *A. gemmosus* O'Shaughnessy 1875, *A. maculigula* Williams 1984, *A. megalopithecus* Rueda-Almonacid 1989, *A. otongae* Ayala-Varela and Velasco 2010, *A. podocarpus* Ayala-Varela and Torres-Carvajal 2010, *A. poei* Ayala-Varela et al. 2014, and *A. ventrimaculatus* Boulenger 1911).

Specimens examined for comparisons are housed in the herpetological collections of the Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ); Museo de Herpetología de la Universidad de Antioquia, Antioquia, Colombia (MHUA); Colección de Herpetología, Universidad del Valle, Santiago de Cali, Colombia (UVC); and Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN). External character terminology follows Williams et al. (1995) and Poe and Yañez-Miranda (2008). Lamellar number was counted using the method of Williams et al. (1995), i.e., only on phalanges III and IV of the

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fourth toe. Measurements were made with digital calipers on preserved specimens and are given in millimeters (mm), usually to the nearest 0.1 mm. Snout-vent length (SVL) was measured from tip of snout to anterior edge of cloaca. Femoral length was measured from midline of venter to knee, with limb bent at a 90-degree angle. Tail length was measured from anterior edge of cloaca to distal point.

Herein we report the first records of *Anolis ventrimaculatus* (Fig. 1) for Ecuador based on specimens collected at three localities. Four specimens (QCAZ 3284–3286, 8934) were collected on 16 September 1992 in Lita (0.87°, -78.45°), Provincia Imbabura; four specimens (QCAZ 2666, 3923, 3924, 3929) were collected on August 1994 in El Cristal (0.83°, -78.49°, 1,200–1,250 m), Reserva Ecológica Cotacachi-Cayapas, Provincia Esmeraldas; and four specimens (QCAZ 4376, 4378, 4389, 4390) were collected on 03 July 2011 in Río San Pablo, near Chical (0.90°, -78.16°, 1,399–1,451 m), Provincia Carchi. The last locality lies approximately 16 km S from the nearest record (ICN 11981-85, 11987-989, 12097, Ñambí, Barbacoas municipality, Department Nariño, Colombia) (Fig. 1, Table 1).

The individuals from Chical (Provincia Carchi) were captured in secondary forest; all individuals were found on leaves, branches, or ferns from 50–150 cm above ground; a male were found head-down, while two females were found head-down and head-up. The smallest specimen (QCAZ 8934, juvenile, SVL = 31.4 mm) was collected on 16 September 1992. An adult female (QCAZ 4378) collected in July 2011 deposited one white egg (17.11 mm × 6.44 mm). Our collections of *Anolis ventrimaculatus* in Ecuador were found from 1,200 to 1,451 m above sea level. In Ecuador, this species occurs in sympatry with *A. aequatorialis*, *A. gemmosus*, and *A. maculiventris* in Chical (Provincia Carchi); with *A. lynchi*, *A. maculiventris*, and *A. princeps* (pers. obs. Sebastián Valverde) in Lita (Provincia Imbabura), and with *A. lynchi* in El Cristal (Provincia Esmeraldas).

Scalation and morphometric characters of *Anolis ventrimaculatus* are presented in Table 2. Scale counts are similar between Ecuadorian and Colombian specimens. Our Ecuadorian specimens of *Anolis ventrimaculatus* are smaller than those from Colombia (maximum SVL 62 mm and 80 mm, respectively).

Coloration in life of specimens from Ecuador was recorded as follows:

Adult female (QCAZ 4390, Figs. 2 A, B): dorsal surfaces of head, body and tail dark brown; dorsal surface of body with a pale brown longitudinal stripe extending from occipital region to base of tail; limbs pale brown with dark brown reticulation; tail pale brown; lateral surface of head with two stripes, one dark brown and extending posteriorly from loreal region, through subocular region, above tympanum to level of the hind limb, the other stripe is pale green and extending posteriorly from loreal region, through subocular region, above tym-
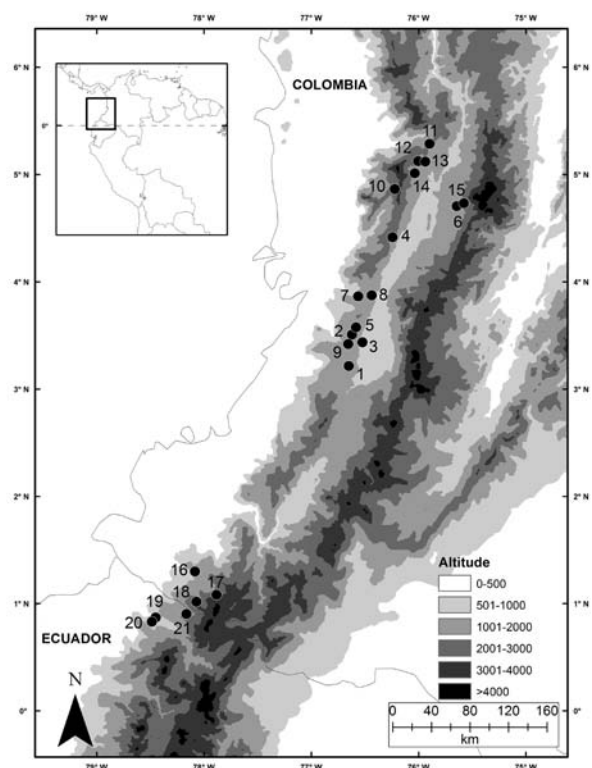
Fig. 1. Distribution of *Anolis ventrimaculatus* in South America (locality numbers are listed in Table 1).

panum to level of neck; lateral surface of body brown anteriorly and olive-green near inguinal region; ventral surface of head yellowish green with pale brown reticulations; ventral surface of body cream; ventral surface of limbs dark cream with dark brown reticulations; ventral surface of tail dark cream.

Adult female QCAZ 4378 (Figs. 2 C, D) differs from the previous pattern in having the dorsal surface of body brown, with seven dark brown blotches arranged longitudinally along the midline.

Adult male (QCAZ 4389, Figs. 2 E, F, G): When stressed, the background of head, body, limbs and tail was yellowish brown; dorsal surface of the neck with two dark brown bands; dorsal surface of body with nine dark brown blotches arranged longitudinally; limbs with dark brown bands; dorsal surface of tail with dark brown transversal bands, and with three dark brown blotches in the proximal part of tail; lateral surface of head with a darker brown first stripe, extending posteriorly from loreal region, through subocular region, above the tympanum and bifurcating into branches that continue on nuchal crest and shoulder, respectively; a yellowish-green second stripe, extending posteriorly from loreal region, through subocular region, above the tympanum to the shoulder; black ocelli with white centers on the shoulder; lateral surface of body with reddish-brown bands oriented ventroposteriorly; ventral surface of head yellowish green with pale brown reticulations; ventral surface of neck pale green; ventral surface of body cream; ventral surface of limbs pale brown with dark brown re-

Table 1. Localities of *Anolis ventrimaculatus* in Ecuador and Colombia.

Site number	Country	Locality	Latitude	Longitude	Source
1	Colombia	Bosque de San Antonio, km 18 vía Cali-Buenaventura, Valle del Cauca	3.22	-76.65	JAV pers. obs
2	Colombia	Bosque de San Antonio, km 18 vía Cali-Buenaventura, Valle del Cauca	3.51	-76.62	UVC 9737, 9749, 9896, MHUA 1671-79, JAV pers. obs.
3	Colombia	Antena, Cerro La Horqueta, ca. 28 km de Cali, Valle del Cauca	3.44	-76.52	JAV pers. Obs, ICN 3567
4	Colombia	Vereda La Tulia, Mpio. Bolívar, Valle del Cauca	4.42	-76.24	JAV pers. obs
5	Colombia	Vereda Chicoral, La Cumbre, Valle del Cauca	3.58	-76.58	JAV pers. obs, UVC 10223
6	Colombia	PRN Barbas-Bremen, Mpio. Filandia, Quindio	4.71	-75.64	JAV pers. obs
7	Colombia	Alrededores Lago Calima, Mpio. Darien, Valle del Cauca	3.86	-76.56	JAV pers. obs; UVC 5189-96, ICN 3553-54
8	Colombia	Reserva Faunística Bosque de Yotoco, Valle del Cauca	3.88	-76.44	JAV pers. obs
9	Colombia	Peñas Blancas, Pichindé, Valle del Cauca	3.42	-76.66	UVC 223, 224
10	Colombia	Alto de Galápagos, carretera Cartago-San José del Palmar, Límite Valle-Chocó	4.86	-76.22	UVC 9366, UVC 8489-95
11	Colombia	PMN Arrayanal, Mpio. Apia, Risaralda	5.29	-75.90	JAV pers. obs
12	Colombia	PMN Planes de San Rafael, Mpio. Santuario, Risaralda	5.13	-76.00	JAV pers. obs
13	Colombia	PMN Agualinda, Mpio. Mistrató, Risaralda	5.12	-75.94	JAV pers. obs
14	Colombia	PMN Verdum, vereda La Secreta, Risaralda	5.01	-76.03	JAV pers. obs
15	Colombia	Vereda Buenos Aires, Cuenca Rio Barbo, Pereira, Risaralda	4.73	-75.58	JAV pers. obs
16	Colombia	Rio Ñambi, Nariño	1.30	-78.08	JAV pers. obs
17	Colombia	Reserva La Planada, Nariño	1.08	-77.88	JAV pers. obs
18	Colombia	Ñambí, Nariño	1.02	-78.07	ICN 11981-85, 11987-889, 12097
19	Ecuador	Lita, Imbabura	0.87	-78.45	QCAZ
20	Ecuador	El Cristal, Reserva Ecológica Cotacachi Cayapas, Esmeraldas	0.83	-78.49	QCAZ
21	Ecuador	Río San Pablo, cerca de Chical, Carchi	0.90	-78.16	QCAZ

ticulations; ventral surface of tail pale brown with small dark brown reticulations; dewlap skin yellowish brown; gorgetals pale green; marginals and sternals yellowish green; iris dark brown with yellowish-brown inner ring.

The coloration of populations of *Anolis ventrimaculatus* from Colombia display a dorsal surface of the body that is bright emerald green, or greenish-brown with slight darker oblique bars and yellow spots on each side of the dorsal midline; yellow spots fuse forming a series of saddle-shaped bars that cross the back and tail, more visible in the stressed phase. At their stressed phase, dorsal and lateral surface of body brown with tiny yellow spots; lateral surface of head with a yellow line under the eye and with a prominent pale yellow or green line over the lips extending back over the ear opening and along the sides of the neck; ventral surface of head yellow-green, sometimes with reticulations; ventral surface of body cream to yellow-green, with dark brown spots on the sides; ventral surface of tail orange in male adults. Some females have a dorsal surface of body with a tan longitudinal stripe and dark edges.

Anolis ventrimaculatus has a wide range of distribution, approximately 570 km in airline between the northern and southernmost localities. However, there is a huge distributional gap between central and southern Colombian populations (approximately 265 km airline between Bosque de San Antonio, Department Valle del Cauca and the Rio Ñambi, Department Nariño). One of the main reasons for this gap is the lack of extensive herpetological inventories in these areas, particularly in both foothills of the Andes cordilleras. More sampling effort should addressed to these areas with the aim to fill distributional gaps in several species, including *Anolis* lizards.
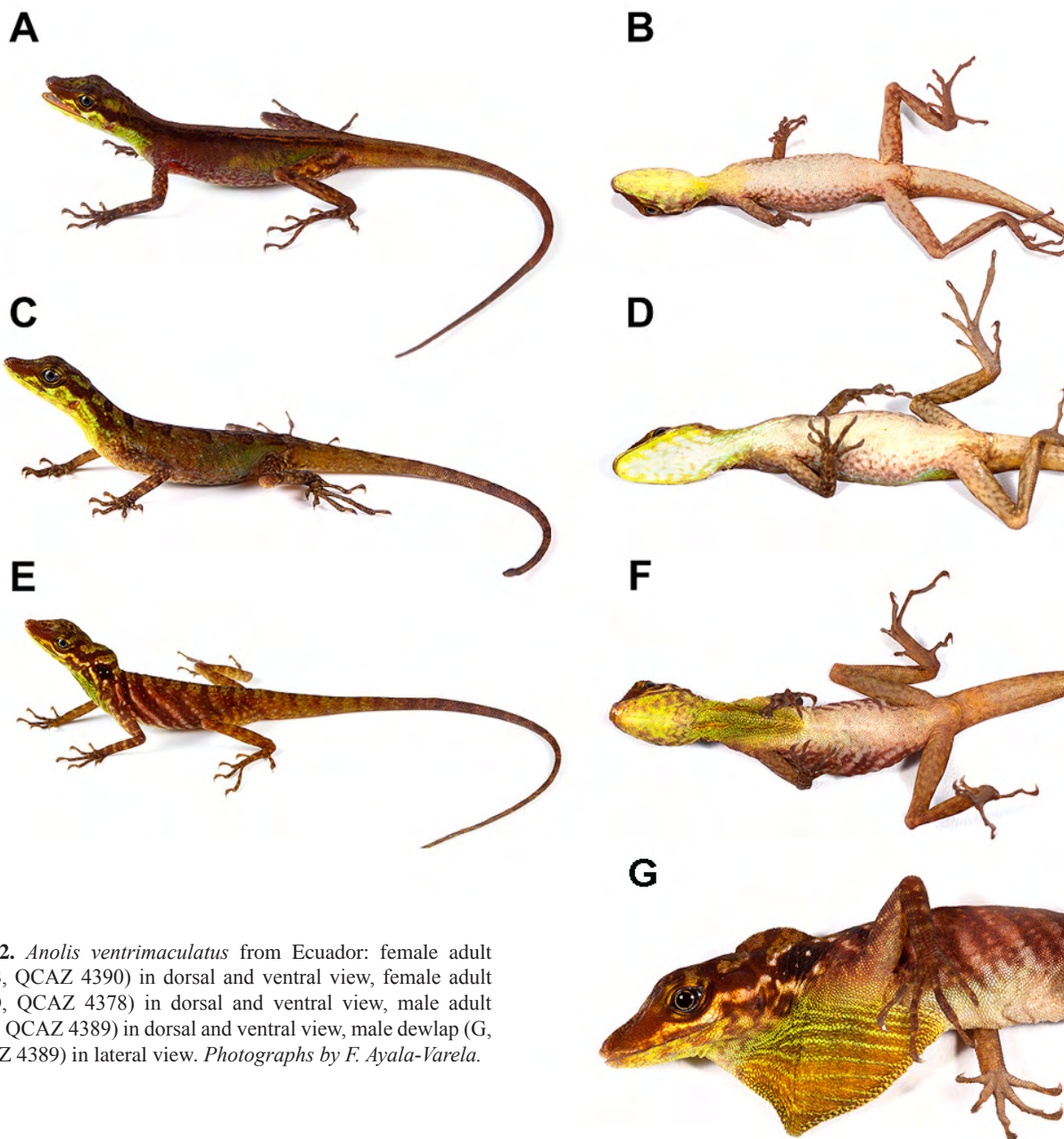
Fig. 2. *Anolis ventrimaculatus* from Ecuador: female adult (A−B, QCAZ 4390) in dorsal and ventral view, female adult (C−D, QCAZ 4378) in dorsal and ventral view, male adult (E−F, QCAZ 4389) in dorsal and ventral view, male dewlap (G, QCAZ 4389) in lateral view. *Photographs by F. Ayala-Varela.*

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Table 2. Scale count and measurements (mm) of specimens of *Anolis ventrimaculatus* from Ecuador and Colombia. Range (sample size) and mean. SVL = snout-vent length.

	Ecuador QCAZ	Colombia UVC, ICN, MHUA	Colombia Williams et al. 1995
Number of scales between second canthals	14−17 (10) 15.7	12−17 (18) 14.6	11−21 (20)
Number of scales bordering rostral	5−7 (10) 6.4	6−8 (18) 6.8	6−10 (20)
Number of scales between supraorbital semicircles	2−5 (10) 3.6	4−6 (18) 4.8	2−6 (20)
Number of scales between interparietal and supraorbital semicircles	7−11 (8) 6.9	6−11 (18) 8.0	5−16 (20)
Interparietal	+/−	+/very small	(+/?)
Number of loreal rows	5−8 (9) 7.9	7−9 (18) 8.2	7−11 (20)
Number of supralabials to center of eye	6−8 (10) 7.2	6−8 (18) 7.4	6−8 (20)
Number of postmentals	6−8 (9) 6.3	6−9 (18) 6.6	4−8 (20)
Number of sublabials in contact with infralabials	0−2 (9) 0.5	1−3 (18) 2.5	0−2 (20)
Lamellar number	16−18 (10) 17.2	17−22 (18) 19.4	16−22 (20)
Number of middorsals in 5% SVL	12−15 (10) 12.9	14−19 (18) 16.6	−
Number of midventrals in 5% SVL	7−11 (10) 9.2	9−14 (18) 11.9	−
Femur length	16.0−20.6 (10) 18.5	15.4−23.1 (16) 18.9	−
Maximum SVL (male/female)	62/57	75/69	80/62

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Fernando Ayala-Varela is the director of the herpetology collection at the Pontificia Universidad Católica del Ecuador in Quito. He received his diploma at the Pontificia Universidad Católica del Ecuador, Quito in 2004. He has been interested in herpetology since childhood and has dedicated a lot of time studying the lizards of Ecuador, specifically the taxonomy and ecology of *Anolis* species. His current research interests include reproductive biology and ecology of lizards and snakes in Ecuador.

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Alejandro Arteaga is a wildlife photographer and undergraduate biology student from Venezuela. In 2009, he co-founded Tropical Herping, an institution striving to preserve tropical reptiles and amphibians through tourism, photography, research, and education. Alejandro is author of *The Amphibians and Reptiles of Mindo* and several scientific articles. He has described three species new to science and his photographic work has been featured in National Geographic, Anima Mundi, and the Discovery Channel.

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Book Review

The Amphibians and Reptiles of Mindo: Life in the Cloudforest

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Title: *The Amphibians and Reptiles of Mindo: Life in the Cloudforest*
Authors: Alejandro Arteaga, Lucas Bustamante, Juan M. Guayasamin
Copyright: 2013
ISBN: 978-9942-13-496-7
Publisher: Universidad Technológica Indoamérica
Pages: 258; **Price:** $49.00 (US)

The authors have produced a much needed local field guide for the Mindo parish, located in northwestern Ecuador and set a high standard for future field guides to follow. The book fills a void with great detail and care. It begins with the Table of Contents, a Forward, and Preface. A brief Symbols and Abbreviation page is followed by the Introduction, which leads into a helpful section on locating and observing reptiles and amphibians in the Mindo region. The authors recommend that those interested in exploring Mindo's herpetofauna should do some homework: understand the habitats and environments where herpetofauna can be found, know your subject, keep a low profile, and try not to disturb the sensitive microhabitats in which these unique species are found. Page 11 illustrates some principal identification features of the amphibians of Mindo (as a diagram figure; p. 11). On the next few pages are additional figures that show the groin pattern and color of the Mindo rainfrogs (*Pristimantis*), principal scale types of Mindo lizards, dewlap color of Mindo anoles, and basic terminology for snake scalation. In total the guide features 20 charts and figures, and 228 superb photographs and art work (a special feature and highlight of the book). These figures allow the future explorer of Ecuadoran cloudforests (particularly Mindo) to prepare for an informed and exciting field trip.

The crux of the book are the accounts. The guide features 101 species accounts of Mindo's unique reptiles and amphibians, with each account accompanied with, as mentioned above, outstanding photos and in addition, a range map. The 228 photos are adequate for identifying the target species and have been photographed with a white background, eliminating distracting clutter so the reader can focus on key marks, characteristics, and colors of each species (see Figure 1 for examples). The range maps are up-to-date and reflect the most current research (in total, 4,000 locality records are featured). Each species account has been peer-reviewed by two or more experts (71 total reviewers and hundreds of personal communications from experts). The accounts are divided into several key sections: English and Spanish common names, Latin name with describing author and year, recognition information, natural history, distribution, conservation status, etymology, notes, reviewer and contributor information, and references. Prior to the species account sections is the "Plan of the Book"—this section is a must read in that it explains how species accounts are set up and discusses the rational of account structure. Additionally, pages 27–29 discusses the Mindo parish; why the area is worthy of continual conservation, and describes the unique characteristics of the region that is home to more than 100 species of reptiles and amphibians in an area smaller than the state of Nevada.

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After the species accounts the book presents a description of a new species of *Pristimantis* found in Mindo (Arteaga et al. 2013). This account illustrates that new species are continually being discovered and regions like Mindo may harbor other new species.

Following the new species description is the Glossary, Reference section (the book lists 1,935 references throughout), and the final section is "about the authors," listing the scientific illustrators and geographers (Rita Hidalgo, Silvia Cevallos, and Belén Baus).

Overall, the field guide of Mindo is an outstanding contribution to the ever-growing field of herpetology, will help conservation efforts, encourage ecotourism and nature observation, be a high standard for other field guides to follow, among other positive allied outcomes, while linking conservation efforts through its publication. The guide is pleasing to read and should inspire others to write and publish regional guides in species rich areas of the Americas, and, as mentioned several times already, sets a high standard for others to follow. The book emphasizes a warning that species extinction is real, is primarily a result of habitat loss, and areas like Mindo are not necessarily safe. The future is unknown and with the advent of climate change, disease, encroachment, as well as many other detrimental factors not mentioned, we may be witnessing the last sanctuaries for these one-of-a-kind species. We need to do our part to spread the word and conserve what's left. The publication of a field guide such as this is very important in bringing attention to the great variety of unique species and lending impetus to conservation efforts. Field guides like this one are also synergistic in bringing about increased conservation efforts and making a positive impact to curb the unprecedented rate of habitat loss. We recommend that you support the conservation of Mindo by purchasing the book, learning about Mindo's amphibians and reptiles, and joining in the conservation efforts of the area (or other similar areas throughout the world) through ecotourism, conservation research (e.g., citizen scientists), and other avenues of endeavors and conservation activism via your individual expertise and enthusiasm to conserve all life on earth, including our own species. No matter who we are (average or exceptional, and all other categories as well) we all can make a positive difference in protecting and conserving earth's unique and precious life systems and diverse biological life (see also Conrad and Hilchey
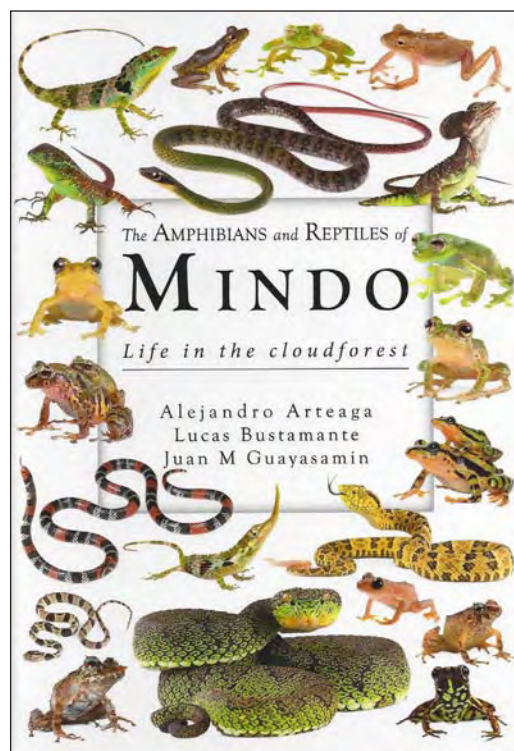
Fig. 1. Book cover of *The Amphibians and Reptiles of Mindo: Life in the Cloudforest. Photo by Howard O. Clark, Jr.*

2011; Johnson et al. 2014), for which amphibians and reptiles form an exciting component.

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Lepidoblepharis sanctaemartae, Sabanas de San Ángel, Magdalena, Colombia. *Photo by Juan Manuel Renjifo.*

Amphibians and reptiles of an agroforestry system in the Colombian Caribbean

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Abstract.—Land-use change is a factor that may alter the assembly of herpetofaunal communities. To determine the effects of land use change, we characterized the herpetofaunal community of "La Gloria Project" in Magdalena, Colombia. Agroforestry crops (Red Gum, Pink Trumpet Tree, Beechwood, and Teak), native forest, wetlands, and built-up zones composing the site. From March to October 2012, we performed eleven field trips, of ten days (eight hours each) for a total sampling effort of 880 hours per observer. We implemented visual encounter surveys and pitfall traps for herpetofauna detection. We recorded 23 amphibian (3,555 individuals) and 37 reptile species (1,088 individuals); the highest diversity for both amphibians and reptiles were found in native forest. Comparing disturbed areas, Teak agroforest presented the highest diversity for both taxa relative to non-natural environments, by factors such as big leaf size, generating conditions to sustenance of some species. However, we demonstrated that short-term differences between natural and non-natural habitats are significant, since there has not been enough time for generalist species to displace the susceptible species and occupy their niches in all vegetation coverages in the study area.

Key words. Agroforest, Caribbean lowlands, habitat fragmentation, herpetofaunal communities, tropical dry forest, lower Magdalena River

Resumen.—El cambio de usos del suelo es un factor que puede afectar el ensamblaje de las comunidades de herpetofauna. Para determinar los efectos del cambio del uso de suelo, caracterizamos la comunidad de herpetofauna del "Proyecto La Gloria" en el departamento del Magdalena, Colombia. Cultivos agroforestales (eucalipto rojo, roble rosado, melina y teca), bosques nativos, humedales y zonas con construcción constituyen el área de estudio. De marzo a octubre de 2012, desarrollamos once salidas de campo de diez días (cada uno de ocho horas) por un esfuerzo total de muestreo total de 880 horas × observador. Utilizamos búsqueda libre por encuentro casual y trampas de caída para la detección de herpetofauna. Registramos 23 anfibios (3,555 individuos) y 37 reptiles (1,088 individuos); La mayor diversidad tanto para anfibios como reptiles la encontramos en los bosques nativos. Comparando las áreas intervenidas, el agrobosque de teca presentó la mayor diversidad de ambos taxones con respecto a los otros ambientes no naturales, por factores como el gran tamaño de sus hojas, que generan condiciones para el sostenimiento de algunas especies. Empero, se demuestra que a corto plazo, las diferencias entre los hábitats naturales y no naturales son significativas, pues no ha pasado suficiente tiempo para que las especies generalistas desplacen a la especies sensibles y ocupen sus nichos.

Palabras clave. Agrobosques, bajo río Magdalena, bosque seco tropical, comunidades de herpetofauna, fragmentación de hábitat, tierras bajas del Caribe

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Introduction

Colombia ranks second in taxonomic diversity of amphibians (785 species) and third in reptiles (593 species) (Acosta-Galvis 2014; Andrade-C. 2011). In the Caribbean lowlands 167 reptiles species and 55 amphibians are recorded (Romero-Martínez and Lynch 2012; Carvajal-Cogollo et al 2012). The low diversity of amphibians in the Colombian Caribbean is due to drier conditions of the region, however, the small number of species have morphological, physiological, and behavioral adaptations to tolerate drought (Cuentas et al. 2002). Existing surveys include checklists, inventories, and diversity of amphibians and reptiles for the entire region (Dugand 1975; Carvajal-Cogollo et al. 2012; Romero-Martínez and Lynch 2012); as well as the states of Córdoba (Renjifo and Lundberg 1999; Carvajal-Cogollo et al. 2007; Carvajal-Cogollo and Urbina-Cardona 2008; Romero-Martínez et al. 2008; Romero-Martínez and Lynch 2010), Sucre (Galván-Guevara and de la Ossa-Velásquez 2009; Acosta-Galvis 2012b), Bolivar and Atlántico (Cuentas et al. 2002), Cesar (Rueda-Almonacid et al 2008a; b; Medina-Rangel 2011; Medina-Rangel et al. 2011), La Guajira (Galvis et al. 2011; Blanco-Torres et al. 2013), and Magdalena (Ruthven 1922; Dueñez-Gómez et al. 2004; Rueda-Solano and Castellanos-Barliza 2010; Montes-Correa et al. 2015). Many studies were performed in natural areas with wetlands or native forests, with different levels of anthropogenic intervention. Nonetheless, the information on the herpetofauna of dry spots is scarce, and most of the available literature are species descriptions, taxonomic reviews of specific groups, or national lists (Acosta-Galvis, 2012a).

Deforestation and changes in land-use modify the assembly of amphibian and reptile communities (Castro and Kattan 1991; Garden et al, 2007). The physical transformation of natural environments can cause drastic changes in humidity and temperature, having significant effects in these organisms (Herrera et al. 2004). However, dryland amphibians have several adaptations to survive the lack of water, as the changes in activity patterns and development of wide ranges of dehydration (Thorson 1995; Cuentas et al. 2002).

Moreover, reptiles are more resistant to disturbance as their skin is covered by keratinized scales. Amniotic eggs make reptiles more tolerant to dehydration and sunstroke (Vargas-Salinas and Bolaños 1999). Even so, the canopy cover, leaf litter cover, and understory density are important factors for the establishment and distribution of both taxa, since it can determine the movement patterns of these ectothermic animals (Urbina-Cardona et al. 2006).

Our goal was to determine the diversity of herpetofauna in "La Gloria Project" (Sabanas de San Ángel, Magdalena, Colombia), and assess the characteristics and variations of herpetofaunal communities among the various vegetation coverages (Agroforestry crops—Red Gum, Pink Trumpet Tree, Beechwood, and Teak—native

forest, wetlands, and built-up zones [any area inhabited by humans] composing the area).

Materials and Methods

Study site: "La Gloria project" is part of "Reforestadora de la Costa (REFOCOSTA S.A.S.)" organization, within the jurisdiction of the municipality of Sabanas de San Ángel, Magdalena department, 30 km from the county seat (10°10'29.2"N; 74°19'38.052"W) (Fig. 1). The study area includes 7,288 hectares, and corresponds to "zonobioma tropical alternohígrico" (tropical dry forest) proposed by Hernández-Camacho and Sánchez (1992). This locality has a unimodal biseasonal climate with an average annual rainfall of 1,157 mm (Rangel-Ch. and Carvajal-Cogollo 2012). The oldest agroforest is about about 20 years old. Timber is grown in the middle extension of the La Gloria project. The main crop is Teak (*Tectona grandis*) (21% of the total extent of study area), followed by Red Gum (*Eucalyptus tereticornis*) (18%). Also grown to a lesser extent is, Pink Trumpet Tree (*Tabebuia rosea*) (7%) and Beechwood (*Gmelina* sp.) (2%),
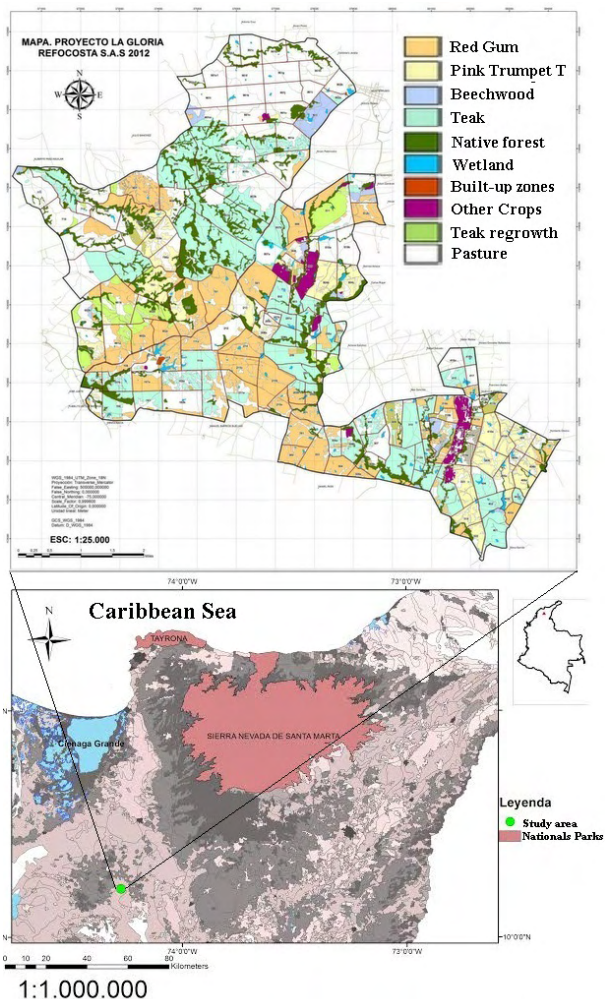
Fig. 1. Map of La Gloria Project (taken and modified from Refocosta 2012). *Map developed by HD Granda-Rodríguez.*

while the remaining 2% consists of other crops. In addition to agroforests, there is an area of regrowth of Teak (5%), pasture (34%), native forest (10%), and wetlands (1%) (Refocosta 2012). There are also small and scattered built-up zones within "La Gloria Project." Surveys for this study were carried out in areas with agroforest, native forests, wetlands, and urbanized sites.

Fieldwork: from March to October of 2012, we made 11 field trips, each one lasting ten days. We used Visual Encounter Surveys (VES) (Crump and Scott 1994). Daily, a single person did random walks for eight hours (09:00–12:00, 14:00–17:00, and 19:00–22:00 h), for a total sampling effort of 880 hours × observer. In addition, we captured cryptic species with terrestrial, semifosorial, and fossorial habits with pitfall traps (Vogt and Hine 1982), eight trap systems per habitat during each survey (56 in total). These traps system consist of two 3.78 liters buckets, and a two m interception net between them. Traps remained open for ten days.

We used a 10% chlorobutanol solution to euthanize all amphibians captured and intrathoracic lidocaine injections for euthanizing reptiles. No turtles or crocodilians were sacrificed for this study. All vouchered specimens were deposited in the Centro de Colecciones Biológicas de la Universidad del Magdalena (CBUMAG:REP and CBUMAG:ANF acronym). The scientific nomenclature used in this contribution is that accepted by Uetz et al. (2014) and Frost (2014).

Data analysis: Relative abundance was calculated as the number of individuals in each sample relative to capture effort, expressed in individuals/hours × observer (RA= Ind/h × obs.) (Lips 1999). Species were qualified according to their relative abundance in "very rare" (VR) if it was observed between 0.1–0.24 individuals per hour × observer; "rare" (R) if it was observed between 0.25–0.49; "common" (C) if it was observed between 0.50–0.74; "abundant" if it was observed between 0.75–0.99; and "very abundant" if it was observed between 1.0 or more (Rueda-Solano and Castellanos-Barliza 2010). Using PRIMER 6 (v 6.1.11) (Clarke and Gorley 2001) we calculated Margalef Richness Index (d), Pielou Uniformity Index (J'), Shannon-Wiener Diversity Index (H'), and Simpson Dominance Index (λ) for each vegetal coverage. We built a Bray-Curtis Similarity Matrix of non-transformed amphibian and reptile abundance data, to generate a nonparametric one-way similarity analysis (ANOSIM) (999 permutations), in order to refute a null hypothesis when there were no significant differences between diversity of amphibians and reptiles among sites. We made dendrograms with the same Bray-Curtis Matrix, to evaluate the similarity among vegetal coverages within the study area; likewise, the similarity between La Gloria project and other localities with published inventories of amphibians and reptiles in the Colombian Caribbean. It should be noted that if the similarity was greater

than 50%, it was considered a homogenous cluster. We used the software EstimateS (v 9.1.0) (Coldwell 2013) to create a species accumulation curve from non-parametric qualitative estimators Chao 2, Bootstraps, Jacknife 1, and Jacknife 2 (randomized 999 times for each case) to quantify the representativeness of the sample. We also calculated the unique and duplicates species.

Results and Discussion

Representativeness of survey: Bootstraps, Chao 2, Jacknife 1, and Jacknife 2 estimators show that amphibian survey had representativeness among 24.83% to 28.95%. The Chao 2 curve was only one who got stabilization. The unique and duplicated species were not reduced during the survey (Fig. 2a). Furthermore, the reptile surveys had more representativeness, since the estimators reached among 39.79% to 45.94%. The Chao 2 and Jacknife 2 curve obtained asymptote. In this case, unique and duplicates species neither decreased (Fig. 2b). Jacknife 1 and Jacknife 2 estimators have higher values, suggesting that surveys had a low representativeness in both taxa (Carvajal-Cogollo and Urbina-Cardona 2008). Bootstraps estimator obtained close values with observed species. Taking this as a reliable algorithm to estimate total richness, amphibian and reptile surveys reached a representativeness of 24.83% and 39.79% respectively. A comparison of survey methods used (observational surveys [VES] and trapping) results in a greater number of species and abundance being obtained through VES. (Fig. 3a, b). Using this technique, we detected 92.31%
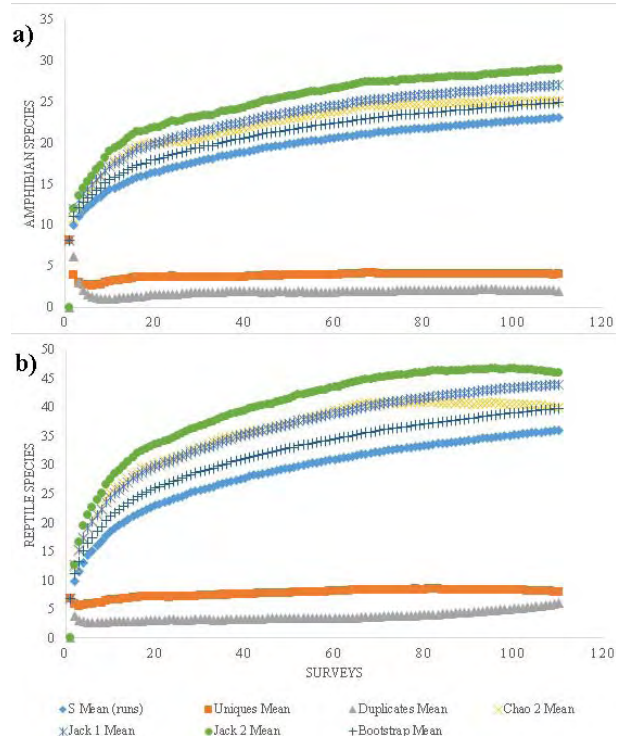
Fig. 2. Cumulative curve species of la Gloria project.

Table 1. Relative abundance = RA, VA = very abundant, A = abundant, C = common, R = rare, VR= very rare, NA = not available, and vegetation coverage, RG = Red Gum, PTT = Pink Trumpet Tree, BW= Beechwood, T = Teak, NF = native forest, WL = wetland, BZ = built-up zones. CBUMAG = Centro de Colecciones Biológicas de la Universidad del Magdalena (ANF = amphibian; REP = reptile).

TAXA	GT	PTT	BW	T	NF	WL	BZ	RA	Voucher
CLASS AMPHIBIA									CBUMAG:ANF
Order Anura									
Family Bufonidae									
Rhinella marina (Linnaeus 1758)	X		X	X	X	X	X	R	699
Rhinella humboldti (Gallardo 1965)	X		X		X	X	X	VR	701
Family Ceratophrydae									
Ceratophrys calcarata (Boulenger 1890)					X			VR	672
Family Hylidae									
Dendropsophus microcephalus (Cope 1886)					X	X		VA	713
Dendropsophus ebraccatus (Cope 1874)						X		VR	00666-67
Hypsiboas pugnax (Schmidt 1857)	X	X			X			VA	00697-8
Hypsiboas crepitans (Wied-Neuwied 1824)					X			VR	30
Scarthyla vigilans (Solano 1971)					X	X		VA	718
Scinax rostratus (Peters 1863)					X	X		VR	00031-32, 49
Scinax "x-signatus" (Spix 1824)	X	X		X	X	X		R	15
Trachycephalus typhonius (Linnaeus 1758)					X	X		VR	696
Phyllomedusa venusta Duellman and Trueb 1967					X			VR	676
Pseudis paradoxa (Linnaeus 1758)						X		VR	
Family Leptodactylidae									
Leptodactylus fuscus (Schneider 1799)	X		X	X	X	X		R	00703-4
Leptodactylus insularum Barbour 1906	X		X		X	X		R	00693, 695, 700
Leptodactylus poecilochilus (Cope 1862)					X	X		VR	348
Leptodactylus fragilis (Brocchi 1877)						X		VR	
Engystomops pustulosus (Cope 1864)	X	X		X	X	X		R	00708, 711, 716
Pleurodema brachyops (Cope 1869)		X		X	X	X		C	00702, 705
Pseudopaludicola pusilla (Ruthven 1916)	X			X	X	X		C	00709, 717
Family Microhylidae									
Elachistocleis panamensis (Dunn, Trapido, and Evans 1948)	X					X		VR	719
Elachistocleis pearsei (Ruthven 1914)	X	X		X	X			VR	00710, 720
Order Gymnophiona									
Family Caecilidae									
Caecilia subnigricans Dunn 1942							X	VR	634
CLASS REPTILIA									CBUMAG:REP
Order Squamata									
Family Sphaerodactylidae									
Gonatodes albogularis (Duméril and Bibron 1836)	X	X	X	X	X			VR	236
Lepidoblepharis sanctaemartae (Ruthven, 1916)					X	X		VR	
Family Gekkonidae									
Hemidactylus frenatus (Duméril and Bibron 1836)							X	VR	237
Family Phyllodactylidae									
Thecadactylus rapicauda (Houttuyn 1782)	X				X			VR	
Family Iguanidae									
Iguana iguana (Linnaeus 1758)					X			VR	
Family Dactyloidae									
Anolis auratus Daudin 1802					X			VR	231

Table 1 (Continued). Relative abundance = RA, VA = very abundant, A = abundant, C = common, R = rare, VR = very rare, NA = not available), and vegetation coverage, RG = Red Gum, PTT = Pink Trumpet Tree, BW = Beechwood, T = Teak, NF = native forest, WL = wetland, BZ = built-up zones. CBUMAG = Centro de Colecciones Biológicas de la Universidad del Magdalena.

TAXA	GT	PTT	BW	T	NF	WL	BZ	RA	Voucher
Family Corytophanidae									
Basiliscus basilicus (Linnaeus 1758)						X		VR	
Family Scincidae									
Maracaiba zuliae (Miralles, Rivas, Bonillo, Schargel, Barros, García-Pérez, and Barrio-Amorós 2009)		X	X	X	X			VR	235
Family Gymnophthalmidae									
Leposoma rugiceps (Cope 1869)		X		X	X			VR	239
Tretioscincus bifasciatus (Duméril 1851)		X	X	X	X			VR	00232-33
Family Teiidae									
Cnemidophorus gaigei Ruthven 1915				X	X			R	
Ameiva praesignis (Baird and Girard 1852)				X	X			R	
Ameiva bifrontata Cope 1862					X			R	
Family Anomalepididae									
Liotyphlops albirostris (Peters 1857)							X	VR	194
Family Boidae									
Boa constrictor Linnaeus 1758					X			VR	
Epicrates maurus Gray 1849					X			VR	234
Family Colubridae									
Chironius spixii (Hallowell 1845)				X	X			VR	120
Tantilla melanocephala (Linnaeus 1758)				X				VR	00208, 210
Leptophis ahaetulla (Linnaeus 1758)					X			VR	10
Family Dipsadidae									
Leptodeira annulata (Linnaeus 1758)				X	X			VR	34
Leptodeira septentrionalis (Kennicott 1859)		X		X	X			VR	
Lygophis lineatus (Linnaeus 1758)	X				X			VR	
Pseudoboa neuwiedii (Duméril, Bibron, and Duméril 1854)				X	X			VR	91
Imantodes cenchoa (Linnaeus 1758)				X	X			VR	16
Thamnodynastes gambotensis Pérez-Santos and Moreno 1989					X	X		NA	232
Thamnodynastes paraguanae Bailey and Thomas 2007					X	X		NA	38
Helicops danieli Amaral 1938						X		VR	128
Oxyrhopus petolarius (Linnaeus 1758)					X			VR	238
Xenodon rabdocephalus (Wied 1824)					X			VR	00170-71
Family Viperidae									
Crotalus durissus Linnaeus 1758					X			VR	
Porthidium lansbergii (Schlegel 1841)				X				VR	74
Bothrops asper (Garman 1883)				X	X			VR	165
Family Elapidae									
Micrurus dissoleucus (Cope 1860)				X				VR	
Order Testudines									
Family Chelidae									
Mesoclemmys dahli (Zangerl and Medem 1957)						X		VR	
Family Emydidae									
Trachemys callirostris (Gray 1855)						X		VR	
Family Testudinidae									
Chelonoidis carbonarius (Spix 1824)		X			X			VR	
Order Crocodylia									
Family Alligatoridae									
Caiman crocodilus (Linnaeus 1758)						X		A	

of amphibian and 68.48% of reptile individuals, respectively. With VES, we recorded 21 amphibian species and 35 reptile species. With this method we recorded 25 exclusive species (10 amphibians and 15 reptiles), that are strictly arboreal or aquatic. Conversely, we captured 7.69% and 31.52% of amphibian and reptile individuals respectively, using pitfall traps. This method recorded 12 amphibian species and 19 reptile species. We only found two fossorial species (*Elachistocleis pearsei* and *Micrurus dissoleucus*) with pitfall traps.

Amphibians: A total of 3,555 individuals, corresponding to two orders, six families, and 23 species (Table 1), were recorded. Anurans found represented five families and 22 species (37% of the total herpetofauna of the area) (Fig 4); a single caecilian specie was encountered (Fig. 4). Forty-two percent (41.8%) of lowland amphibian species occurring in the Colombian Caribbean were observed at La Gloria Project. The absence of expected species is due to a lack of specialized capture methods. For example, *Typhlonectes natans* is rarely observed due to its cryptic aquatic habits despite being distributed throughout the Caribbean region of the upper Magdalena-Cauca River (Tapley and Acosta-Galvis 2010). However, in this study we report the first record of the Clown Treefrog (*Dendropsophus ebraccatus*) in the lower Magdalena River, for which the nearest known distribution is in Rio Manso, Cordoba (Cochran and Goin 1970). In this contribution, we prefer to name *Scinax* "*x-signatus*" instead *Scinax* "*ruber*" (as was known previously Renjifo and Lundberg 1999; Cuentas et al. 2002). This is due to unresolved controversy regarding its taxonomy and biogeography (Barrio-Amorós 2004; Acosta-Galvis et al. 2006; Barrio-Amorós et al. 2011; Acosta-Galvis et al. 2012a). Following Rivero's (1969) criteria, the absence of dark dorsolateral lines and head equally long as wide place the collected specimens within the *x-signatus* and *ruber* groups.

Reptiles: We recorded 1,088 specimens corresponding to three orders, 19 families, and 37 species (Table 1). The most diverse order was Squamata with 15 families and 32 species, the suborder Lacertilia was the richest with nine families and 13 species, 20% of the total herpetofauna of the area. The suborder Serpentes represented six families and 20 species (34%). We observed three families and three species of turtles (5%) and recorded one crocodilian species (2%) (Fig. 3). La Gloria Project harbors 21.8% of lowlands reptile species of the Colombian Caribbean. We found three endemic species from Colombia, *Helicops danieli*, *Thamnodynastes gambotensis*, and *M. dahli*, the latter with restricted distribution in the Colombian Caribbean (Rossman 2002; Bailey and Thomas, 2007; Carvajal-Cogollo et al. 2012; Forero-Medina et al. 2013). The presence of *M. dahli* in the study area was unexpected, as species distribution models by Forero-Medina et al. (2012) propose a low probability
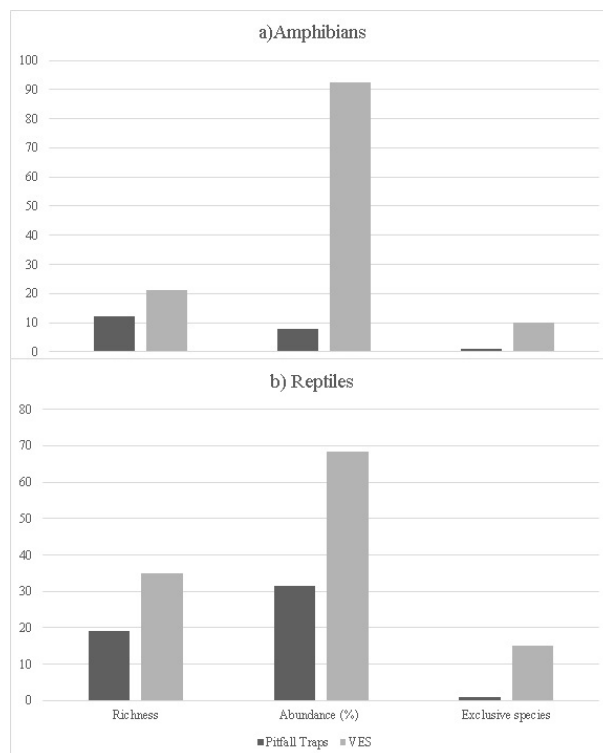
Fig. 3. Comparisons between the methods used for herpetofauna recording and capturing.
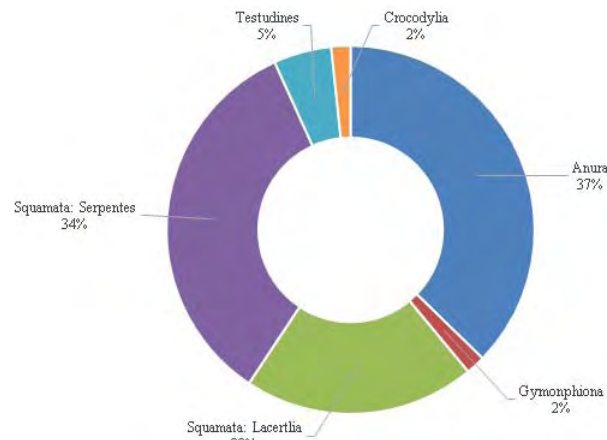
Fig. 4. Herpetofauna composition percentage in la Gloria Project.

of occurrence within this region. However, this area has many first-order streams with abundant riverine vegetation, throughout native forests and agroforests, habitat characteristics of this species (Forero-Medina et al. 2011; Montes-Correa et al. 2014).

In addition, we report the first record of *Maracaiba zuliae* in the lower Magdalena River, an expansion of its currently known distribution. This species was recently reported in Colombia in Reserva Forestal Protectora Montes de Oca, La Guajira state (Galvis et al. 2011). Several records by Ruthven (1922) in the Barbacoas River, the Arenas Stream, and Las Pavas must correspond with this recently described species. Likewise, we report the first record of *Thamnodynastes paraguanae* in the region. In Colombia, this snake is only known from La Gu-
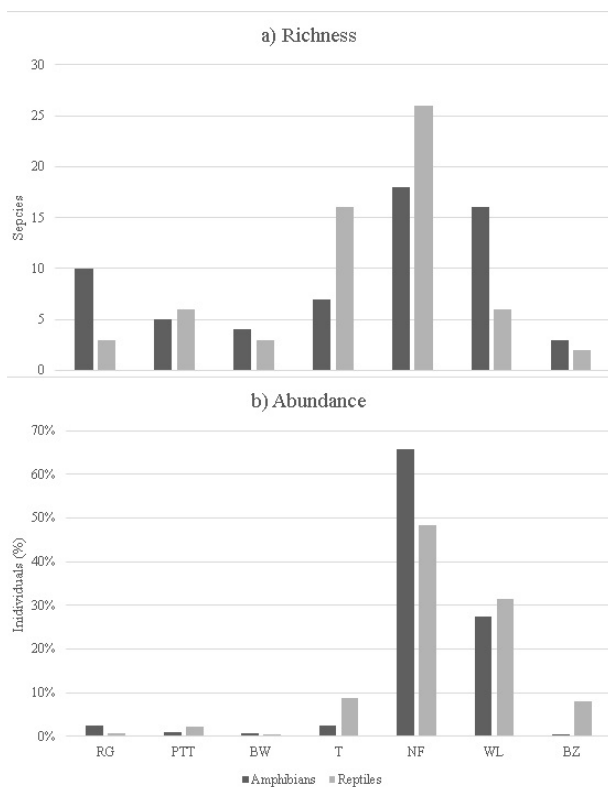
Fig. 5. Richness (a) and abundance (b) of amphibians and reptiles between habitats (RG = Red Gum, PTT = Pink Trumpet Tree, BW = Beechwood, T = Teak, NF = native forest, WL = wetland; BZ = built-up zones).

ajira: Uribia, Riohacha, and Reserva Forestal Protectora Montes de Oca (Bailey and Thomas 2007; Galvis et al. 2011). We must clarify that while the fieldwork was developed, snakes of *Thamnodynastes* genus were treated as one species, and they are not included in this analysis because their relative abundance is not available.

Richness and abundance patterns: in La Gloria Project, the native forest was the habitat that hosted the greatest number of species (Fig. 5a), 18 amphibians and 26 reptiles. The wetlands were the second habitat in amphibian composition, while the Teak agroforest was second in number of reptiles. Remaining habitats had less or equal to 10 species, both for amphibians and reptiles. We verified the greatest abundance in native forest (Fig. 5b), as 65.63% of amphibians and 48.35% of reptiles were detected in this habitat. All non-natural habitats scored an abundance below 10%. Some studies show that abundance patterns of natural and non-natural environments are similar (Gardner et al. 2007; Carvajal-Cogollo and Urbina-Cardona et al. 2008). Over time, composition and abundance tend to homogenize by dominance of the generalist species that displace more sensitive species for their lower habitat requirements and increased tolerance to disturbance (Offerman et al, 1995; Laurance et al. 2002). Surely, La Gloria Project does not present homogenization because agroforests are very recent. Regarding

the qualitative relative abundance in amphibians, we observed three very abundant species, two common, five rare, and 13 very rare. *Dendropsophus microcephalus*, *Scarthyla vigilans*, and *Hypsiboas pugnax* were the most abundant amphibians, while *Caecilia subnigricans* is represented by a single individual. Moreover, in reptiles we observed one abundant species, three rare, 31 very rare, and two not available. The most abundant species of reptiles were *Caiman crocodilus*, *Ameiva bifrontata*, and *Ameiva praesignis*. Furthermore, *Mesoclemmys dahli* and *Micrurus dissoleucus* were observed for a single individual. Similarly, other studies of tropical dry forest herpetofauna, found over half species had low relative abundance (Rueda-Solano and Castellanos-Barliza 2010; Pedroza-Banda and Angarita-Sierra 2011). In addition, snakes present a lower detection, possibly due to their cryptic habits or low abundance. *Leptodeira annulata* and *Leptodeira septentrionalis* were the most common snakes throughout the study area, supporting Scott and Seigel (1992) and Dodd (1993) hypotheses, where small sized snakes are more tolerant to disturbance, therefore, possibly more abundant. As to the community attributes (Table 2), native forest had the highest Margalef Richness and Shannon-Wiener Diversity for amphibians and reptiles and Beechwood agroforest had the greatest Pielou Uniformity Value. For these three attributes, built-up areas showed the lower values, however, this habitat had dominance for the highest values. In this study, the higher value of Margalef Richness, Shannon-Wiener Diversity, and Pielou Uniformity created higher values obtained for the coverage of floristic and structural complexity. A similar pattern was observed in Zapatosa region by Medina-Rangel (2011).

Habitat comparisons and herpetofaunal autoecology: ANOSIM determined there are global composition and abundance differences between seven evaluated habitats (p-value = 0.502). However, there are specific differences between Red Gum agroforest and Pink Trumpet Tree agroforest (*p*-value = 0.006), Red Gum and Beechwood (*p*-value = 0.038), Red Gum and Teak (*p*-value = 0.161), Pink Trumpet Tree and Beechwood (*p*-value = 0.068), Pink Trumpet Tree and Teak (*p*-value = 0.012), and Beechwood and Teak (*p*-value = 0.357). These similarities among agroforests are due to sharing among pioneer and generalist species that are able to tolerate conditions imposed by the new environment (Luja et al. 2008), e.g., Nest-building Frogs (*Leptodactylus*) (Heyer 1969). Some of these can be considered as common colonizers (see also, Dueñez-Gómez et al. 2004).

In La Gloria Project, the herpetofauna composition was quite heterogeneous, thus, all clusters were below 50% similarity (Fig 6). The more similar habitats were the Teak and Pink Trumpet Tree (48.5% similarity). Likewise, native forests and wetlands have a cluster (42.6%) and Beechwood and Red Gum agroforest an-
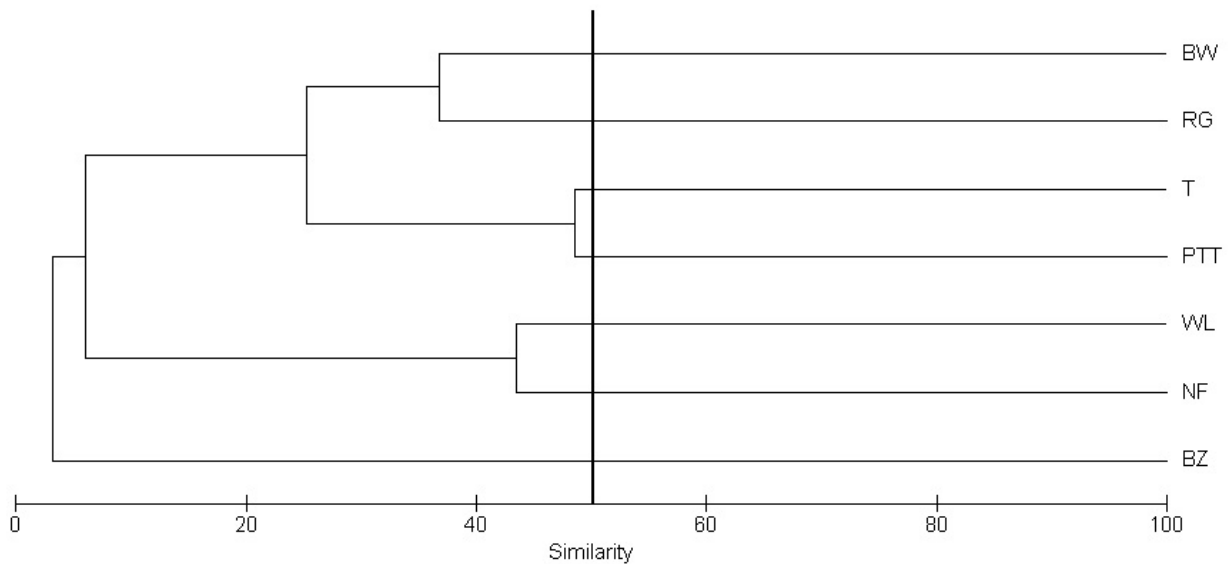
Fig. 6. Bray-Curtis similarity dendrogram between habitats in la Gloria Project (RG = Red Gum, PTT = Pink Trumpet Tree, BW = Beechwood, T = Teak, NF = native forest, WL = wetland; BZ = built-up zones).
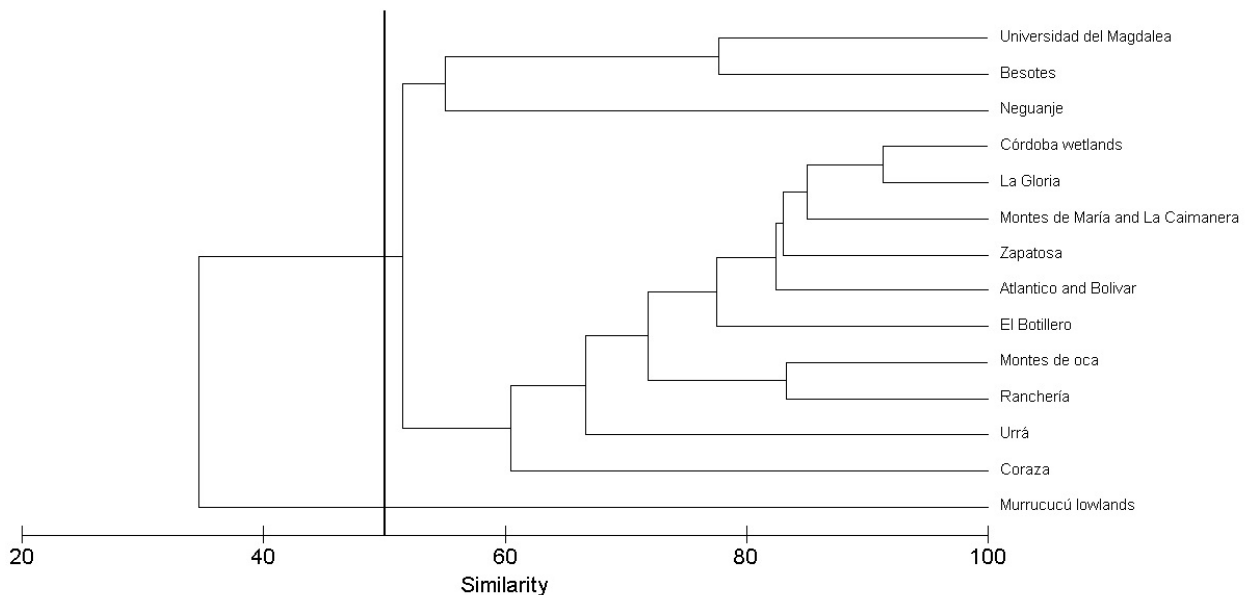
Fig. 7. Similarity of amphibian richness between La Gloria project and others inventories in Caribbean lowlands. Humedales del Córdoba (Romero-Martínez and Lynch 2010); Montes de María and Ciénaga la Caimanera (Acosta-Galvis 2012b); El Botillero (Dueñez-Gómez et al. 2004); Ciénaga del Zapatosa (Medina-Rangel et al. 2011); Atlántico and north Bolivar (Cuentas et al. 2002); Montes de Oca (Galvis et al. 2011); Ranchería (Blanco-Torres et al. 2013); Urrá (Renjifo and Lundberg 1999); Los Besotes (Rueda-Almonacid et al. 2011a); Serranía de Coraza (Galván-Guevara and de la Ossa-Velásquez 2009); Universidad del Magdalena (Montes-Correa et al. 2015); Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); Cerro de Murrucucú lowlands (Romero-Martínez et al. 2008).

other (36.8%). The more dissimilar habitat is the built-up zone with 3.1% similarity with respect to other habitats.

The species with greater frequency of occurrence was *Rhinella marina*, which was present in six of the seven evaluated habitats. This species has ecological plasticity and is able to tolerate highly degraded environments, including benefiting from human activities (Zug and Zug 1979). On the other hand, we found 28 exclusive species from a single cover. For example, *Pseudis paradoxa*, *Caiman crocodilus*, and *Trachemys callirostris* are strictly aquatic species and only found in wetlands. The exclusivity of *Hemidactylus frenatus* is due to its strong

synanthropy (Caicedo-Portilla and Dulcey-Cala 2011). *Phyllomedusa venusta* and *Trachycephalus typhonius* were exclusive of native forests, since these organisms have behavioral adaptations to tolerate prolonged drought in these habitats (Cuentas et al. 2002).

The richness and abundance of amphibians in La Gloria project was higher in native forests and their nearby wetlands. Moreover, in the Red Gum agroforest, amphibian richness and abundance was lower due to the sparse canopy of this tree which allows more sunlight to reach the forest floor, similar to what Gardner et al. (2007) reported for Brazil. In Indonesia, Wanger et al. (2009)
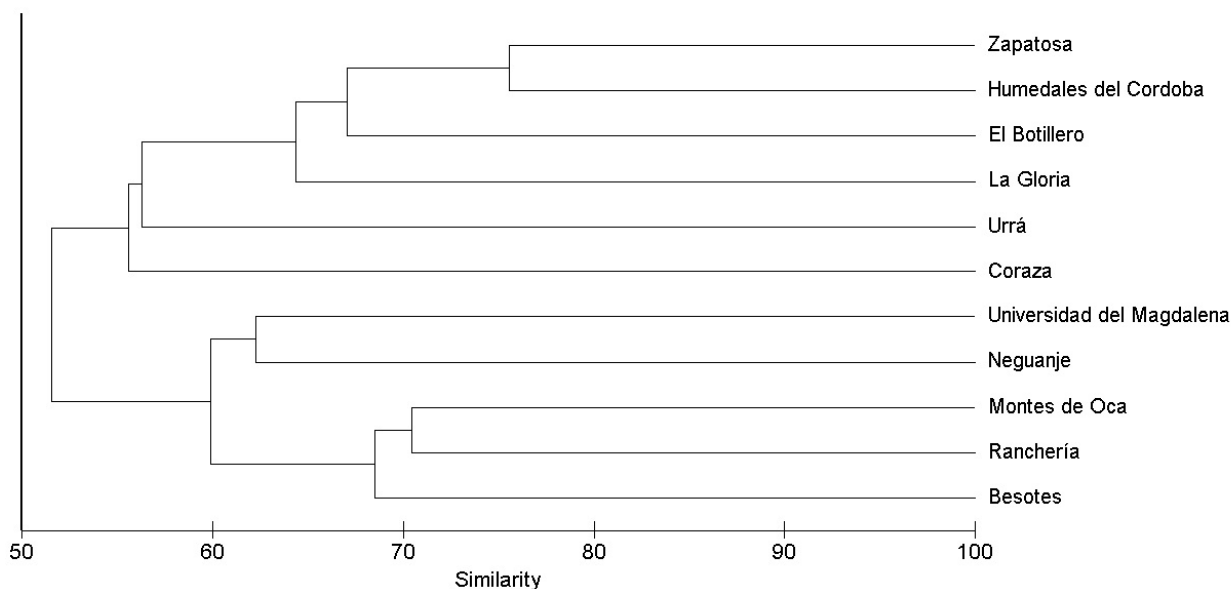
Fig. 8. Similarity of reptile richness between La Gloria project and others inventories in Caribbean lowlands. Humedales del Córdoba (Carvajal-Cogollo et al. 2); El Botillero (Dueñez-Gómez et al. 2004); Ciénaga del Zapatosa (Medina-Rangel et al. 2011); Montes de Oca (Galvis et al. 2011); Ranchería (Blanco-Torres et al. 2013); Urrá (Renjifo and Lundberg 1999); Los Besotes (Rueda-Almonacid et al. 2011b); Serranía de Coraza (Galván-Guevara and de la Ossa-Velásquez 2009); Universidad del Magdalena (Montes-Correa et al. 2015); Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010).

found that amphibians are more abundant in native rainforests than in Cacao Tree agroforest. In Gorgona Island, amphibians were more abundant in little disturbed rainforests than in palm cultivation (Urbina-Cardona and Londoño-Murcia 2003). On the other hand, in mountain rainforest, amphibian composition and abundance were higher in open areas that agroforest and native forests (Hoyos-Hoyos et al. 2012).

Canopy coverage may not be as important to some reptiles. Wanger et al. (2009) found that reptile richness and abundance was similar in Cacao Tree agroforest, native rainforests and open areas, and even these showed greater richness in open areas than in native rainforests. In Gorgona Island, reptile richness was higher in secondary forests; nevertheless, were more abundant in disturbed areas than in primary forests (Urbina-Cardona and Londoño 2003). In La Gloria project, the reptile richness was higher in native forests, although we recorded several species in agroforests, mainly in Teak; this because large leaves of this tree generate heavy shade and leaf-litter layers able to generate favorable microclimatic conditions for herpetofaunal establishment. In other agroforest reptile composition and abundance was low due to thin canopy cover and insufficient leaf-litter depth. In the case of Red Gum agroforest, the leaf-litter layer is very poor, as this tree is perennial. Changes of leaf-litter dynamics can alter amphibian and reptile assembly (Whitfield et al. 2014).

In La Gloria project, there are typical species of forest formations, but not necessarily exclusive of native forest. For example, *Lepidoblepharis sanctaemartae* occurred in native forest and Teak agroforest, being slightly more abundant in the native forest; *L. sanctaemartae*, as other small leaf-litter geckos, requires a leaf-litter layer containing humidity and little light penetration through the canopy, because of their passive thermoregulatory strategy (Vitt et al. 2005). Because of this aspect, *L. sanctaemartae* was not present in Red Gum agroforest. This species is a good model of Garden et al. (2007) hypotheses, since a dense canopy and a humid leaf-litter layer are more important for this species persistence than forest vegetation composition. Therefore, *L. sanctaemartae* is abundant both in preserved native forests as agroforest with sufficient coverage canopy and leaf-litter humidity (Montes-Correa pers. obs.).

The tortoise *Chelonoidis carbonarius* was present almost exclusively in native forest, where there is available fruit, which makes up much of their diet (Rueda-Almonacid et al. 2007). A single individual was recorded in Pink Trumpet Tree agroforest, feeding on flowers of this tree in breeding season, which are also an important part of their diet (Moskovits and Bjorndal 1990). We did not find this species in other agroforests since the timber cultivation does not offer alimentary resources. The slider turtle *Trachemys callirostris* was more abundant in wetlands with open areas on its banks, as these offered sites for nesting (Moll and Legler 1971).

The Spectacled caiman (*C. crocodilus*) was very abundant, being present in all wetlands in the zone. The low metabolic rate and generalist feeding habits allow them to maintain populations in areas with small and disperse wetlands (Castro-Herrera et al. 2013). Likewise, it is possible that the extermination of *Crocodylus acutus* in the lower Magdalena River has favored the increasing

Table 2. Attributes of amphibians and reptiles communities in the habitats of La Gloria project (d = Margalef richness, J' = Pielou Uniformity, H' = Shannon-Wiener Diversity, λ = Simpson Dominance).

		Red Gum	Pink Trumpet Tree	Beechwood	Teak	Native Forest	Wetlands	Built-up Zone
Amphibians	d	2.03	1.11	0.92	1.33	2.19	2.18	0.8
	J'	0.85	0.81	0.93	0.77	0.68	0.55	0.75
	H'	0.85	0.57	0.56	0.65	0.85	0.67	0.36
	λ	0.17	0.31	0.3	0.27	0.2	0.32	0.51
Reptiles	d	0.96	1.55	1.12	3.3	3.83	0.69	0.22
	J'	0.82	0.92	0.92	0.84	0.7	0.23	0.31
	H'	0.39	0.72	0.44	1.01	0.98	0.16	0.09
	λ	0.47	0.21	0.39	0.12	0.17	0.85	0.89

populations of *C. crocodilus*. A similar situation occurred in Venezuelan Llanos with *Crocodylus intermedius* extermination (Medem 1981).

Compared to other inventory studies in the Colombian Caribbean lowlands. La Gloria project presented similarity in richness of amphibians with other inventory studies in areas with abundant wetlands (Fig. 6); it showed the highest similarity with the Humedales del Córdoba (Romero and Lynch 2010) (85.7% similarity). Although they agreed in many lowlands species, forest formations are scarce in Córdoba Wetlands, thus, in La Gloria project forest species such as *Phyllomedusa venusta* were present, while in Córdoba Wetlands it was not reported. There is another great cluster with the localities of La Guajira. Studies made in Urrá (Renjifo and Lundberg, 1999), Coraza (Galván-Guevara and de la Ossa-Velásquez 2009), and Murrucucú (Romero et al. 2008) suggest the area of influence of the Sinú River has many common elements with the Cordillera Occidental, biogeographic Chocó, and Central America. (v. gr. *Colostethus pratti*, *Strabomantis bufoniformis*, *Bolitoglossa biseriata*, and *Oscaecilia polizona*). Clustering between Neguanje (Rueda-Solano and Castellanos-Barliza 2010), and Universidad del Magdalena (Montes-Correa et al. 2015) and Besotes (Rueda-Almonacid et al. 2008a) is due to the typical elements of tropical dry forest and the Sierra Nevada de Santa Marta (as *Colostethus ruthveni*, *Cryptobatrachus boulengeri*, and *Allobates* sp.).

In reptiles, La Gloria project is very similar to other areas of lowlands with wetlands, presenting the most similarity between Humedales del Córdoba (Carvajal-Cogollo et al. 2007) and Ciénaga del Zapatosa (Medina-Rangel et al. 2011) (69.8% similarity) (Fig. 7). This evident clustering of the lowlands is very similar to the localities in La Guajira but differs from typical elements from northeastern Caribbean, as *Gonatodes vittatus*, *Bachia talpa*, and *Thamnodynastes paraguanae*. The western regions are very dissimilar to La Gloria project by having typical elements of biogeographic Chocó, as *Chelydra acutirostris* and *Anolis vittigerus* (Medem 1977; Castro-Herrera and Vargas-Salinas 2008).

Conclusions

This study shows that communities of amphibians and reptiles are affected by structural changes in forests, since cultivated timber does not provide the necessary microhabitats to sustain many elements of herpetofauna species. The introduction of agroforests results in alterations of the spatial distribution of species, restricting them to small remnants of native forest.

A greater problem of studies of amphibians and reptiles in the Colombian Caribbean is that the predominant information is unpublished literature and the methodologies unclear (Blanco-Torres et al. 2013). This study contributes to the state of knowledge of amphibian and reptile richness in the lower Magdalena River, providing three new records for the region and establishes a list from a standardized inventory.

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Appendix I. Amphibian Caribbean lowlands inventories used for Bray-Curtis Similarity Analyses. A = La Gloria Project; B = El Botillero (Dueñez-Gómez et al. 2004); C = Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); D = Medio Ranchería (Blanco-Torres et al. 2013); E = Reserva Forestal Protectora Montes de Oca (Galvis et al. 2011); F = Serranía de Coraza (Galván-Guevara and De la Ossa-Velásquez 2011); G = los Montes de María y la Ciénaga La Caimanera (Acosta-Galvis 2012b); H = Represa de Urrá (Renjifo and Lundberg 1999); I = Murrucucú lowlands (sensu Romero-Martínez et al. 2008); J = Humedales del Córdoba (Romero-Martínez and Lynch 2010); K = Atlántico and North Bolívar (Cuentas et al. 2002); L = Santuario de Vida Silvestre Los Besotes (Rueda-Almonacid et al. 2008a); M = Ciénaga del Zapatosa (Medina-Rangel et al. 2011); N = Universidad del Magdalena (Montes-Correa et al. 2015).

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Rhinella humboldti	1	1	0	1	1	1	1	1	0	1	1	1	1	1
Rhinella margaritifera	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Rhinella marina	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Rhinella sternosignata	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Rhaebo haematiticus	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Hyalinobatrachium collymbiphyllum	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Hyalinobatrachium fleischmanni	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Ceratophrys calcarata	1	1	0	1	1	1	1	1	0	1	1	1	1	0
Craugastor raniformis	0	0	0	0	0	0	1	1	1	1	1	0	1	0
Pristimantis taeniatus	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pristimantis viejas	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Strabomantis bufoniformis	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Eleutherodactylus johnstonei	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Colostethus pratti	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Colostethus ruthveni	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Dendrobates truncatus	0	0	1	0	0	1	1	1	1	1	1	0	1	0
Cryptobatrachus boulengeri	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Dendropsophus ebraccatus	1	0	0	0	0	0	0	1	0	1	0	0	0	0
Dendropsophus microcephalus	1	1	0	1	1	1	1	1	0	1	1	0	1	0
Hypsiboas boans	0	0	0	0	0	1	0	1	1	0	1	0	1	0
Hypsiboas crepitans	1	1	1	0	1	0	1	0	0	1	1	1	1	0
Hypsiboas pugnax	1	0	1	1	1	1	1	1	1	1	1	1	1	1
Hypsiboas rosenbergi	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Phyllomedusa venusta	1	1	0	0	0	0	1	1	1	0	1	1	0	0
Pseudis paradoxa	1	1	0	0	0	0	1	1	0	1	1	0	0	0
Scarthyla vigilans	1	1	0	1	0	1	1	1	0	1	1	0	1	0
Scinax boulengeri	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Scinax elaeochrous	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Scinax rostratus	1	1	0	0	0	0	1	0	0	1	0	0	1	0
Scinax ruber	0	1	0	1	1	1	1	1	1	1	1	0	0	0
Scinax x-signatus	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Smilisca phaeota	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Smilisca sila	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Trachycephalus typhonius	1	0	0	1	1	1	1	1	0	1	1	0	1	0
Engystomops pustulosus	1	1	1	1	1	0	1	1	1	1	1	1	1	1
Pleurodema brachyops	1	1	1	1	1	0	1	1	0	1	1	1	1	1
Pseudopaludicola pusilla	1	1	0	1	1	0	1	1	0	1	1	0	1	0
Leptodactylus colombiensis	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Leptodactylus fragilis	1	1	1	1	0	0	1	1	0	1	1	0	1	0
Leptodactylus fuscus	1	1	0	1	1	1	1	1	0	1	1	1	1	1
Leptodactylus insularum	1	1	1	1	1	1	1	1	0	1	1	1	1	1
Leptodactylus poecilochilus	1	1	0	1	1	0	1	0	1	1	1	1	1	0

Appendix I (Continued). Amphibian Caribbean lowlands inventories used for Bray-Curtis Similarity Analyses. A = La Gloria Project; B = El Botillero (Dueñez-Gómez et al. 2004); C = Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); D = Medio Ranchería (Blanco-Torres et al. 2013); E = Reserva Forestal Protectora Montes de Oca (Galvis et al. 2011); F = Serranía de Coraza (Galván-Guevara and De la Ossa-Velásquez 2011); G = los Montes de María y la Ciénaga La Caimanera (Acosta-Galvis 2012b); H = Represa de Urrá (Renjifo and Lundberg 1999); I = Murrucucú lowlands (sensu Romero-Martínez et al. 2008); J = Humedales del Córdoba (Romero-Martínez and Lynch 2010); K = Atlántico and North Bolívar (Cuentas et al. 2002); L = Santuario de Vida Silvestre Los Besotes (Rueda-Almonacid et al. 2008a); M = Ciénaga del Zapatosa (Medina-Rangel et al. 2011); N = Universidad del Magdalena (Montes-Correa et al. 2015).

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Leptodactylus savagei	0	0	0	0	0	0	0	1	1	0	1	0	0	0
Lithodites lineatus	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Elachistocleis panamensis	1	0	1	1	1	0	0	1	0	1	1	0	1	0
Elachistocleis pearsei	1	0	0	0	0	1	1	1	0	1	1	0	1	0
Pipa parva	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Lithobates vaillanti	0	0	0	0	1	0	0	1	0	0	1	1	1	0
Caecilia isthmica	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Caecilia caribea	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Caecilia subnigricans	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Oscaecilia polyzona	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Typhlonectes natans	0	1	0	0	0	1	1	1	0	1	0	0	0	0
Bolitoglossa biseriata	0	0	0	0	0	1	0	1	1	0	0	0	0	0

Appendix II. Reptile Caribbean lowlands inventories used for Bray-Curtis Similarity Analyses. A = La Gloria Project; B = El Botillero (Dueñez-Gómez et al. 2004); C = Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); D = Medio Ranchería (Blanco-Torres et al. 2013); E = Reserva Forestal Protectora Montes de Oca (Galvis et al. 2011); F = Serranía de Coraza (Galván-Guevara and De la Ossa-Velásquez 2011); G = Represa de Urrá (Renjifo and Lundberg 1999); H = Humedales del Córdoba (Carvajal-Cogollo et al. 2007); I = Santuario de Vida Silvestre Los Besotes (Rueda-Almonacid et al. 2008b); J = Ciénaga del Zapatosa (Medina-Rangel et al. 2011); K = Universidad del Magdalena (Montes-Correa et al. 2015).

Species	A	B	C	D	E	F	G	H	I	J	K
Amphisbaena alba	0	0	0	0	1	0	0	0	1	0	0
Amphisbaena fuliginosa	0	0	0	0	1	0	0	0	1	0	0
Amphisbaena medemi	0	0	0	1	0	0	0	0	1	0	0
Gonatodes albogularis	1	1	1	1	1	1	1	1	1	1	1
Gonatodes vittatus	0	0	0	1	1	0	0	0	0	0	0
Lepidoblepharis sanctaemartae	1	0	1	1	1	0	0	1	1	1	1
Sphaerodactylus heliconiae	0	0	0	0	0	0	0	0	0	1	0
Phyllodactylus ventralis	0	0	1	1	1	0	0	0	2	0	1
Thecadactylus rapicauda	1	1	1	1	1	0	1	1	0	1	1
Hemidactylus brookii	0	0	1	1	1	1	1	1	1	0	1
Hemidactylus frenatus	1	0	0	0	1	0	0	0	0	1	1
Basiliscus basilicus	1	1	0	1	1	1	1	1	0	1	0
Basiliscus galeritus	0	0	0	0	0	0	0	1	0	0	0
Corytophanes cristatus	0	0	0	0	0	0	1	0	0	0	0
Anolis auratus	1	1	1	1	1	1	1	1	1	1	1
Anolis biporcatus	0	0	0	0	1	0	0	0	0	0	0
Anolis pentaprion	0	0	0	0	0	0	1	0	0	0	0
Anolis onca	0	0	0	0	1	0	0	0	0	0	0
Anolis tropidogaster	0	0	0	0	1	0	1	1	0	1	0
Anolis vittigerus	0	0	0	0	0	1	0	1	0	0	0
Iguana iguana	1	1	1	1	1	1	1	1	1	1	1
Polychrus marmoratus	0	0	1	1	1	0	0	0	1	1	0
Stenocercus erythrogaster	0	0	1	0	1	0	0	0	1	1	0
Maracaiba zuliae	1	0	0	0	1	0	0	0	0	0	0
Mabuya sp.	0	1	1	1	0	1	1	1	1	1	0
Bachia bicolor	0	0	1	0	0	0	0	0	0	1	1
Bachia talpa	0	0	0	1	1	0	0	0	1	0	0
Gymnophthalmus speciosus	0	0	0	1	1	0	0	1	1	1	1
Leposomoma rugiceps	1	1	1	0	0	0	1	1	0	1	0
Tretioscincus bifasciatus	1	1	1	1	1	0	0	1	1	1	1
Ameiva praesignis	1	1	1	1	1	1	1	1	1	1	1
Ameiva bifrontata	1	0	1	1	1	0	0	0	0	0	1
Cnemidophorus lemniscatus	1	1	1	1	1	1	1	1	1	1	1
Holcosus festivus	0	0	0	0	0	1	1	1	0	1	0
Tupinambis teguixin	0	1	0	1	1	1	1	1	1	1	0
Liotyphlops albirostris	1	0	1	1	1	1	1	1	0	0	1
Epictia goudotii	0	0	0	1	0	0	0	0	1	0	1
Trilepida macrolepis	0	0	0	0	0	0	1	0	0	0	0
Trilepida dugandi	0	0	0	0	0	0	0	0	1	0	0
Boa constrictor	1	1	1	1	1	1	1	1	1	1	1
Corallus batesi	0	0	0	0	0	0	1	1	0	0	0
Corallus ruschenbergerii	0	0	1	1	1	1	1	1	0	1	0
Epicrates maurus	1	1	0	1	1	1	1	1	0	1	0
Chironius carinatus	1	0	0	0	1	1	1	1	0	1	0

Appendix II (continued). Reptile Caribbean lowlands inventories used for Bray-Curtis Similarity Analyses. A = La Gloria Project; B = El Botillero (Dueñez-Gómez et al. 2004); C = Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); D = Medio Ranchería (Blanco-Torres et al. 2013); E = Reserva Forestal Protectora Montes de Oca (Galvis et al. 2011); F = Serranía de Coraza (Galván-Guevara and De la Ossa-Velásquez 2011); G = Represa de Urrá (Renjifo and Lundberg 1999); H = Humedales del Córdoba (Carvajal-Cogollo et al. 2007); I = Santuario de Vida Silvestre Los Besotes (Rueda-Almonacid et al. 2008b); J = Ciénaga del Zapatosa (Medina-Rangel et al. 2011); K = Universidad del Magdalena (Montes-Correa et al. 2015).

Species	A	B	C	D	E	F	G	H	I	J	K
Coluber mentovarius	0	0	0	0	1	0	0	0	0	0	0
Dendrophidion bivittatus	0	0	0	0	0	1	1	0	0	0	0
Dendrophidion percarinatus	0	0	0	0	0	0	1	0	0	0	0
Drymarchon caudomaculatus	0	0	0	1	0	0	0	0	0	0	0
Drymarchon melanurus	0	0	0	0	1	0	0	0	1	0	0
Leptophis ahaetulla	1	0	0	1	1	1	0	1	1	1	0
Mastigodryas boddaertii	0	0	0	1	1	0	1	0	1	0	1
Mastigodryas pleei	0	1	1	1	1	0	1	1	1	1	0
Oxybelis aeneus	0	1	1	1	1	1	1	0	0	1	1
Oxybelis fulgidus	0	0	1	0	1	0	0	0	1	0	0
Pliocercus euryzonus	0	0	0	0	0	0	1	0	0	0	0
Pseustes poecilonotus	0	0	0	0	0	0	1	0	0	0	0
Pseustes shropshieri	0	0	0	0	0	0	1	0	0	0	0
Spillotes pullatus	0	1	0	1	0	1	1	1	1	1	0
Stenorrhina degenhardtii	0	0	0	0	0	0	1	0	0	0	0
Tantilla melanocephala	1	0	0	0	1	0	0	1	1	0	1
Tantilla semicincta	0	0	0	1	1	0	0	0	1	0	1
Clelia Clelia	0	0	0	0	1	1	1	1	1	0	0
Enulius flavitorques	0	0	0	0	1	0	0	1	1	1	1
Erythrolamprus melanotus	0	0	0	1	1	0	1	1	1	0	0
Erythrolamprus bizona	0	0	0	0	0	0	0	0	1	0	0
Helicops danieli	1	1	1	0	0	1	1	1	0	1	0
Imantodes cenchoa	1	0	0	0	1	1	1	0	1	1	0
Leptodeira annulata	1	0	1	1	1	0	0	1	1	0	1
Leptodeira septentrionalis	1	1	0	0	0	1	0	0	0	1	1
Lygophis lineatus	1	0	0	0	1	1	1	1	1	1	0
Ninia atrata	0	0	0	0	0	0	1	0	0	0	0
Oxyrhopus petolarius	1	0	0	0	1	0	1	0	0	0	0
Phimophis guianensis	0	1	1	1	1	0	0	0	1	1	1
Pseudoboa neuwiedii	1	1	1	1	1	0	1	1	0	1	0
Sibon nebulatus	0	0	0	0	1	0	1	0	0	0	0
Thamnodynastes paraguanae	1	0	0	1	1	0	0	0	0	0	0
Thamnodynastes gambotensis	1	1	0	0	0	0	1	1	0	1	0
Xenodon severus	0	0	0	1	1	0	0	0	0	0	0
Xenodon rabdocephalus	1	0	0	0	1	0	0	0	0	0	0
Micrurus camilae	0	0	0	0	0	0	1	0	0	0	0
Micrurus dissoleucus	1	0	0	1	1	0	1	0	1	0	1
Micrurus dumerili	0	0	0	0	1	0	0	1	1	0	0
Bothriechis schlegelii	0	0	0	0	0	0	1	0	0	0	0
Bothrops asper	1	1	1	0	1	0	1	1	1	1	0
Crotalus durissus	1	0	1	1	1	0	0	0	1	1	1
Porthidium lansbergii	1	1	1	1	1	0	0	1	1	1	1
Porthidium nasutum	0	0	0	0	0	0	1	0	0	0	0
Mesoclemmys dahli	1	0	0	0	0	0	0	1	0	1	0

Appendix II (continued). Reptile Caribbean lowlands inventories used for Bray-Curtis Similarity Analyses. A = La Gloria Project; B = El Botillero (Dueñez-Gómez et al. 2004); C = Ensenada Neguanje (Rueda-Solano and Castellanos-Barliza 2010); D = Medio Ranchería (Blanco-Torres et al. 2013); E = Reserva Forestal Protectora Montes de Oca (Galvis et al. 2011); F = Serranía de Coraza (Galván-Guevara and De la Ossa-Velásquez 2011); G = Represa de Urrá (Renjifo and Lundberg 1999); H = Humedales del Córdoba (Carvajal-Cogollo et al. 2007); I = Santuario de Vida Silvestre Los Besotes (Rueda-Almonacid et al. 2008b); J = Ciénaga del Zapatosa (Medina-Rangel et al. 2011); K = Universidad del Magdalena (Montes-Correa et al. 2015).

Species	A	B	C	D	E	F	G	H	I	J	K
Podocnemis lewyana	0	0	0	0	0	0	1	0	0	1	1
Chelydra acutirostris	0	0	0	0	0	0	1	0	0	0	0
Cryptochelys leucostomum	0	0	0	0	1	0	1	0	0	0	0
Kinosternon scorpioides	0	0	0	0	1	0	1	1	1	1	1
Rhinoclemmys melanosterna	0	0	0	0	1	0	0	1	0	1	0
Trachemys callirostris	1	1	0	1	1	0	1	1	0	1	1
Chelonoidis carbonaria	1	1	0	1	1	0	1	1	1	1	1
Crocodylus acutus	0	0	0	0	1	0	1	0	0	1	0

A survey for the Chinese giant salamander (*Andrias davidianus*; Blanchard, 1871) in the Qinghai Province

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Abstract.—**The Chinese giant salamander (*Andrias davidianus*) was once common, but it has declined precipitously in the past several decades. An enigmatic specimen collected in 1966 represents the only historical record of the species from the Qinghai-Tibetan Plateau. From June–July 2012, we conducted opportunistic community inquiries and field surveys in Qinghai to attempt to locate *Andrias*. We received anecdotal evidence that additional *Andrias* have been found in recent years, but we failed to discover any *Andrias* during our field surveys. We suspect that relict populations persist in Qinghai, but the significant degradation of stream quality in the region likely threatens the long-term survival of any remaining *Andrias*. Here, we provide a brief overview of *Andrias* conservation, a summary of our surveys, and emphasize the importance of continued searches for this geographically disjunct population.**

Key words. Cryptobranchidae, Qinghai-Tibetan Plateau, conservation

Introduction

The Chinese Giant Salamander (*Andrias davidianus*) was once widely distributed throughout the Yangtze, Yellow, and Pearl River drainages. However, dramatic declines since 1950 have restricted the species to twelve fragmented regions across seventeen provinces (Zhang et al. 2002). These declines are due largely to habitat degradation and harvest for food (Dai et al. 2009). In response to these declines in the wild, the 2004 International Union for Conservation of Nature Red List evaluated *A. davidianus* as Critically Endangered, and the recognition of the conservation needs of the species has attracted national and international attention. Additionally, at least thirty preserves have been established in China to conserve *A. davidianus*, and captive breeding for human consumption has increased in prevalence and success (Dai et al. 2009; Zhang et al. 2002). One challenge for the conservation of *A. davidianus* is the preservation of genetic diversity, and several studies have examined variation between and among populations of *A. davidianus*. Significant substructuring exists among populations (Murphy et al. 2000; Tao et al. 2006), although results may be confounded by translocations of animals through the food trade. However, the overall genetic diversity of *A. davidianus* is relatively low compared to other salamanders (Tao et al. 2005; Yang et al. 2011).

A single specimen of *A. davidianus* was collected in the headwaters of the Yangtze River in the Qinghai Province in August 1966 (33.898 96.522; Fig. 1; Trap Location 9, Figure 2; Fig. 3). The specimen was a gravid female caught on hook-and-line near the town of Bagan at approximately 4,200 m, representing the highest known distribution record of *A. davidianus* by more than 2,000 m and a greatly disjunct population (Chen 1989). The geography and the geological history of this region (Yin 2010) suggest the possibility that the gap between this Qinghai record and other known localities for *A. davidianus* represents a true biogeographical break, and this population may be important for conservation purposes.

From 6 June to 2 July 2012, we used a variety of methods to survey Qinghai for *A. davidianus*. We were unsuccessful in locating any *Andrias*, but here we report the environmental conditions of the historic locality and others, anecdotal reports of *Andrias* from locals, and suggestions for future efforts to locate *Andrias* in Qinghai.

Methods

Throughout our stay in Qinghai, we frequently talked to officials from the Bureau of Forestry to obtain permission to search for *Andrias*. During this process, we also inquired about anecdotal *Andrias* sightings from fishermen. This amounted to discussions with approximately

fifteen government officials and five local fishermen. After talking with government officials and locals of Qinghai, we selected sites to survey based on historical and anecdotal records. We trapped in three general regions—Bagan, Zhiduo, and Yushu (Table 1; Fig. 2)—including the exact locality of the collection of the 1966 specimen from Bagan (Trap Location 9, Figure 2).

Browne et al. (2011) reviewed and evaluated survey techniques for cryptobranchid salamanders. Because of religious and cultural beliefs about the sanctity of fish, local people in Qinghai are overwhelmingly unsupportive of any attempts by biologists to survey aquatic organisms. Due to these limitations, some methods (e.g., electroshocking) were not possible, and our field surveys were conducted primarily through trapping, which has been shown to be reasonably effective for surveying for *Cryptobranchus a. alleganiensis* and *A. japonicus* (Foster et al. 2008; Briggler et al. 2013). Even so, we were restricted to trapping discretely, had several traps stolen, and were actively discouraged from actually entering the streams by both locals and governmental officials. These practical challenges significantly limited our trapping efforts. We primarily used two sizes of custom-made, mesh-net rectangular crab traps (approximately 81 × 61 × 28 cm; 61 × 46 × 20 cm) designed to catch *Andrias* of varying sizes. The traps were baited alternatingly with sardines, fishmeal, liver, and sponges soaked in fish oil

Fig. 1. The adult female *Andrias* captured in Qinghai, China in 1966. This specimen now resides at the Northwest Plateau Institute of Biology in Xining.

held in bait containers. Traps were weighted with stones, anchored to shore, and entirely submerged in 0.3 – >5 m of water in suitable habitat. Typically, the traps were placed in still pools along rocky bluffs at the edge of the river and checked after approximately 24 hours. Additionally, baited hook-and-line and manual searches of rocky habitat were used opportunistically when the habitat was suitable.

Results and Discussion

During our discussions with local people and government officials, we heard several anecdotal reports of *Andrias* being caught in recent years. Local Bureau of Forestry officials and one layman in Qumalai told of an adult *Andrias* that had been caught and thrown back by a fisherman at the same locality as the original record (Trap Locality 9, Figure 2) around 1992. The same officials in Qumalai and several officials in Zhiduo told of an *Andrias* that had been caught in the Nieqia River at its confluence with the Tongtian River in Qumalai (34.016, 95.817) between 1996–1997. This individual was reportedly sent to Xian and sold for food. An official from Zhiduo also reported that this fisherman's brother had caught an *Andrias* in a slow part of the Tongtian River between Zhiduo and Yushu earlier in 2012. Finally, two residents of Yushu reported seeing dead *Andrias* in the Tongtian River after the earthquake of 2010. Only one other species of caudate (*Batrachuperus tibetanus*) is

Table 1. Trapping effort in Qinghai. Numbers to right of the location indicate the corresponding points on Fig. 2.

Date Placed	Traps	Location
13 June	12	Four tributaries of De Qu River near Bagan (1–4)
13 June	4	De Qu River on the road to Bagan (5)
14 June	5	Bo Qu River near Bagan (6–8)
14 June	4	De Qu River at the bridge in Bagan (9)
15 June	12	Four tributaries of De Qu River near Bagan (1–4)
15 June	1	De Qu River on the road to Bagan (5)
16 June	12	Four tributaries of De Qu River near Bagan (1–4)
16 June	5	Upper De Qu River outside of Bagan
17 June	5	De Qu River at the bridge in Bagan (9)
19 June	4	Tribuatries of Hie Qu River near Zhiduo (10–11)
20 June	14	Tribuatries of Hie Qu River near Zhiduo (10–11)
21 June	14	Tribuatries of Hie Qu River near Zhiduo (10–11)
26 June	10	Tributaries of Tongtian River near Yushu (12)
28 June	14	Tributaries of Tongtian River near Yushu (12)
TOTAL	116	
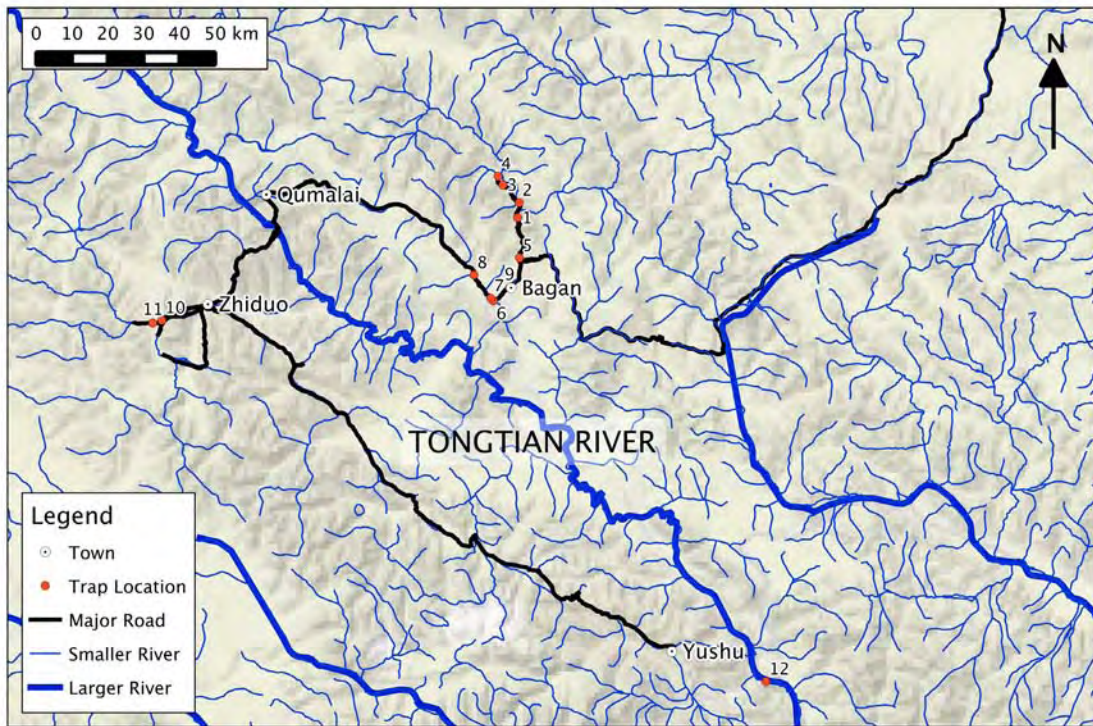
Fig. 2. Map of trapping localities and nearby towns in Qinghai.

present in the region, but its limited distribution in Qinghai and small size make it unlikely to have been misidentified as *Andrias* in locals' reports. Although there is no hard evidence to substantiate the reports we heard, when taken in aggregate, they seem credible.

We trapped for 106 trap-nights (Table 1) and were not able to discover any *Andrias* during our field survey of Qinghai. Foster et al. (2008) used a similar trapping protocol and caught *Cryptobranchus a. alleganiensis* at a rate of 0.01–0.10 captures/trap-night. Briggler et al. (2013) trapped for *C. a. alleganiensis* in deeper and more turbid water and reported an average capture rate of 0.042 captures/trap-night with net-mesh traps. We acknowledge that our limited number of trap-nights prevents us from making definitive conclusions about the presence or absence of *A. davidianus* at our trapping sites.

Virtually all of the streams in which we trapped were turbid and swollen with silted water (Fig. 3), which is a major threat to *Andrias* conservation. While it is possible that some of this turbidity was due to seasonal snowmelt, it is more likely that anthropogenic causes are primarily responsible. Since the collection of the lone specimen in 1966, mining for gold and other valuable commodities has become prevalent throughout the Qinghai-Tibetan Plateau. Furthermore, dozens of active sand and gravel mining operations were stationed throughout the rivers we sampled (Fig. 4). Locals in Yushu reported an increase in mining activity in response to construction and reparation needs following the major earthquake of 2010. Additionally, some streamside microhabitats for *Andrias* have been degraded due to road and bridge construction

(Fig. 5). Another contributor to the siltation of Qinghai streams may be grassland degradation and desertification driven by climate change that has been demonstrated in the region (Cui and Graf 2009).

Conclusion

Despite our inability to locate *Andrias* in Qinghai, anecdotal reports suggest that relict populations may still exist throughout the former range of the species. However, the apparent dramatic declines in stream quality in the region probably threaten the persistence of these populations. Although more remote regions further west of Bagan have fewer roads and present more practical challenges to fieldwork, they hold large headwaters of the Yangtze upstream of significant mining activity and may represent the most suitable remaining habitat. While we were not able to survey these regions during our expedition, they should be prioritized in future searches. Because Qinghai is at such a high elevation, suitable conditions for searching occur in a small window each year. We recommend that efforts be focused in August or September, after seasonal flooding from snowmelt has passed, but before winter has returned. In addition to the continued use of trapping, hook-and-line, and manual searches, we recommend the possibility of using environmental DNA, which has been demonstrated to be an effective tool for detecting populations of other cryptobranchids (e.g., Olson et al. 2012; S. Spear, pers. comm.).

Because of the potential importance of this geographically isolated population of *Andrias* in Qinghai, its redis-

Fig. 3. The locality where the first and only *Andrias* was collected from Bagan, Qinghai in 1966. Today, the water is turbid and appears largely unsuitable for *Andrias*.

Fig. 4. A mining operation on the banks of the Tongtian River, near Qumalai, Qinghai.

Fig. 5. Stream bank degradation caused by road construction along the Tongtian River.

covery should continue to be a top priority for *Andrias* conservation.

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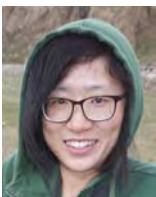
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REVIEW

The importance of enrichment for advancing amphibian welfare and conservation goals: A review of a neglected topic

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Abstract.—**Enrichment, broadly the provision of stimuli to improve the welfare of captive animals, is known to be important in husbandry practice and in the success of *ex situ* conservation and reintroduction programs. Practical evidence of the importance of enrichment exists for a number of taxa, yet amphibians are poorly represented. There is no reason to assume *a priori* that amphibians would not benefit from enrichment and, given their increasing prominence in captive programs, their requirements in captivity beyond basic husbandry should be the focus of more intense study. We review the existing body of research on enrichment for amphibians, as well as that for fish and reptiles, which may be regarded as behaviorally and neurologically broadly similar to amphibians. We also briefly discuss mechanisms by which enrichment might affect amphibian fitness and, therefore, reintroduction success. Our review supports the contention that there may be important consequences of enrichment for both captive welfare and *ex situ* conservation success in amphibians and that amphibian enrichment effects may be highly variable taxonomically. In the face of increasing numbers of captive amphibian species and the importance of *ex situ* populations in ensuring their species level persistence, enrichment for amphibians may be an increasingly important research area.**

Key words. Behavior, conservation, environmental enrichment, re-introduction, welfare, *ex situ*, fish, reptiles

Introduction

A wide range of amphibian species is currently maintained in captivity. Some species are used as models in laboratory research, including the ubiquitous *Xenopus laevis* and the dendrobatid frogs used to study skin peptides (reviewed by Daly 1998) and caecilians used in biomechanics research (e.g., Summers and O'Reilly 1997) and leaf frogs involved in conservation research (Ogilvy et al. 2012a, b). Several species are farmed (in addition to the many collected from the wild) for food or other products and others are maintained by private individuals as hobby or pet animals (Gascon et al. 2005). In addition, the *ex situ* conservation response to the on-going global amphibian extinction crisis (e.g., Gagliardo et al. 2008; Lee et al. 2006; Norris 2007) has drawn much publicity to the growing number of amphibians maintained for conservation breeding and education in zoos and similar institutions. This increase in captive amphibians (both

in actual numbers and species held) and their mounting conservation importance, has highlighted the need for a more thorough understanding of amphibian captive husbandry (Gascon et al. 2005), particularly for species that have no history in captivity and for those that are intended for release into the wild (Gagliardo et al. 2008; Gascon et al. 2005).

For many other taxa, the importance of enrichment has been identified for not only the welfare, or the physical and psychological wellbeing, of individual animals in captivity or those destined for release, but also for the overall/long-term success of reintroduction projects (Crane and Mathis 2010; Shepherdson et al. 1998; Young 2003). However, the implications of past work on the value of enrichment schemes for captive species currently has limited scope because enrichment has neither explicitly used nor well researched in amphibians (de Azevedo et al. 2007; Burghardt 2013). The objective of this paper is to draw attention to this lack of knowledge

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Table 1. Studies of enrichment in amphibians.

Species	Origin	Type of enrichment investigated	Findings	Notes	Reference
Xenopus laevis	Unknown	Shelter provision	No effect on growth rate. Frogs provided with shelter reluctant to leave it, even when provided with food.	Small sample size; unknown origins and genetics (see Chum et al. 2013)	Hilken et al. (1995)
Xenopus laevis	Laboratory bred	Shelter provision	Frogs use any shelter provided, but prefer plastic tubes to plants, rocks and wood. Frogs prefer tanks with shelter to tanks with no shelter. Frogs showed increased activity and reduced panic in tanks with shelter.	—	Brown and Nixon (2004)
Xenopus laevis	Laboratory bred	Shelter provision	Provision of plastic tubes reduced aggressive encounters, wounds and/or cannibalisation events.	—	Toreilles and Green (2007)
Xenopus laevis	Laboratory bred	Shelter provision	No effect on growth rates. Reluctant to leave shelter.	—	Gouchie et al. (2008)
Xenopus laevis	Laboratory bred	Shelter provision	No effect on growth rates or body condition (fat bodies). Higher propensity to clump together without shelter.	—	Archard (2012)
Xenopus laevis	Laboratory bred tadpoles	1. Surface area size 2. Water depth 3. Aquatic partitioning/ maze	1. Reduced surface area increased air-breathing behavior 2. Shallow water reduced growth rates and caused abnormal floating behavior (tadpoles could not surface to breath properly) 3. Tadpoles avoided narrower passages (2 cm) and preferred wider ones (4 cm)	Enrichments are not ecologically relevant to this species; this work may have limited implications for captive husbandry	Calich and Wassersug (2012)
Xenopus laevis	Laboratory bred females	1. Shelter provision 2. Conspecific provision (always with shelter)	1. Refuge provision reduced daytime activity and animals used shelter when provided 2. Addition of conspecific further reduced daytime activity in increased refuge use. No aggression observed and refuges were shared	—	Archard (2013)
Lithobates catesbeianus	Farmed/wild-caught	Environmental complexity (ramps, perches and caves)	Improved general welfare (general aspect and condition of animals)	High density laboratory condition	Bang and Mack (1998)
Lithobates catesbeianus	Farmed/wild-caught	Shelter provision	Reduction in mortality and improvement in condition	High density laboratory condition	Hedge and Saunders (2002)
Dendrobates tinctorius *D. azureus* *D. auratus* *D. leucomelas* Mainly reported as aggregate data across species	Zoo bred	1. Feeding enrichment (control vs. insect dispenser vs. broadcast feed/aphid stem) 2. Enclosure switch	1. Some effects on behavior (mainly activity) 2. Effect on activity levels (enclosure switch lead to higher activity levels)	Very small sample sizes. Issues with experimental design, including few replicates and unexplained measures	Hurme et al. (2003)
Oophaga pumilio	Zoo bred	Feeding enrichment (feeding dish control vs. feeding dish with leaf cover to allow insects to disperse)	Increased foraging duration, increased duration between prey capture events and reduced rapid feeding	—	Campbell-Palmer et al. (2006)

Table 1. Studies of enrichment in amphibians (continued).

Species	Origin	Type of enrichment investigated	Findings	Notes	Reference
Mannophryne trinitatis	Wild collected as tadpoles	1. Shelter provision 2. Substrate type	1. Strong, positive effect on growth rates. No effect on behavior (weak effect on time spent jumping) 2. Preferred shallow water	Substrate preference predicted by habitat	Walsh and Downie (2005)
Physalaemus pustulosus	Wild collected as spawn		1. No/weak effect on growth or behavior 2. Preferred dig-able (sand or gravel) substrate		
Leptodactylus fuscus					
Agalychnis callidryas	Laboratory bred juveniles and adults	Shelter provision	Frogs prefer planted to non-planted enclosures. This preference increases when animals are deprived of plants before choice test. Froglets reared with plants grow faster and are in better condition than those reared without. Frogs reared with plants have more diverse and more abundant cutaneous bacterial communities.	—	Michaels et al. (2014b)
Cryptobranchus alleganiensis	Wild collected as eggs (head-starting program)	Pre-release anti-predator training	Hellbenders were able to learn to exhibit a fright response to trout scent after classical conditioning; control animals showed no such improvement.	—	Crane and Mathis (2010)

and to call for more research in order to better understand the importance of enrichment for this taxon. We will explore the meaning of enrichment for amphibians, review the body of existing research (Table 1), and discuss the neglect of this field as well as how and why enrichment may be important as a focus for both amphibian conservation and welfare research activity. Finally, we will suggest a potential structure and goals for future research in this area (Table 2).

Concepts of enrichment

Enrichment for captive animals has been defined in various ways, but in general, is any intervention designed to improve animal welfare beyond the basic requirements for survival, usually taking the form of modifications to enclosures or husbandry protocols. Well known examples include the provision of bamboo stems filled with grubs for captive Aye-aye (*Daubentonia madagascariensis*) (Quinn and Wilson 2004), running wheels for captive rodents (Hutchinson et al. 2005) and the spraying of unfamiliar scents on parts of the enclosures for big cats; e.g., Szokalski et al. 2012 in tigers (*Panthera tigris*).

Enrichment is often sub-divided into environmental, behavioral, and social categories. Shepherdson (1998) defined environmental enrichment as any intervention that provides "the environmental stimuli necessary for optimal psychological and physiological well-being." This is distinct from behavioral enrichment, which is designed to elicit or allow the expression of specific behav-

iors or behavioral repertoires (Shepherdson 1994). Social enrichment, the provision of access to other individuals (usually, but not always, conspecifics) to cater for social interaction needs (including both environmental and behavioral components), has also been identified as important for a number of taxa (Berejikian et al. 2001; Lantermann 1993; Miranda de la Lama and Mattiello 2010; Polverino et al. 2012; Saxby et al. 2010; Sloman et al. 2011; reviewed by Hayes et al. 1998 and Young 2003; see below).

Enrichment can influence behavioral repertoires and stress levels beyond addressing stereotypical behavior and physical health problems (reviewed by Young 2003) and can affect physical brain structure in species as diverse as mice (*Mus musculus*) and crickets (*Acheta domestica*) (Lomassese et al. 2000; van Praag et al. 2000). These findings have led to a current view of enrichment, which recognizes the importance of all three categories for the psychological as well as the physical welfare of captive animals (Dawkins 2006; Young 2003).

The three forms of enrichment can be used to improve conservation success by training animals with the aim of improving survivorship upon release; e.g., anti-predator training in the black footed ferret (*Mustela nigripes*; Dobson and Lyles 2000). Although some forms of training may be beneficial, the use of enrichment may result in conflict between maximizing individual welfare in captivity and equipping animals destined for release with the most appropriate survival skills (Caro and Sherman 2013; Harrington et al. 2013), and both objectives should

Table 2. Key areas of species biology knowledge required for effective enrichment research, potential tools for assessing enrichment needs and effects and areas of amphibian captive husbandry for which enrichment may be important.

Key areas of amphibian biology, to be integrated into enrichment research			Potential measures of welfare and fitness		Potential areas of captive husbandry for enrichment research focus

Cognition	Learned and hard-wired behavioral components	Inter- and Intra- individual, phenotypic and genetic variation	Catalogue existing issues in captive amphibians and their husbandry	Comparisons against wild populations, where appropriate	Enclosure design
					• Size
					• Complexity
					  - Permanent (furniture and decor)
					  - Temporal (novel objects, timed misting)
					• Refuges
					• Lighting
					  - Wavelength
					  - Photoperiod
					  - Intensity

	Perception of environment		Behavior and behavioral assays		
Behavior	Natural behavioral repertoires and activity levels of species		Foraging success		Environmental parameters • Gradients • Fluctuation (seasonal and diel)
	Foraging strategies and dietary composition		Growth and development		
	Reproductive behavior • Breeding strategies • Mate choice • Competition for mates/breeding sites		Body condition		Threat stimuli • Predation • Competition • Environmental stressors (e.g., drying ponds)
	Migration and home ranges		Hormones • Stress • Reproductive		Encouraging specific behavioral responses
	Antipredator behavior		Micro- and macro-biotas associated with animals • Beneficial communities (mainly skin and gut) • Parasite and pathogen loads		Nutrition and food presentation • Nutritional content • Temporal variation • Variation in food types (different species of prey animal or algae) • Total abundance
	"Personality" vs behavioral plasticity		Pathologies • Behavioral • Physical (disease, malformation and pathogen susceptibility)		
Interactions	Intra- and inter-specific		Reproductive success		Social enrichment • Presence of conspecifics and non-conspecifics • Stability of social groups • Territory creation and maintenance • Mate choice • Human habituation
	As predators, prey and competitors				
Genetics and evolution	Heritability of traits		Survivorship		
	Potential for selection				

be considered for conservation breeding populations. The ferrets trained for release, for example, although not physically harmed, would have been psychologically distressed by being pursued by muzzled dogs as is a prerequisite of successful aversive training. This topic will continue to be controversial, as it is impossible to objectively resolve the relative importance of individual welfare and the persistence of a species as a whole, or whether the compromise of one is worth the assurance of the other. However, it is important to consider the individual welfare gains of such training post release. Pre-release anti-predator training may compromise welfare of animals in captivity, but may result in a larger welfare gain, when animals avoid predators in the wild.

Burghardt (1996) suggested that the term "controlled deprivation" might be more appropriate than "enrichment." This term acknowledges that it is impossible to provide in captivity the level of stimulation gained by animals in the wild, but rather management strategies should seek to strategically provide stimulation in such a way as to control the effects of general deprivation. The term "enrichment" may suggest a positive increase in

stimulation due to management strategies, when in fact it is not. While "controlled deprivation" is perhaps more honest, the vast majority of work continues to use the term "enrichment." We will therefore continue to do so, but with the caveat that such strategies enrich the life of captive animals compared with captive life devoid of any stimulation, rather than compared with what they might receive in the wild.

The conceptual framework of enrichment has largely focused on birds and mammals, and it may be problematic to apply it consistently when assessing enrichment for amphibians, particularly because the distinction between environmental and behavioral enrichment is blurred. Amphibian behaviors are often linked to specific physiological functions, such as basking, hunting or burrowing, or to reproduction, so we will not differentiate between these two enrichment types. Additionally, the highly specific environmental requirements of captive amphibians mean that many aspects of amphibian husbandry, such as UVB provision (Antwis and Browne 2009) and nutrition (e.g., Antwis et al. 2014; Li et al. 2009; Ogilvy et al. 2012a, b), impact both basic requirements and enrichment as described by Shepherdson (1998). The relative lack of empirical work in this field further hinders differentiation between different enrichment categories. We opt to exclude aspects of husbandry that offer benefits only to "physiological well-being," in order to allow a focus on true enrichment that transcends basic husbandry. Within this category, there is a distinction between enrichment solutions that simply provide animals with things that they have evolved to psychologically rely upon and those that offer specific learning opportunities. The provision of shelter may fall into the former category, for example, while training amphibians to avoid predators may be included in the latter. Both may be important to consider, although learning-oriented enrichment may be of greater significance to animals intended for release.

The neglect of amphibian enrichment research

Within the conservation and animal welfare literature there is a lack of research on amphibians and reptiles compared with the other tetrapod vertebrates (de Azavedo et al. 2007; Bonnet et al. 2002; Griffiths and Pavajeau 2008; Griffiths and Dos Santos 2012) and the body of published work in the area of enrichment for amphibians is limited (Table 1).

Amphibians, like all ectotherms, have historically been perceived as animals that cannot suffer, or do not feel pain, at least to the same degree as mammals and birds (Gross 2003). This bias has meant that the use of anaesthetics and analgesics during amphibian veterinary care and surgical procedures in the laboratory and field is relatively recent (Machin 1999). Although arguments have been made to suggest that amphibians (and fish) do not exhibit consciousness or emotion, while the amniotes do to varying degrees (reviewed by Cabanac, et al.

2009), this is by no means conclusive. The identification of pain pathways shared between amphibians and other amniotes (Stevens 2004) suggests an ability to experience pain, even if in a different and more restricted sense than in amniote taxa. This argument notwithstanding, the capacity to suffer in the presence of pain does not influence the importance of enrichment for conservation purposes.

Additionally, amphibian behavioral motivations, the reasons animals exhibit a particular behavior, are more difficult for humans to intuit than those of mammals and, to a lesser extent, birds, both of which may engage in behaviors more easily recognized by humans. Along with a lack of available, amphibian-specific measures of welfare, the difficulty in instinctively understanding amphibian behavioral motivations may have reduced interest in enrichment for this group as there may be fewer easily noticed welfare problems. Furthermore, the reliance of many amphibian species on highly specific environmental conditions often necessitates more complex and often "naturalistic" environments than would be required to maintain and breed mammals or birds, or even many reptiles. Consequently obvious symptoms of extreme deprivation may be less apparent, unlike in other taxa that may survive and reproduce in confined and bare enclosures, the more complex environmental requirements of some amphibians may be more difficult to disentangle from their basic husbandry. The rapidity with which many amphibians physiologically succumb to poor environmental conditions (Wright and Whitaker 2001) may not allow the development of any potential behavioral abnormalities before an animal dies. Moreover, the reduced activity in many contexts and lower metabolic capacity of many amphibians may reduce or mask the appearance of active behavioral stereotypes in some taxa. Additionally, increased stress hormone levels have been associated with a downregulation of behaviors, including reproduction (Moore and Miller 1984; Moore and Zoeller 1985; Chrousos 1997; Moore and Jessop 2003) and foraging (Crespi and Denver 2005; Carr et al. 2002), in some amphibians and so the effects of poor enrichment may, in some cases, manifest as absences of normal behavior instead deviant or new behaviors.

The relatively innate, "hard-wired" behavior of amphibians is often used to support the idea that enrichment, and consequently research investigating it, is not an important consideration, particularly in *ex situ* conservation (Bloxam and Tonge 1995; Griffiths and Pavajeau 2008). Some forms of enrichment involve learning (e.g., antipredator behavior learning; Dobson and Lyles 2000), whereas others may simply allow the manifestation of behaviors without a learning component. Although amphibians may not rely on captive conditions to develop normal behavioral repertoires as mammals or birds, their behaviors can be complex (reviewed by Burghardt 2013) and the role of learning is more important (reviewed by Bee et al. 2012; Wells 2007) than was previously thought.

Research on enrichment in amphibians, reptiles and fish

Measuring the impact of enrichment on amphibians

Objective measures of amphibian welfare have not been well developed or validated, beyond major issues such as cannibalism and bite trauma (Toreilles and Green 2007). Stereotypical behaviors in amphibians are poorly defined or understood (there is no mention of behavioral problems in Wright and Whitaker's (2001) otherwise comprehensive amphibian medicine and captive husbandry volume), and are usually only recognized in the form of gross trauma. It is likely that abnormal and stereotypical behaviors frequently used to assess welfare in mammals and birds may not be applicable to amphibians. Moreover, a number of commonly used measures are subject to *a priori* assumptions about their interpretation and, although they may seem reasonable, good rationales for the use and interpretation of characters as measures of welfare are rarely given. Activity levels have been used (Archard 2013; Campbell-Palmer et al. 2006; Hurme et al. 2003), but the conclusion that particular effects (e.g., increased foraging time or reduced daytime activity) translate to improved welfare remain largely untested assumptions. Similarly, authors generally interpret faster growth rates and larger fat bodies as indicators of better welfare, as well as being indicative of the production of more robust individuals. Dawkins' (1983; 1990) "consumer demand" methodology to assess animal needs has not been applied to amphibians, although choice chambers have been used to assess preferences (Michaels et al. 2014b; Walsh and Downie 2005). In reptiles, trade-offs between palatable food and cold temperatures have been used to assess the "consumer value" of a food reward to green iguana (*Iguana iguana*; Balasko and Cabanc 1998) and this methodology could be applied to amphibians.

Corticosteroid or "stress" hormone levels have been used to assess welfare in amphibians (Coddington and Cree 1995; Narayan et al. 2010, 2011a, b; Narayan and Hero 2011; Paolucci et al. 1990; Zerani et al. 1991), but beyond easily interpreted contexts such as capture, transport, and toe clipping, they can be problematic. In particular, a lack of baseline data across different contexts for most species makes interpretation, in terms of welfare, of isolated samples difficult. "Stress" is best viewed in its evolutionary, physiological, genetic, ecological, and behavioral contexts (Boonstra 2013) and increased levels are associated with and necessary for normal behaviors including reproduction (Moore and Jessop 2003; Narayan et al. 2010), immune responses (Rollins-Smith 2001), and adaptive plasticity (Denver 1997). "Stress" and "distress" are very different states, with only the latter having negative impacts on animal fitness and welfare, and these must be considered separately (Linklater and Gedir 2011). However, non-endocrine, unambiguous measures of welfare must be developed in order to properly distinguish between stress, which may be normally physiologically elevated in certain contexts, and distress in amphibians. Measurements of suites, instead of isolated, characters (e.g., Michaels et al. 2014b) will help to build a more easily interpreted picture of the effects of enrichment. Assessment of symbiotic or mutualistic bacterial communities on the physiologically active skin of amphibians may provide a new measure of welfare. These communities are sensitive to facets of enclosure design that can also be shown to impact other "traditional" measures of welfare and fitness including growth rates, body condition, behavior, and reproductive output (Antwis et al. 2014; Michaels et al. 2014b) as well as corticosteroid levels following challenges (R. Antwis, unpublished data). Although these communities do not allow distinction between stress and distress, they provide an additional line of enquiry in this area. Given the important impact of microbial communities on disease resistance (Bletz et al. 2013), this field can provide strong links between enrichment and the likelihood of reintroduction success.

Importantly, any evidence must be interpreted in the context of the focal species (Michaels et al. 2014a). Increased activity levels, for example, are more likely to be beneficial in actively hunting species than in ambush predators that do not typically engage in extended locomotion. Comparison between wild and captive conspecifics may provide guide "targets" for developmental and physiological measures, such as body condition, as well as a means to establish natural behavioral repertoires.

Existing enrichment research in amphibians

We identified 14 primary research articles on amphibian enrichment, summarized in Table 1, all but one (Crane and Mathis' (2010) hellbender training study; see below) of which were concerned primarily with improving individual welfare of captive animals, as opposed to improving breeding or release success. In some cases, the impact of enrichment has not been investigated beyond a subjective assessment of "appreciation" by people and practicality (e.g., Hanley 1993; Kirkland and Poole 2002) and such work has not been included in this count. Burghardt (2013) reviewed evidence for the effects of enrichment in both reptiles and amphibians, but did not include some of the studies discussed here. Furthermore, the focus of his review was on cognition and its implications for the understanding of enrichment for reptiles and amphibians, as well as a consideration of evidence for consciousness, play, and emotion in these groups. There was no discussion of pre-release training or the role of enrichment in conservation for amphibians.

Shelter provision is the most investigated form of enrichment for amphibians, including the common model organism *Xenopus laevis* (reviewed by Chum et al. 2013; Tinsley 2010; see Table 1), and in five other species (*Physalaemus pustulosus*, *Leptodactylus fuscus*, *Man-*

nophryne trinitatis, *Agalychnis callidryas*, and *Lithobates catesbeianus*; Table 1). Although shelter provision undoubtedly has physiological benefits for amphibians (Michaels et al. 2014b; Walsh and Downie 2005), behavioral tests (see Table 1) have suggested a psychological element to the effects of shelter provision, implying that it falls within our definition of enrichment for amphibians. However, more comprehensive investigations of this are warranted.

The conclusions of this literature are somewhat mixed, particularly for *Xenopus* but in general support the importance of shelter provision for frogs studied (Archard 2013; Chum et al. 2013; Bang and Mack 1998; Hedge and Saunders 2002; Michaels et al. 2014b; Tinsley 2010; Walsh and Downie 2005; Table 1). In non-*Xenopus* species, multiple measures of welfare and fitness all show improvements in the presence of enrichment. In *Xenopus*, changes in behavior do not seem to be reflected in growth rates or body condition, nor are these negatively affected by enrichment. These differences between taxa in response to the same type of enrichment (shelter provision) are indicative of the limited degree to which findings from one species can be applied to others, and the need for the development of species-specific measures of welfare. They also highlight the importance of measuring a number of variables in response to enrichment.

Two studies investigate enrichment through environmental complexity beyond shelter provision. Bang and Mack (1998) showed that increased general environmental complexity in the form of ramps, perches, and caves positively affected the welfare of captive bullfrogs (*Lithobates catesbeianus*; Table 1), although it is unclear if this extended beyond the effects of shelter alone (Hedge and Saunders 2002). Calich and Wassersug (2012) found impacts of water depth, surface-area size and aquatic partitioning on the behavior of *X. laevis* tadpoles, but the enclosure modifications were not ecologically relevant to this open-water species (Tinsley and Kobel 1996) and the findings are perhaps of limited use in developing husbandry protocols.

Food-delivery enrichment affects behavior and activity levels in dendrobatid frogs (Campbell-Palmer et al. 2006; Hurme et al. 2003), whereas introduction of frogs to novel environments also increased activity levels (Hurme et al. 2003). Archard (2013) investigated the effect of social enrichment, through the provision of conspecifics, in an enclosure containing a refuge, as well as the effect of shelter *per se* (see above). The author found that *X. laevis* exhibited reduced daytime activity, beyond the reduction seen when refugia are provided, when conspecifics are present in tanks with shelter. This result was interpreted as an improvement in welfare, but such and interpretation may be viewed as ambiguous, particularly in a species known to show a degree of territoriality in the wild (Tinsley and Kobel 1996).

One study has investigated the use of enrichment to train hellbenders (*Cryptobranchus alleganiensis*) for release into the wild. Crane and Mathis (2010) used a combination of trout-scented water and conspecific distress secretions to train hellbender larvae in head-starting programs to avoid predation by predatory trout. This pre-release training may be classed as a form of enrichment for these salamanders, encouraging them to express normal anti-predator behavior, but manipulating this to improve future survival in the face of invasive alien predators. Several classes of amphibian behavior have now been shown to include learned components, including predator avoidance (Crane and Mathis 2010 in *Cryptobranchus alleganiensis*; Epp and Gabor 2008 in *Eurycea nana*; Ferrari and Chivers 2008 in several species of anuran larvae), territoriality (Dawson and Ryan 2009; 2012 in *Physalaemus pustulosus*), foraging (Sontag et al. 2006 in anuran larvae) and other aspects of social behavior (Bee et al. 2012; Wells 2007). Moreover, complexity and cognition, whereby behavioral processes exceed simple responses to stimuli, have been detected in a range of amphibian behaviors, including spatial learning and homing (Brattstrom, 1990; Shoop 1965) and individual recognition (Gauthier and Miaud 2003). Amphibians are also capable of visual discrimination learning, identifying objects based on visual characteristics, (Jenkin and Laberge 2010) and even rudimentary quantity learning, showing the capacity to compare quantities, (Krusche et al. 2010; Uller et al. 2003). Although these findings have implications for all areas of enrichment for amphibians, they suggest that enrichment in captivity might have particular applications in pre-release training. However, applying this increased knowledge of amphibian learning and behavioral complexity to enrichment has not been empirically tested (apart from the aforementioned hellbender study). Furthermore, in the context of predation the ethics of any compromise between welfare and long-term reintroduction success must be carefully considered (Caro and Sherman 2013; Harrington et al. 2013).

Some of the research investigating enrichment for amphibians is problematic in terms of sample size and experimental design. Hurme et al. (2003) could not detect significance in some effects due to extremely limited sample size. Walsh and Downie (2005) used a sample size suitable for statistical analysis, but in their cover provision experiments, fossorial or semi-fossorial anuran species (*Leptodactylus fuscus* and *Physalaemus pustulosus*) were provided with a soft substrate in enclosures both with and without cover. As the authors admit, it is likely that the effects of cover provision in these species were weaker in comparison with the non-fossorial third study species (*Mannophryne trinitatis*) due to this soft substrate acting as "cover" for the frogs, which could simply burrow in order to hide.

Enrichment research in amphibians is subject to strong taxonomic bias in addition to bias towards shelter provision. Half of the articles (seven of 15, Table 1) used *X. laevis* as a study species, while of the other species used, six of eleven were dendrobatoid frogs and only one

caudate was represented (Table 1). To our best knowledge, there has been, to date, no explicit research on enrichment for caecilians (Gymnophiona). However, one biomechanics study (Ducey et al. 1993) may be relevant to caecilian enrichment, as it demonstrates that caecilians of four fossorial species (*Ichthyophis kohtaoensis*, *Dermophis mexicanus*, *Gymnopis syntrema*, and *Schistometopum thomense*) preferred and were most capable of digging in uncompacted soil, and that they use existing burrows rather than constructing new ones if given the choice. This concurs with field studies, which have generally found terrestrial caecilians in looser, more friable soil and leaf-litter in established burrow systems (Kupfer et al. 2005; Malonza and Measey 2005; Measey 2004; Oomen et al. 2000; Habidata.co.uk).

Comparison with fish and reptile literature

For amphibians, given the narrowness of enrichment types investigated and the limited range of focal species (both taxonomically and ecologically), it is difficult to extrapolate current evidence to other amphibians and to other enrichment types. In order to predict the importance of enrichment for amphibians, therefore, we examined evidence from the two vertebrate taxa most similar to amphibians: reptiles and fish. Despite the fact that mammals and birds are better studied (de Azevedo et al. 2007), reptiles and fish are generally more similar to amphibians in neurological complexity, cognitive ability, physiology, and ecology. The literature for fish is much larger than for amphibians and that for reptiles is both larger and includes a wider range of enrichment types (de Azevedo et al. 2007). We do not suggest that these groups are identical in their needs, but until advances in amphibian enrichment research are forthcoming, inference from these taxa may be important to consider. Furthermore, methodologies used to assess enrichment in reptiles and fish may easily transfer to the study of amphibians.

Research on fish has focused largely on the commercial improvement of fisheries, the improvement of fitness in animals intended for release to the wild, and to a lesser degree on the welfare of fish species commonly used in biomedical research. Enrichment through environmental complexity generally improves cognitive and learning ability in fish (Brown and Braithwaite 2005 in *Brachyraphis episcope*; reviewed by Strand et al. 2010), reduce stress and stress-related behavior and metabolic activity (Batzina and Karakatsouli 2012 in *Sparus auratus*; Finstad et al. 2007 in *Salmo salar*; Millidine et al. 2006 in *S. salar*; Zimmerman et al. 2012 in *Gadus morhua*), increases behavioral plasticity (Berejikian et al. 2001 in *Onychorhyncus mykiss*), increases territory holding power (Nijmen and Heuts 2000 in a variety of species) and improves foraging, risk assessment, and predator-avoidance behavior (Braithwaite and Salvanes 2005 in *G. morhua*; Brown et al. 1998 in *O. mykiss*; Brown et al. 2003 in *S. salar*; Lee and Berejikian 2008

in *O. mykiss*; Roberts et al. 2011 in *S. salar*). Moberg et al. (2011) found increased timidity of *G. morhua* reared in enriched hatchery conditions once exposed to a novel arena, possibly due to less developed coping strategies in animals reared with shelter. This body of evidence should stimulate interest in similar phenomena linked to environmental complexity in amphibians, which could have important implications for the success of release or reintroduction projects. It seems that innate, "hard-wired" fish behavior can be enhanced and honed by enrichment in the form of exposure to simulated predator disturbance (Berejikian et al. 2003 in *O. tshawytscha*) or by social learning (Vilhunen et al. 2005 in *Salvelinus alpinus*; reviewed by Brown and Laland 2001). The similarity to the limited literature on comparable phenomena in amphibians (Crane and Mathis 2010; Epp and Gabor 2008; Ferrari and Chivers 2008; Sontag et al. 2006) suggests that there is much to learn about the application of amphibian learning to captive husbandry and pre-release training.

Enrichment in fish farms also improves growth rates, similar to the effects of shelter provision in amphibian species (Archard 2013; Chum et al. 2013; Tinsley 2010; Walsh and Downie 2005), increases potential stocking densities and reduces aggression (Batzina and Karakatsouli 2012; Finstad et al. 2007), as does enrichment in amphibians (*X. laevis*; Toreilles and Green 2007). The impacts of enrichment may be trans-generational; Evans et al. (2014) found that adult farmed salmon (*S. salar*) in enclosures enriched by exposure to wild conditions while in captivity produced offspring with a two-fold increase in survivorship compared with fish maintained under standard farm conditions. Given the normal use of pre-release training only in the individuals to be exposed to predation (Crane and Mathis 2010), it may be important to investigate trans-generational effects of enrichment in amphibians.

A few studies have focused on individual welfare in laboratory and aquarium fish species, but as for amphibians these have mainly investigated cover provision. This work has, surprisingly, found little benefit to providing enrichment in laboratory aquaria, in the form of cover/environmental complexity, with fish often showing no differences in growth rates or stress-hormone levels (Brydges and Braithwaite 2009 in *Gasterosteus aculeatus*; Wilkes et al. 2012 in *Danio rerio*), although these are perhaps not comprehensive measures of welfare. Kistler et al. (2011), however, found a preference for structured, rather than barren, environments in both *D. rerio* and the barb *Puntius oligolepis*. These contradictory results may partly be due to the highly constrained nature of enrichment solutions within strictly controlled laboratory conditions. The glass rods provided as enrichment for zebrafish by Wilkes et al. (2012) may not have been sufficient to generate a beneficial effect, whereas the plants and hides provided in the preference study of Kistler et al. (2011) may have been complex enough to generate a detectable behavioral response in the same species.

Furthermore, as neither study analyzed both behavioral and developmental/endocrine data, it is possible that any improvement to welfare did not translate to all measures.

Saxby et al. (2010) and Sloman et al. (2011) found evidence for welfare and behavioral benefits of social enrichment in terms of both increased group size and mixed species assemblages in a variety of fish species commonly kept in home aquaria. Similarly, schooling and mixed species assemblages are common in anuran tadpoles in the wild and may have implications for learning (Ferrari and Chivers 2008; Sontag et al. 2006); the application of this for conservation breeding may be important to consider.

Reptiles have been better studied than amphibians in terms of enrichment research (de Azevedo 2007; Hayes et al. 1998) and attempts have been made in reptiles to identify and define stereotypical behavior and to suggest aetiologies (Bels 1989; Hayes et al. 1998; Warwick 1990). This literature is more focused on individual welfare of captive animals than is the fish literature and has involved zoo animals, as opposed to farms. Small sample sizes and anecdotal reports are a common problem in the reptile enrichment literature and much of it includes reasoned suggestions for enrichment, rather than empirical evidence of its efficacy (Burghardt 2013; Hayes et al. 1998). For this reason, enrichment solutions are, in general, more suitable for short-term use by a small group of animals, in contrast to the types of larger-scale enrichment often investigated in fish.

Captive conditions alter and reverse wild patterns of antipredator behavior of reptiles (Hennig and Dunlap 1978; Hennig 1979, both in *Anolis carolinensis*) and strike-induced chemosensory searching ("scent-trailing;" Marmie et al. 1990 in *Crotalus enyo*). The provision of a complex environment in captivity improves cognitive behavior (Almli and Burghardt 2006 in *Elaphe obsoleta*) and reduces stress hormone levels and stress-related escape behavior (Case et al. 2005 in *Terrapene carolina*). Blue-tongue skinks (*Tiliqua scincoides*) show alterations to activity patterns and exhibit reduced weight gain and obesity when provided with larger enclosures and the opportunity to hunt for insect prey (Phillips et al. 2011). Complex environments are also actively sought out by reptiles (Case et al. 2005 in *T. carolina*), while individuals of cryptic species may also seek out and prefer appropriately colored refugia (Garrett and Smith 1994 in *Morelia viridis*), as do wild amphibians (Pacific treefrogs, *Pseudacris regilla*; Morey 1990). Furthermore, although sometimes controversial (Burghardt 2005), some reptiles have been reported to engage in divertive, play behavior when provided with novel objects (Burghardt et al. 1966 and Burghardt 2005 in *Trionyx triunguis*; Hill 1946, Murphy 2002 and Burghardt 2005 in Varanus komodoensis; Lazell and Spitzer 1977 in *Alligator mississippiensis*). Animals have also exhibited a reduction in self-mutilation (Burghardt et al. 1996) and engaged in normal behavioral repertoires instead of apathy or ste-

reotyping when provided with such enrichment (Therrien et al. 2007 in *Caretta caretta* and *Chelonia mydas*). Also, monitors (*Varanus albigularis* and *V. rudicollis*) and anoles (*Anolis evermanni*) were capable of rapidly learning to solve cognitively demanding tasks (Gaalema 2011; Leal and Powell 2012; Manrod et al. 2008).

In contrast, Marmie et al. (1990) found no differences between groups of rattlesnakes (*Crotalus enyo*) raised in large or small enclosures, and wild conspecifics, in their ability to explore novel environments. Likewise, Rosier and Langkilde (2011) found no differences in *Sceloporus undulatus* behavior, stress hormone levels, survivorship and growth when a complex environment (climbing space) was provided. However, the small size and relative simplicity of the enclosures utilized in these cases may not have provided the degree of complexity required to provide effective enrichment for these animals: there has been some discussion of the validity of experimental design (see Burghardt 2013 for a summary of this exchange).

Finally, a few studies in reptiles examined the relationship between enrichment and survival in reintroduced animals, with encouraging results. Cook et al. (1978) reported the use of enrichment in the form of pre-release desert survival training of captive desert tortoises (*Gopherus agassizii*) in California and suggested that this approach improved survival from 0% in earlier release trials to 70% in trained tortoises. Although pre-release enrichment and training may have improved release success, rehabilitation centers also treated tortoises for a host of diseases that do not seem to have been addressed in earlier reintroduction attempts (the documentation is unclear), so the true impact of training is difficult to ascertain. Price-Rees et al. (2013) reported a similar training effort in blue-tongue skinks (*Tiliqua scincoides intermedia*), where aversive training was used to prevent lizards from eating lethally toxic cane toads (*Rhinella marina*), with large improvements in survivorship compared with control skinks. These findings reinforce the need for further investigation into the role of enrichment in pre-release training for amphibians. They also highlight the potential for such slightly aversive training to significantly improve both the welfare of individuals released into the wild and the success of conservation initiatives.

What impacts might enrichment have for captive amphibians?

Impacts on welfare

Enrichment has been demonstrated to reduce mortality and injury in some amphibians and to improve growth rates and body condition in others (Table 1). Furthermore, the majority of amphibian diseases found in captive populations and regularly treated by specialist veterinarians are related to improper husbandry (Wright and

Whitaker 2001). Obesity, metabolic bone disease (MBD) and related nutritional disorders are common problems in captivity (Gagliardo et al. 2008; Lee et al. 2006; Wright and Whitaker 2001). Enrichment designed to increase the effort required to forage for food (alongside a balanced diet; Li et al. 2009) increased activity levels (Campbell-Palmer et al. 2006) and, for actively foraging species (see below), should re-balance energy budgets while allowing animals to satiate their hunger, as has been demonstrated in skinks (Phillips et al. 2011) and cats (Clarke et al. 2005). Likewise, enrichment to encourage basking behavior in appropriate species (e.g., *Pelophylax lessonae*, which spend considerable portions of the day in the wild basking in sunlight; Michaels and Preziosi 2013), alongside the provision of Ultraviolet B radiation in suitable doses and gradients, is likely to be important in facilitating calcium uptake from the gut in many species, thus avoiding clinical and subclinical Metabolic Bone Disease (MBD) (Antwis and Browne 2009; Verschooren et al. 2011). Alongside basic facilitation via perches and basking sites, the provision of shelter and environmental complexity may alleviate perceived predation pressure and encourage basking behavior.

Beyond effects on the health and physical welfare of captive amphibians, enrichment may also have implications for psychological welfare. Enrichment may improve the cognitive engagement and capacity of amphibians, as has been shown in both reptiles and fish, as well as allowing animals to avoid perceived predation pressure (Michaels et al. 2014b). Further work is needed, however, to address these issues and to establish how enrichment may influence psychological well-being.

Implications for conservation

Enrichment may improve the success of reintroduction and head-starting programs in amphibian conservation. Evidence from amphibians, reptiles and fish strongly suggests that enrichment can influence a suite of characteristics, from growth rates to anti-predator behavior, which may influence the success of reintroductions. Furthermore, the potential for trans-generational effects warrants investigation in captivity. The provision of enrichment may influence survival and reproduction and consequently the genetic changes that occur over multiple generations, generating animals adapted to a captive environment (Frankham 2008). Genetic adaptation to captivity, or domestication, occurs due to differences between the wild and captive environment via genetic drift, founder effects, the unintentional selection for animals suited to the captive environment rather than the wild habitat into which they will eventually be released, or a combination these forces (Frankham 2008). Evidence for this phenomenon has been found in a wide range of breeding programs (reviewed Witzenberger and Hochkirch 2011) may be evident in a single generation (Christie et al. 2012). Amphibians are no exception, and adap-

tation to captivity has been detected in this group. For example, lack of exposure to predator cues and predation pressure resulted in loss of anti-predator behavior in the tadpoles of *Alytes muletensis* after 8−12 generations in captivity in association with a reduction in genetic diversity (Kraaijeveld-Smit et al. 2006). Although many captive breeding programs run studbooks to preserve genetic diversity and avoid genetic adaptation to captivity, these may fail due to unrealistic model assumptions (Witzenberger and Hochkirch 2011). In amphibian studbooks, tadpoles do not tend to be included as individuals and so populations may suffer non-random mortality before allele frequency changes can be prevented. The high fecundity of many amphibians means that most larvae cannot be raised to adulthood and necessary culls often remove tadpoles or metamorphs perceived to be weaker or smaller (C. Michaels, per. observ.). The use of enrichment to sort behaviorally fit and less fit animals, for example in response to predator cues, may be a more valid basis for culls than, for example, body size, although this idea is inevitably a source of ethical controversy (Caro and Sherman 2013; Harrington et al. 2013). Appropriately applied enrichment may also prevent more domesticated animals from gaining reproductive advantages in captivity. For example, animals that are unable to hunt effectively, but are capable of producing large numbers of young and readily reproduce in captivity may contribute disproportionately to programs unless animals are forced to forage more naturally for prey. Similarly, the use of enrichment may allow less domesticated animals to thrive in captivity, where they may be lost from breeding programs if housed without appropriate stimulation.

Finally, non-genetic inherited traits ("maternal" or "parental" effects) are becoming increasingly recognized as important in evolutionary terms. The genetic or environmental background of parents can influence offspring phenotype regardless of the genetic correlation between parents and offspring (Marshall and Uller 2007; Mousseau and Fox 1998). Epigenetic effects may improve or reduce offspring fitness, depending on the system and circumstances and can influence a wide range of characters in most plant and animal taxa (Franklin and Mansuy 2010; Marshall and Uller 2007; Mousseau and Dingle 1991; Mousseau and Fox 1998; Roach and Wulff 1987). Epigenetic effects have been reported in a number of amphibian taxa (including Kaplan 1987; Kaplan and Philips 2006; Pakkasmaa et al. 2003; Parichy and Kaplan 1992; Räsänen et al. 2003) and are of increasing importance in the consideration of animal behavior and welfare (reviewed Jensen 2014). They may be linked to the degree of enrichment in the captive environment, although this has not been studied in amphibians. McCormick (2006), for example, found that crowding in a number of marine fish species resulted in decreased fitness, regardless of their genotype, of offspring, independent of genotype, even when offspring were raised under identical, spacious conditions. Similarly, Evans et al. (2014)

demonstrated trans-generational effects of enrichment in salmon bred for conservation, such that enriching parental enclosures improved post-release survivorship in offspring. Enrichment for captive amphibians therefore has the potential to influence the fitness of future generations through both epigenetic and genetic effects. Importantly, the phenotype (and therefore chance of survival in the wild) of an individual is determined by the interaction between genes and the environment (including both direct and epigenetic/parental components), both of which can be partially determined by the enrichment strategies employed in captivity.

As these effects cannot be controlled through studbooks, it may be of great importance to provide a degree of enrichment that does not encourage epigenetic changes in captive amphibians.

Future directions for research

Being at the early stage of enrichment research in amphibians means that little is known of its impact on welfare and fitness or which types of enrichment may be important. Amphibian captive welfare and methods suitable for measuring it are poorly understood or underdeveloped in comparison with other taxa. Given the urgency to provide answers for *ex situ* conservation projects (Gascon et al. 2005) it is important to develop enrichment research goals and priorities. Table 2 outlines a potential structure for enrichment research in amphibians. Most areas of amphibian husbandry are strongly constrained by the natural history of the species in question (Michaels et al. 2014a) and needs and responses to captive stimuli vary greatly among taxa and sometimes between populations (e.g., Tidwell et al. 2013). A more thorough understanding of the biology of focal species can aid in the design of meaningful enrichment and experiments. Consequently, we recommend that researchers first develop a good understanding of the biology of focal species before attempting to develop and evaluate enrichment activities. Based on this knowledge, experimental methods and measures of welfare can be developed and areas both already identified as important in amphibians, and those highlighted by work in fish and reptiles, can be investigated. It is important to develop objective measures of welfare, including identification of stereotypical or abnormal behaviors in captive amphibians. Ideally, researchers should aim to use as many different measures of welfare and fitness as possible in order to develop the best possible picture of the effects of enrichment. Comparisons between wild and captive conspecifics may also help with this process, particularly where enrichment is intended to improve the suitability of animals for release. Objective measures of welfare may also aid in addressing conflicts between training required for improved reintroduction success and ensuring that animals are not distressed while in human care.

Collaboration between research institutions, which have the experimental expertise to carry out meaningful research, and zoological collections, which have access to animals and species-specific knowledge may expedite research. With these tools, research could better determine the need for and impact of enrichment for both individual captive welfare and long-term conservation success in amphibians. Such knowledge could help to successfully and humanely maintain these animals in captivity and to successfully release them into the wild.

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Caatinga Ethnoherpetology: Relationships between herpetofauna and people in a semiarid region of northeastern Brazil

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Abstract.—**We investigated the interactions between humans and herpetofauna in the semiarid region of Paraíba State, Brazil. Data were obtained by means of interviews with 124 hunters or ex-hunters using semi-structured questionnaires, complemented by informal conversations. We recorded 18 species (17 reptiles and one amphibian) that local human populations interact with because they have some utilitarian value or because of conflicting relations with local inhabitants. Implementation of conservation measures aimed at the herpetofauna in this region is particularly difficult due to the aversion that local people hold toward many of these species. Therefore, environmental education strategies should be adopted. These efforts should not be solely directed at species subject to hunting, but should be all-inclusive and take into consideration the cultural, social, and utilitarian role that governs the interactions of human populations and the herpetofauna of the Caatinga.**

Key words. Caatinga, conservation, ethnobiology, ethnozoology, hunting, reptiles, wildlife use

Introduction

Humans and herpetofauna (amphibians and reptiles) have interacted for millennia, virtually wherever they have been in contact (Alves et al. 2013b). As a result, interactions between humans and these animals are quite varied, encompassing utilitarian, symbolic, and conflicting aspects (Alves et al. 2008, 2009a, 2012b, c; Fernandes-Ferreira et al. 2012a; Franke and Telecky 2001; Klemens and Thorbjarnarson 1995; Morris and Morris 1965; Moura et al. 2010; Schlaepfer et al. 2005). Such interactions can be studied through ethnoherpetology, a subdivision of ethnozoology, which examines the relationships between human cultures and herpetofauna (Bertrand 1997; Das 1998; Goodman and Hobbs 1994; Speck 1946). Ethnozoological studies can aid in the evaluation of the impacts human populations have on native animal species and in the development of sustainable management plans, and thus, they are essential to conservation efforts (Alves 2012; Alves and Souto 2011).

Caatinga is the name given to the semiarid region that occupies the largest portion of Northeast Brazil and represents one of the major examples of a semiarid environment in the Neotropical region (Albuquerque et al. 2012; Alves et al. 2012b). In this biome, 205 herpetofaunal species have been recorded (65 amphibians, 66 lizards,

12 amphisbaenids, 53 snakes, five testudines, and four crocodilians), many of which interact with local human populations, where they furnish products exploited by the local people or are hunted and killed due to conflicting relations with people (Alves et al. 2009b, 2012a, b, c; Barbosa et al. 2011; Fernandes-Ferreira et al. 2013). In this context, understanding of the relations between humans and the herpetofauna of the region is an important step in designing strategies for management and sustainable use, and should consider the ecological, economic, and cultural aspects associated with these interactions.

Ethnoherpetological studies have only recently begun in Caatinga, although general ethnozoological research indicates that reptiles and amphibians are hunted by rural and urban populations of the region (Albuquerque et al. 2012; Alves et al. 2012b; Fernandes-Ferreira et al. 2012a). In an effort to contribute to our ethnoherpetological knowledge and its implications in the semiarid region of northeastern Brazil, we investigated the interactions between humans and herpetofauna in the municipality of Pocinhos in the semi-arid region of Paraíba State (PB). Our aim was to record the patterns of interactions of the local people with representatives of this animal group in the region. This information may be used to enhance conservation of the Caatinga's herpetofauna.

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Materials and Methods

Study area

The present study was carried out in the municipality of Pocinhos, located in the semi-arid region of Paraíba State, Brazil (Fig. 1; Ribeiro 2003). Pocinhos is 630 km² in area, with approximately 17,032 inhabitants. Average annual temperature is 23 °C, which varies little throughout the year. The region has a very low rainfall rate, fluctuating annually between 400 and 600 mm. The climate is hot, semi-arid, with rainfall in the autumn and winter months (Ribeiro 2003) and the vegetation is dominated by sub-deciduous and deciduous forests typical of semi-arid regions (Alves et al. 2009b; Ribeiro 2003).

Procedures

The study was conducted in the period of June 2010 to June 2011. The information was obtained by means of interviews with hunters or ex-hunters using semistructured questionnaires, complemented by informal conversations (Bernard 1994; Huntington 2000). The selection of informants was done by the "snowball" sampling technique (Bailey 1994), where from the initial contact, an informant indicates another who in turn indicates still another and so forth. Before each interview, the nature and objectives of the research were explained, and the interviewees gave their permission to record the information, by signing an informed consent form.

The questionnaires were applied to 124 hunters from the municipality, of which 98 (79%) live in urban areas but frequently travel to rural areas to hunt, while 26 (21%) live in the rural zone. The ethical approval for the study was obtained from the Ethics committee of Hospital Lauro Wanderley (protocol number: CEP/HULW nº 103/10).

Vernacular names of the specimens cited were recorded and the animals identified in the following ways: (1) analysis of the specimens or parts thereof donated by the interviewees; (2) analysis of photographs of animals taken during the interviews and during the accompaniment of hunting activities; (3) use of identifications by taxonomists familiar with the fauna of the study area and use of vernacular names; and (4) information from previous ethnozoological studies carried out in the study area (Alves et al. 2009b; Confessor et al. 2009; Mendonça et al. 2011). The scientific nomenclature of the species that are cited in this study follows the guidelines of the Brazilian Society of Herpetology (http://www.sbherpetologia.org.br/).

After analysis, specimens were deposited at the zoological collections of the Universidade Federal da Paraíba. Samples were collected with the permission of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the Sistema de Autorização e Informação em Biodiversidade (SISBIO), license number 25926-2.

Data Analysis

An accumulation curve of the herpetofaunal species cited by interviewees was prepared. In an accumulation curve for ethnobiological data, the X-axis corresponds to the number of individuals interviewed and Y-axis the number of species cited by the respondents. The curve was randomized 1,000 times and the means were calculated using the software EstimateS© version 8.2 (Colwell 2009). EstimateS© permits the statistical analysis of species richness (for this work, species richness can be interpreted as the richness of species locally exploited) of samples by determination of the Chao2 index (Colwell and Coddington 1994). This index has been used in pre-
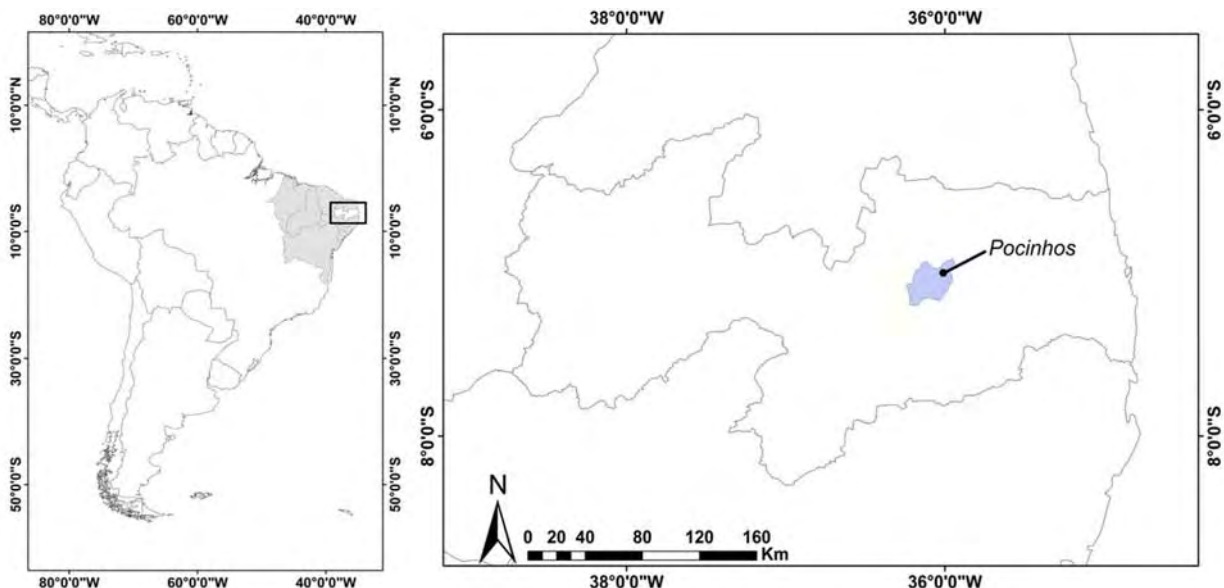

Fig. 1. Location of the municipality of Pocinhos (Paraíba State, Northeast Brazil), where the study was conducted.

vious ethnozoological studies (Ferreira et al. 2012; Souto et al. 2011; Whiting et al. 2011).

The non-parametric estimator Chao2 (Chao 1987) is calculated by the following formula:

$$Chao2 = Sobs + \left(\frac{L^2}{2M}\right)$$

where: Sobs corresponds to the number of species in a given sample, L is the number of species in only one sample ("uniques"), and M is the number of species that occur exactly in two samples. The utilization of the Chao2 estimator is recommended for ethnozoological studies since it is a non-parametric estimator based on data of incidence.

The data were entered in EstimateS© using a spreadsheet of type of respondent (rows) x type of species (columns). In preparing the spreadsheet, a value of 1 was given for each species mentioned by an interviewee and 0 for those that were not recorded.

For each species we calculated the Use-Value [adapted from the proposal of Phillips et al. (1994)], a quantitative method that demonstrates the relative importance of species known locally. This value was calculated using the following formula: UV = ∑ U/n, where: UV = Use-Value of the species; U = number of citations per species; n = number of informants. The calculations of the Use-Values of any species is based objectively on the importance attributed by the informants themselves, and does not depend on the opinion of the researcher.

Results

We recorded 18 species of herpetofauna (17 reptiles and 1 amphibian) that interacted with people in the surveyed area, either because they have some utilitarian value or because they are involved in conflicting relations with local inhabitants (Table 1). Products derived from herpetofauna were used for the following purposes: food (n = 7 species), medicinal use (n = 7 species), pets (n = 4 species), ornamental use (n = 4 species), and commerce (n = 2 species). Additionally, 13 species were hunted because they are considered harmful (particularly snakes), although some of these also provide products of utilitarian value.

Based on the data collected, the mean number of species observed (Sobs) was compared with that expected to be cited in the surveyed area (Fig. 2). The results demonstrated that the sampling efficiency was adequate, since 78.4% of all species of the herpetofauna of ethnozoological importance for the study area (Chao2 = 22.96 ± 5.07) were recorded. The species accumulation curve showed a tendency to stabilize.

When we considered the utilitarian value of the herpetofauna in the area studied, a greater number of species

were cited for their utilization as food (n = 7 species), where lizards were the principal group cited for this purpose, mainly the White tegu (*Salvator merianae*, Duméril and Bibron 1839; Use-Value = 0.66). Other lizards reported as being used for food were the Green iguana (*Iguana iguana*, Linnaeus 1758) and the whiptail lizard (*Ameivula ocellifera*, Spix 1825), with the latter being rarely consumed, as it was cited by only two interviewees. In relation to snakes, only three hunters cited species useful as food: rattlesnake (*Crotalus durissus*, Linnaeus 1758) and Rainbow boa (*Epicrates assisi*, Machado 1945). The Northeastern pepper frog (*Leptodactylus vastus*, Lutz 1930) is the only amphibian used for food according to interviewees.

The medicinal use of herpetofauna, reported by 28 hunters, appears to be the most common form of utilization for this animal group. The species most utilized for this purpose, according to the interviewees, are the White tegu (n = 28 citations), Green iguana (n = 14 citations), and rattlesnake (n = 8 citations; Table 2). From the animals cited as useful in popular medicine, various parts or medicinal subproducts are extracted, especially the fat and hide, which are used in the treatment of various diseases and are administered in various ways (Table 2).

Use of reptiles as pets was recorded in only three of the homes visited, suggesting that the use of herpetofauna as pets is not a common practice in the study area. Species used as pets were: Red footed tortoise (*Chelonoidis carbonaria*, Spix 1824; raised by three hunters), Tuberculate toadhead turtle (*Mesoclemmys tuberculata*, Lüederwaldt 1926), White tegu, and Boa snake (*Boa constrictor*, Linnaeus 1758; cited by only one hunter). The hunter who mentioned this last species stated that he captured the animal by hand on a hunting trip, but that he did not keep the animal long at his home because he was unable to feed it adequately, thus letting it go in the forest.
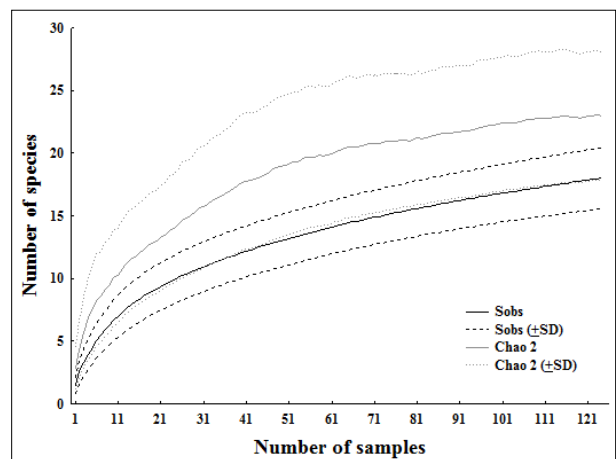
Fig. 2. Graphs showing the values obtained with the richness estimators of herpetofaunal species hunted in surveyed area. Number of Species Observed (Sobs = 18 ± 2.44), Number of species estimated (Chao2 = 22.96 ± 5.07).

Table 1. Hunted herpetofaunal species with their respective popular names in the surveyed area. Legend: **F** = food resource, **M** = medicinal, **C** = commerce, **P** = pets, **O** = ornamentation and decoration, and **CR** = conflicting relationships.

Family/species/popular name	Use-Value	F	M	C	P	O	CR
Leptodactylidae							
Leptodactylus vastus (Lutz, 1930) – "Jia," Northeastern pepper frog	0.01	X	X				
Testudinidae							
Chelonoidis carbonaria (Spix, 1824) –"Jabuti," Red footed tortoise tortoise	0.01		X		X		
Chelidae							
Mesoclemmys tuberculata (Luederwaldt, 1926) – "Cágado d'água," Tuberculate toadhead turtle	0.008		X	X			
Iguanidae							
Iguana iguana (Linnaeus, 1758) – "Camaleão," Common green iguana	0.20	X	X	X			
Teiidae							
Ameivula ocellifera (Spix, 1825) – "Calango," Spix's whiptail	0.008	X					
Salvator merianae (Duméril and Bibron, 1839) – "Teju," White tegu	0.66	X	X	X	X	X	X
Boidae							
Boa constrictor (Linnaeus, 1758) – "Cobra de veado," "jibóia," Boa snake	0.03	X	X			X	X
Epicrates assisi (Linnaeus, 1758) – "Salamanta," Rainbow boa	0.16	X				X	X
Colubridae							
Oxybelis aeneus (Wagler, 1824) – "Cobra de cipó," Brown vine snake	0.008						X
Dipsadidae							
Boiruna sertaneja (Zaher, 1996) –"Cobra preta," Black snake	0.02						X
Philodryas olfersii (Linchtestein, 1823) – "Cobra verde," Lichtenstein's Green racer	0.02						X
Leptodeira annulata (Linnaeus, 1758) – "Jararaca," Salamanta de parede, Banded cat-eyed snake	0.11						X
Philodryas nattereri (Steindachner, 1870) – "Cobra corre campo," Paraguay green racer	0.04						X
Pseudoboa nigra (Duméril, Bibron e Duméril, 1854) – "Cobra de leite," Black false boa	0.01						X
Xenodon merremii (Wagler, 1824) – "Jararaquinha," "Goipeba," Wagler's snake	0.01						X
Elapidae							
Micrurus ibiboboca (Merrem, 1820) – "Cobra coral," Caatinga coral snake	0.11						X
Viperidae							
Bothrops erythromelas (Amaral, 1923) – "Malha de cascavel," Jararaca da seca, Caatinga lancehead	0.02						X
Crotalus durissus (Linnaeus, 1758) – "Cascavel," South American rattlesnake	0.20	X	X	X		X	X

The use of herpetofauna to make artisanal products was mentioned by only three interviewees, where the hide is the principal product used for this purpose. This product is used mainly in the manufacture of accessories (belts, purses, and key chains). The species used for this purpose are: rattlesnake, whose rattle is used in the manufacture of key chains by some hunters and the hide, which can be used to make belts; and Boa snake, Rainbow boa, and White tegu, whose hide is used in the manufacture of accessories.

Despite being sources of products used for different purposes, the main motivation for the hunting and killing of the herpetofauna in the study region is that many of the species cited are considered harmful, particularly the snakes, considered venomous and efficient predators that pose a risk to humans and their domestic animals. Forty (32.2%) hunters interviewed affirmed having killed some type of reptile while hunting or during daily activities in the countryside. Meanwhile, the hunters were unanimous in stating that they kill whatever snake they encounter. The most persecuted species are the rattlesnake (n = 26 citations), Rainbow boa (n = 21 citations), Caatinga lancehead (*Bothrops erythromelas*, Amaral 1923; n = 3 citations), and coral snake (*Micrurus ibiboboca*, Merrem 1820; n = 14 citations).

Besides snakes, the White tegu can be killed by some hunters (n = 4) of rural areas because they do damage, since this lizard feeds on chicks and chicken eggs. The latter are important food for local families, besides being a source of income when sold.

Table 2. Herpetofauna used for medicinal purposes cited by hunters in the Pocinhos city, Paraiba State, Brazil.

Species / vernacular name	Citations	Medicinal use (Treated diseases)	Parts
Chelonoidis carbonaria (Spix, 1824)	2	Rheumatism and swelling	Shell and fat
Mesoclemmys tuberculata (Luederwaldt, 1926)	2	Sore throat, cough, asthma, earache, wounds, rheumatism, haemorrhoids, shortness of breath, bronchitis	Fat
Iguana iguana (Linnaeus, 1758)	14	Suck a splinter out of skin or flesh, snakebite, choking, boils, rheumatism, earache, sore throat, and wounds	Skin, fat, and bone
Salvator merianae (Duméril e Bibron, 1839)	28	Sore throat, earache, choking, deafness, boils, wounds, arthritis, asthma, rheumatism, headache, tumor; suck a splinter out of skin or flesh, cough, and swelling	Fat, tongue, and skin
Crotalus durissus (Linnaeus, 1758)	8	Asthma, sore throat, skin problems, cancer, rheumatism, urinary problems, arthritis, toothache, haemorrhoids, backache, mycoses, wounds, deafness, and varicose veins	Rattle and fat
Leptodactylus vastus (Lutz, 1930)	1	Sore throat	Meat

Discussion

Our results reveal that the people of the surveyed area establish a greater interaction with reptiles than amphibians. This finding can be related to the greater richness of reptiles that occurs in the Caatinga (140 reptiles and 65 amphibians) and also among the reptiles there are larger-sized species, which can offer larger amounts of products for use. Snakes are feared animals in all of the semiarid northeast and in other places in Brazil, calling extra attention associated with the prevention of potential accidents (Alves et al. 2010b, 2012b, c; Moura et al. 2010).

Despite the negative view related to the many species of reptiles in the area studied, there are many species (even those killed because of conflicts) that supply products used by the local inhabitants. These observations are in agreement with Marques (1995), who noted that the link between humans and animals is fraught with contradictions and ambiguities, as the native fauna can represent either a resource or a risk to the local people.

The small number of species of herpetofauna used as food is not surprising, since traditionally, this group does not play an important role as a protein source for the populations living in the Caatinga. The principal groups of wild vertebrates used as a source of protein in the region are birds and mammals (Alves et al. 2009b; Bezerra et al. 2011, 2012a, b, 2013; Fernandes-Ferreira et al. 2012b). However, the game importance of the White tegu should be pointed out, as its meat is used as a source of protein in the Caatinga. Such observation can be substantiated in a parallel study on the consumption of bushmeat in the same area as the present study (Mendonça 2012), which monitored the consumption of meat by local families during a year and within the local herpetofauna, only recording the consumption of two species of reptiles: *S. merianeae* and *I. iguana*, with greater frequency for the former. The use of these two species for food also has been recorded in other localities of the semiarid northeast, including urban areas (Alves et al. 2012a; Marques and Guerreiro 2007). Considering the cultural and utilitarian importance of the lizard *S. merianae*, we do not exaggerate when we suggest that this animal represents one of the animals of greatest ethnozoological importance in the Brazilian Caatinga. This can be due to its size, since it is the largest species of lizard of the semiarid region (Vanzolini et al. 1980) and corroborates the findings of Alves et al. (2012b), which pointed out that *S. merianeae* represents the main game reptile of the semi-arid region of Brazil.

Corroborating a tendency observed in other studies (Alves et al. 2012c; Marques and Guerreiro 2007; Santos-Fita et al. 2010), the consumption of snakes was little cited by the hunters in the study area. In Brazil, only five snake species have been reported as being used for human consumption: *Boa constrictor*, *Eunectes murinus*, *Lachesis muta*, *Crotalus durissus*, and *Epicrates assisi* (Alves et al. 2012c; Fernandes-Ferreira et al. 2013). Alves et al. (2012c) highlighted that the small numbers of snake species currently used as food in Brazil is not surprising given the negative images attributed to these animals in myths, legends, and popular beliefs. Reinforcing this notion, Rea (1981) noted that not only are snakes rejected because of their disagreeable nature but also any other creature with a similar shape or behavior. A study undertaken among human populations living along the banks of the Rio Negro (Amazonas State, Brazil) indicated that the electric eel (*Electrophorus electricus*) was one of the least favored meats because of its strong smell and the shape of its body—"it looks just like a snake" (Silva 2007).

Although the herpetofauna does not play an important role as a source of protein in the region studied, this group stands out when considering the popular medicine of the region. Despite having been cited less as medicinal species ($n = 6$ species) than as those used for food ($n = 7$ species), medicinal use showed a higher number of citations, suggesting its greater dissemination among the interviewees. In this context, the tegu was also featured with regard to number of citations as well its broad

medicinal applicability. Studies in various localities have already indicated the importance of this species of lizard in popular medicine in Brazil (Alves 2009; Alves et al. 2007, 2009a, 2011; Ferreira et al. 2012; Oliveira et al. 2010), even in urban areas, where the sale of products derived from *S. merianeae* (as well as other species of reptiles recorded in this work) is common in public markets in various cities in northeast Brazil (Alves and Rosa 2007; Alves and Rosa 2010; Ferreira et al. 2012).

Raising wild animals as pets, particularly wild birds (Alves et al. 2010a, 2013a; Bezerra et al. 2001, 2013; Fernandes-Ferreira et al. 2012b; Nobrega et al. 2012) is a very common practice in the semiarid northeastern region, but few species of the herpetofauna are utilized for this reason, in accordance with our finding presented here. Among the reptiles of the Caatinga, the Jabuti (*C. carbonaria*) is one of the species of the most popular pets, probably because it is considered docile and easy to capture and keep in captivity. Additionally, there is also a popular belief that its presence helps avoid illnesses such as bronchitis and asthma (Alves et al. 2009a).

The strong aversion to reptiles, especially snakes, is common in various places in Brazil (Alves et al. 2012b, c; Moura et al. 2010; Santos-Fita et al. 2010), and was also recorded in our study. This aversion serves as a strong motivation for hunters and the public in general to kill snakes indiscriminately, where they are persecuted and killed whenever they are encountered. People are used to killing not only venomous snakes but also the non-venomous species, and even those amphibians that have a similar body shape as snakes. Similarly, Santos-Fita et al. (2010) documented that all inhabitants of a semiarid area of the state of Bahia have strong negative reactions in relation to snakes, always killing them if possible. It should be emphasized that these conflicts involve other groups besides snakes. In our study, we recorded that even species of reptiles with high utilitarian value, such as the tegu, can also be killed for feeding on chicken eggs, causing financial losses for farmers.

Our data, together with previous findings of other ethnozoological studies carried out in the semiarid region of the northeast, allow us to suggest some patterns of interactions between the people and herpetofauna of the Caatinga: (1) there are more frequent interactions between the people and reptiles than with amphibians; (2) lizards comprise the group with the most important species for food, particularly the White tegu; (3) products from herpetofauna play an important role in popular medicine in the semiarid, northeastern region; (4) besides food and medicinal use, products from herpetofauna can be used in handicrafts and jewelry; and, (5) various reptile species, especially snakes, are hunted and killed because of cultural aversion to these animals and the risks they pose to people and domestic animals.

Information from previous studies and that obtained here demonstrate that in the semiarid region of Brazil's northeast, reptiles and amphibians are hunted because they are useful or considered dangerous, and sometimes for both reasons. The implementation of conservation measures aimed at the herpetofauna in this region is particularly difficult due to the aversion of the people to a good part of the species of this group. Therefore, strategies of environmental education should be adopted, besides specific actions directed at species of high game value, taking into consideration the cultural, social, and utilitarian role that governs the interactions of human populations and the herpetofauna of the Caatinga.

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CONTENTS

COVER: An adult individual of the Tandayapa Andean Toad (*Rhaebo olallai*, syn. *Andinophryne olallai*) from an extant population discovered in Reserva Río Manduriacu, Imbabura Province, Ecuador. Prior to the discovery of the population in Reserva Río Manduriacu, the species had not been documented since its original description in 1970 and was presumed Extinct. The rediscovery of the species in 2014 resulted in the collection of the first color photographs of the species, information on its ontogeny and natural history, the species conservation status, and led to a taxonomic update of the genus *Andinophryne*. Photography: Ryan L. Lynch.