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Variation, Systematics, and Relationships of the Leptodactylus bolivianus Complex (Amphibia: Anura: Leptodactylidae)

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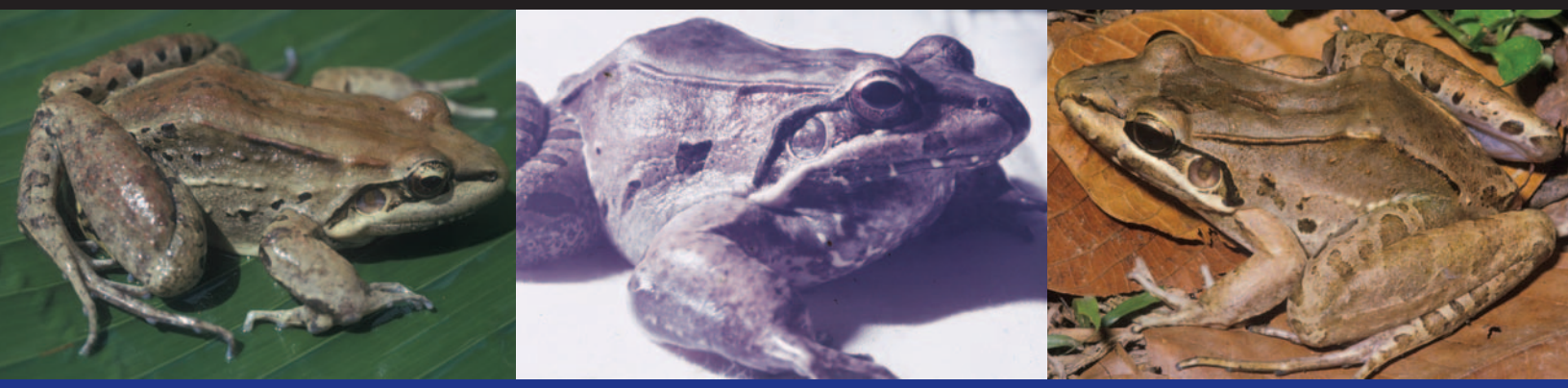

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Variation, Systematics, and Relationships of the *Leptodactylus bolivianus* Complex (Amphibia: Anura: Leptodactylidae)

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ABSTRACT

Heyer, W. Ronald, and Rafael O. de Sá. Variation, Systematics, and Relationships of the *Leptodactylus bolivianus* Complex (Amphibia: Anura: Leptodactylidae). *Smithsonian Contributions to Zoology*, number 635, viii + 58 pages, 21 figures, 20 tables, 2011.—The *Leptodactylus bolivianus* complex has been considered to consist of one or two species, *L. bolivianus* alone or *L. bolivianus* and *L. insularum*. Detailed morphological analyses were undertaken to evaluate variation in the complex, which ranges from Costa Rica through Panama, across northern South America in the river valleys draining to the Caribbean, and throughout much of the Amazon basin with southern limits in Bolivia. Members of the complex also occur on several islands off Nicaragua, Panama, and Colombia. Analyses of morphological and advertisement call data indicate that there are either two or three species comprising the complex. Analysis of molecular data strongly supports recognition of three species, one of which is described as a new species, *Leptodactylus guianensis*. The three species comprising the *L. bolivianus* clade are most closely related to the *L. ocellatus* clade within the genus *Leptodactylus*.

Cover photos, from left to right: *Leptodactylus insularum* (Figure 17, courtesy of R. W. McDiarmid), *L. guianensis* (Figure 15, courtesy of M. S. Hoogmoed), and *L. bolivianus* (Figure 12, courtesy of R. B. Cocroft).

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Variation, Systematics, and Relationships of the *Leptodactylus bolivianus* Complex (Amphibia: Anura: Leptodactylidae)

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INTRODUCTION

A cluster of morphologically similar frogs of the genus *Leptodactylus* having a pair of distinct dorsolateral folds on the dorsum and well-developed lateral fringes on the toes has never been systematically evaluated by examining materials from throughout its geographic range. The species involved are herein referred to as members of the *Leptodactylus bolivianus* complex. There have been three names proposed for members of this complex: *Leptodactylus bolivianus* Boulenger, 1898; *Leptodactylus insularum* Barbour, 1906; and *Leptodactylus romani* Melin, 1941. The collective range for the *L. bolivianus* complex is from Costa Rica southward through Panama, extending across northern South America (east of the Andes) in the river valleys draining to the Caribbean, and throughout much of the Amazon basin with southernmost limits in the Bolivian departments of La Paz, Cochabamba, and Santa Cruz.

We analyze variation in this complex of frogs throughout its geographic range to understand inherent patterns of differentiation and to interpret those patterns in terms of species-level recognition, distributions, and relationships.

METHODS AND MATERIALS

We follow Fabrezi and Alberch (1996) in numbering the fingers II–V (considering finger I as lost in frogs). Herein, we use the name *Leptodactylus latrans* following the recent elucidation of the nomenclature of the widely spread taxon previously referred to as *L. ocellatus* (Lavilla et al., 2010).

The primary materials examined for this study are juvenile and adult museum specimens; only this data set provides extensive locality records for the members of this complex. The juvenile and adult morphological data are

complemented by available data on larval morphology, advertisement calls, and molecular sequences.

The names of the institutions and collections from which specimen data were obtained are abbreviated here (after Sabaj Pérez, 2010) for subsequent mention in the text.

AMNH	American Museum of Natural History
CM	Carnegie Museum of Natural History
FMNH	Field Museum of Natural History
ICNMHN	Instituto de Ciencias Naturales, Museo de Historia Natural, Universidad Nacional de Colombia
IND	Instituto Nacional de Recursos Naturales Renovables y del Ambiente, Bogota
INPA	Instituto Nacional de Pesquisas da Amazonia, Brazil
KU	University of Kansas Natural History Museum
LSUMZ	Louisiana Museum of Natural History
MCZ	Museum of Comparative Zoology, Harvard University
MNCN	Museo Nacional de Ciencias Naturales, Madrid
MNRJ	Museu Nacional, Universidade Federal do Rio de Janeiro
MPEG	Museu Paraense "Emilio Goeldi," Belém, Brazil
MPUJ	Museo de Pontificia Universidade de Javeriana, Bogotá
MZUSP	Museo de Zoologia da Universidade de São Paulo, São Paulo
NRM	Naturhistoriska Riksmuseet, Department of Vertebrate Zoology, Stockholm
TCWC	Texas Cooperative Wildlife Collection, Texas A&M University, College Station, Texas
TNHC	Texas Natural History Collections, Texas Natural Science Center, Texas Memorial Museum, University of Texas at Austin
UMMZ	University of Michigan Museum of Zoology, Ann Arbor, Michigan
USNM	National Museum of Natural History, Smithsonian Institution, Washington, D.C.
UTA	University of Texas at Arlington, Department of Biology, Texas

The following collections are not included in Sabaj Pérez (2010).

AL	Adolfo Lutz collection housed at MNRJ
ULABG	Universidad de Los Andes, Laboratorio de Biogeografía, Mérida, Venezuela

The following are field tag abbreviations, not yet incorporated into collections.

AMU	Abraham Mijares
ELM	Enrique La Marca
JC	Jay Cole
RdS	Rafael de Sá
UC	Ulisses Caramaschi
USNM-FS	National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Juvenile and Adult Morphological Data

The data involved were taken and analyzed by the first author (WRH) for consistency (see Hayek et al., 2001). Because of the moderately large size of adult specimens of this complex and new restrictions on transportation of alcohol-stored specimens, it was necessary to take data on the specimens in the collections involved rather than borrowing the specimens. Thus only the USNM specimens, plus a few other specimens on loan to WRH, were available for reexamination during this study.

Specimen data from the following collections were taken in situ: AMNH, FMNH, ICNMHN, IND, KU, MCZ, MNRJ, Museo Universidad Javariana–Bogota, MZUSP, UMMZ, and USNM.

Geographic samples are analyzed to evaluate the nature of morphological variation. The samples are indicated by italics and leading capital letters.

In 2005 an evaluation of the assembled locality data was undertaken to determine if there were any obvious geographic gaps for which specimens should be sought in other collections. There was only one obvious gap—no data were available from the Brazilian State of Pará, even though members of the complex occur in extensive portions of Amazonia. The MZUSP and USNM collections contain rather extensive amphibian collections from Pará, suggesting that members of the complex really do not occur in Pará. The institution housing the most herpetological specimens from Pará is the MPEG. Dr. Marinus Hoogmoed reported that there were no specimens of the *L. bolivianus* complex catalogued in the MPEG collection (M. Hoogmoed, personal communication [e-mail] to WRH, 30 March 2005). At the VI World Congress of Herpetology in July 2008, Dr. Hoogmoed (pers. comm.) informed WRH that he had reexamined frogs from the state of Pará, near the state of Amapá and found them to be members of the *Leptodactylus bolivianus* complex.

Subsequently (e-mail message of 20 December 2008 to WRH) Dr. Hoogmoed wrote, "During the last fieldwork again 4 specimens of *Leptodactylus bolivianus* [new species as recognized in this paper]. Thus the species is well distributed in Para north of the Amazon."

Specimens from the collections indicated above should be adequate to evaluate patterns of morphological variation throughout the distribution of the species complex.

After examination of materials from the AMNH, ICNMHN, IND, MCZ, MNRJ, MZUSP, and USNM, it seemed that data taken to that point on surface textures of the dorsal shank, outer tibia, and sole of foot did not demonstrate meaningful interpopulational variation. Before visiting further collections, an analysis was made (see Appendix 1) that confirmed the uninformative value of those characters, and no further data were taken for these variables.

The variables recorded for the entire data set for analysis of variation organized by category are as follows:

(1) Sex. Adult males are defined as having vocal slits. Juvenile males lack vocal slits. Gonads of individuals near adult size were examined to determine whether they were juvenile males or females. Adult females are defined as having at least curly oviducts. Juvenile females have straight oviducts. Most small specimens were not dissected to examine for gonads and are classified as juveniles.

(2) Belly, dorsal, lip, posterior thigh, and dorsal shank patterns. Outline drawings were filled in for each of these characters, and these drawings served as pattern standards; additional standards were created as deemed appropriate. For data-recording purposes, each pattern was given a unique letter or combination of a letter and number. Specimens that had patterns intermediate between two standards where creation of a new standard was considered inappropriate were recorded as having both patterns separated by a slash. These slashed pattern notations are treated as separate states. Belly patterns were based on standards for the *Leptodactylus pentadactylus* species group (Heyer, 2005) as a starting point to which standards were added as needed. The dorsal, lip stripe, posterior thigh, and dorsal shank patterns used were initially created on the basis of small samples of specimens of both the *L. bolivianus* and *L. latrans* clusters with additional standards added in this study. The first two collections examined were MZUSP and USNM during which the majority of new standards were created. Subsequently, any new standard created was dated to know when in the study it was available for comparison.

(3) Dorsolateral and lateral folds. Combinations of letters, numbers, and parentheses were used in data coding to express the completeness of the pair of dorsolateral folds extending from the supratympanic fold to the groin along the junction of the dorsal and flank regions and the pair of lateral folds extending from the supratympanic fold to the groin in the midflank region.

(4) Male secondary characteristics. Combinations of numbers, letters, pluses, minuses, and parentheses were used to code variation in male arm hypertrophy, tympanic tubercles, jaw tubercles, chest tubercles, and thumb spines.

(5) Measurements. Measurement data were taken with calipers either with the unaided eye or under a dissecting microscope for snout–vent length (SVL), head length, head width, shank length, and foot length following Heyer (2005). Measurements on MZUSP specimens were recorded to the nearest 0.5 mm; all others were recorded to the nearest 0.1 mm.

Observer error increases when materials are not available for reexamination. Therefore this problem was specifically addressed before the data were analyzed for geographic variation. The methods used to determine observer error are described in Appendix 1.

Larval Morphology

Terminology and methods follow McDiarmid and Altig (1999) including use of Gosner developmental stages. In addition to the examined USNM larval collection for samples of this complex, AMNH, CM, FMNH, ICNMHN, KU, MCZ, and UMMZ were contacted for larvae identified as either *L. bolivianus* or *L. insularum* in their collections.

There are very few larval samples from the above collections. Only KU and USNM had samples identified as belonging to the *L. bolivianus-insularum* complex. The KU samples were borrowed and examined by WRH.

The KU larval specimens from two localities have been described in the literature (Duellman, 1997, 2005). Upon examination of these specimens, WRH determined that the published descriptions were based on misidentified specimens. Duellman (1997) described KU 167784 and 167785 from km 104 on the road from El Dorado to Santa Elena de Uairén, Bolívar, Venezuela, as *L. bolivianus*. No juvenile or adult KU specimens of the *L. bolivianus-insularum* complex were collected at this locality. The tadpoles are in fair condition, very soft, with all teeth missing. Duellman (1997) indicated that seven individuals at Gosner stage 25 had body lengths of 15.5–18.4 mm. Data were taken on five larvae from each sample that visually appeared to cover the size ranges for each sample. Tadpole stages ranged from 26 to 35 with body sizes ranging

from 14.0 to 18.1 mm. Duellman (1997) did not report a tooth row formula for the larvae. Two tadpoles were examined using crystal violet stain to differentiate the tooth row ridges that bear the teeth. The anterior ridges were unclear in one individual, but the other individual has a 3(3)/3(1) formula. Both tadpoles have the same posterior tooth row condition, with a short P3 row, which is unusual for *Leptodactylus* larvae but does occur in a few members of the *L. pentadactylus* species cluster, such as in some individuals of *L. knudseni* (Heyer, 2005). The vent appears to be median, although the condition of preservation makes the evaluation difficult. Given the 3(3)/3(1) tooth row formula, the larvae most likely do not belong to the genus *Leptodactylus*. Duellman (2005) described and illustrated larvae of *L. bolivianus* from Cusco Amazonico, Madre de Dios, Peru. The specimen illustrated in Duellman (2005:177, fig. 13.10A) was placed in a separate container and identified as the illustrated specimen. There is one discrepancy between the specimen, Duellman's description, and the illustration. Duellman (2005:279) described a specimen at stage 25. The illustration shows a limb bud with early differentiated digits, perhaps at stage 35 or 36, but not of normal shape for digits at those stages of development. Duellman (2005) reported the tooth row formula as 2/3(1). Reexamination of the illustrated specimen indicates a formula of 1/3(1), with a very short P3 row. Examination of several specimens from KU 205810 indicates the variable formula 1/2–3(1), with P3 always very short when present. A single tadpole was examined using crystal violet stain. A broadly separated pair of ridges with two lateral extensions is present where an A2 row would be expected. As indicated, none of these ridges bear teeth in the several specimens examined from lot KU 205810. The tadpoles identified as *L. bolivianus* (Duellman, 2005) do not belong to the genus *Leptodactylus*; most likely, they are unidentified larvae of leiuperid, leptodactylid, or dendrobatid frogs.

Advertisement Calls

The same institutions that were contacted for larval holdings were contacted for holdings of any recordings of advertisement calls. Two recordings were available at the Texas Natural History Collection. Both recordings were from the canal zone region of Panama, one of poor quality. The better recording was not requested, as there was another recording available for the same area. In addition, César Barrio and Enrique La Marca reported that they did not know of any recordings of the *L. bolivianus-insularum* complex from Venezuela.

There are three commercially available CDs containing purported recordings of specimens of the *L. bolivianus-insularum* complex.

Márquez et al. (2002) contains two brief recordings of advertisement calls identified as *L. bolivianus*. The recordings were obtained near a pond where several males were calling, and no particular voucher was collected (Ignacio De la Riva, pers. comm. [e-mail], 6 July 2006). These calls from Bolivia are very different from those recorded from Tambopata, Peru, for which there is a museum voucher specimen corresponding to *L. bolivianus* (USNM 343347). Either the Bolivian recording species identification is in error or the Bolivian and Peruvian frogs associated with the calls represent distinct species. The Bolivian recordings have considerable sound in the same broadcast channel as the call of interest and cannot be analyzed in much detail. On the basis of the available information, these Bolivian CD recordings are considered to not belong to a member of the *L. bolivianus-insularum* complex.

The Cusco Amazónico recording KU cassette 104/3 published and figured by Duellman (2005:279) for *L. bolivianus* was requested for analysis. KU cassette 104/3 was mistakenly discarded before it was incorporated into the KU tape archives (W. E. Duellman, pers. comm., e-mail message of 27 June 2006). The description provided (Duellman, 2005:279) could represent either *L. bolivianus* or more probably (on the basis of the statement, "The call consists of a continuous bubbling sound"), *L. leptodactyloides*.

Two Costa Rican recordings were borrowed for analysis: KU 801 and KU 802. The KU 801 recording has minor tape speed problems. The KU 802 recording has major tape speed problems and was not analyzed.

Three recordings were analyzed with Raven 1.2 software (Charif et al., 2004); terminology follows Heyer et al. (1990). Data for beginning, highest, and ending frequencies were taken from audiospectrogram displays. Dominant frequencies were obtained from spectrum analyses of entire calls. Call durations were taken from waveform displays. All calls were filtered around the broadcast frequencies for analysis. Default settings were used for audiospectrograms (window: Type Hann, size 512 samples, 3 dB filter bandwidth 124 Hz; time/grid: overlap 50%, hop size 256 samples; frequency grid: DFT size 512 samples, grid spacing 86.1 Hz; averaging: 1 spectra) and spectrums (window: size 256 samples, 3 dB filter bandwidth 248 Hz; time grid: overlap 50%, hop size 128 samples; frequency grid: DFT size 256 samples, grid spacing 172 Hz; averaging: 1 spectra).

Molecular Sequence Data

Total genomic DNA was extracted using DNAeasy kit (USB) from liver or thigh muscle that was preserved in ethanol 95%. A segment of about 850 base pairs (bp) from the 16S ribosomal RNA (rRNA) gene was polymerase chain reaction (PCR) amplified (Palumbi, 1996) using an MJ Research PTC-200 thermocycler. Double-stranded PCR amplifications were performed using Green Taq Master Mix (Promega).

Amplification primers used were 16S Ar 5′-CGCCTGTTTACCAAAAACAT-3′ and 16S Br 5′-CTCCGGTCTGAACTCAGATCACGTAG-3′ under the following thermal conditions: initial denaturation at 94°C for 2 min followed by 34 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min 30 s. Amplified segments were purified using Exo-Sap (USB) by heating samples at 80°C for 15 min. Purified products were cycle sequenced with the dideoxy chain termination method using the Sequi-Therm Excel II DNA sequencing kit (Epicentre Technologies). Infrared-labeled sequencing primers (same as amplification primers) were used in sequencing reactions (LiCor Biotechnology) under the following thermal conditions: initial denaturation at 95°C for 2 min 30 s, followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 70°C for 30 s. Sequencing products were run in 6% acrylamide, 44 cm in length, gels using a Licor DNA 4300 automatic sequencer. Sequencing reactions were single stranded; double-stranded PCR fragments were sequenced in both directions. Sequences included in the analyses represent a consensus of both DNA strands; GenBank accession numbers for the sequence data are: HQ 232831–232846, HQ259119, HQ259120, AY947857, AY911285, AY669856, AY943235, EF632048, EF632053, and EF632055

Complementary sequence strands for each sample were first aligned and, using the chromatographs created by BaseImagr software (LicorBiotechnology), were inspected visually for mismatches of aligned positions to confirm or manually correct the automatic reading. Consensus sequences were aligned with ClustalX (Thompson et al., 1997) using the multiple-alignment option. Phylogenetic analyses of sequence data were run using maximum parsimony (MP) and maximum likelihood (ML) criteria as implemented in PAUP* 4b6 (Swofford, 2002). Character states were treated as unordered, and gaps were considered as a fifth character (MP analysis); heuristic searches were performed with 100 random additions of sequences and tree bisection-reconnection (TBR) branch swapping. Strict consensus trees were calculated when several equally parsimonious trees resulted from the MP searches. Maximum likelihood analyses used the general time-reversible model with empirical base frequencies, gamma distribution of across-site rate variation, and an estimated proportion of invariable sites (GTR + Γ + I; Swofford et al., 1996) recommended by ModelTest 3.04 (Posada and Crandall, 1998). Clade support of inferred trees was assessed by nonparametric bootstrapping (Felsenstein, 1985) on the basis of 100 pseudoreplicates.

Seventeen available samples from the *L. bolivianus-insularum* complex (additional data provided in Appendix 2) in addition to a sample of *L. latrans* and of *L. chaquensis* were considered as the ingroup taxa (traditionally all included in the *L. latrans* species group). To include variation found in the genus *Leptodactylus* (*sensu stricto*), the following taxa were used as outgroups: *L. podicipinus*, *L. wagneri* (*melanonotus* species group), *L. mystacinus*, *L. gracilis* (*fuscus* species group), *L. paraensis*, and *L. knudseni* (*pentadactylus* species group).

MEASUREMENT DATA

The protocol recommended by Hayek et al. (2001) of measuring a single individual male and female 20 times was followed. For this study, two sets of specimens were remeasured. The first set represents well-preserved specimens USNM 200352 (female) and USNM 200353 (male). Twenty-one data sheets were prepared to record data (to allow for at least one unambiguous error to be discarded and still have 20 data observations). Only a single sheet was filled out on any given day. These data were taken on the same days other data were being taken on USNM specimens. The sheets were filled in such that male data were recorded first on alternate sessions and the sheets were filled out alternately at either the beginning of the data taking session or the end of the session. Data were taken between 19 August and 28 October 2004. The second set represents stiff specimens (presumably yielding larger measurement errors): USNM 145708 (male) and USNM 145711 (female). Twenty-one data sheets were prepared to record data. A single sheet was filled out on any given day. Male data were recorded first on alternate recording sessions. Data were taken between 21 September and 21 October 2005.

Surprisingly, the well-preserved specimens were not notably measured more accurately than the stiff specimens (Table 1). For example, half of the well-preserved specimen variables had the highest coefficient of variation values. As it makes little or no difference which male and female

TABLE 1. Summary statistics for specimens remeasured 21 times to determine observer error. Definitions: Min, minimum measurement; Max, maximum measurement; CV, coefficient of variation; SVL, snout–vent length; HL, head length; HW, head width; EN, eye-nostril distance; TD, tympanum diameter.

	Well-preserved specimens						Stiffly preserved specimens					
	♂ USNM 200353			♀ USNM 200352			♂ USNM 145708			♀ USNM 145711		
Specimen variable	Min	Max	CV	Min	Max	CV	Min	Max	CV	Min	Max	CV
SVL	87.8	88.9	0.004	87.4	89.3	0.007	87.4	88.5	0.003	75.8	76.8	0.003
HL	30.8	33.3	0.019	31.2	32.2	0.011	30.3	32.3	0.016	26.8	29.3	0.026
HW	31.0	32.1	0.010	29.1	30.9	0.015	30.0	31.1	0.008	26.2	27.5	0.013
EN	8.3	8.7	0.013	8.0	8.6	0.018	8.1	8.4	0.011	7.7	8.0	0.011
TD	6.2	6.6	0.014	6.0	6.3	0.018	5.8	6.3	0.020	5.1	5.6	0.026
Thigh	39.0	39.8	0.005	40.5	42.5	0.013	37.6	38.5	0.007	34.0	35.3	0.009
Shank	39.8	40.8	0.006	43.3	43.8	0.003	42.2	43.3	0.008	39.3	40.2	0.007
Foot	42.2	43.6	0.009	44.4	46.2	0.010	42.5	45.3	0.014	40.4	42.1	0.012

data set is used in subsequent analyses, the male data for USNM 145708 and female data for USNM 145711 with the greatest magnitude of differences in coefficient of variation scores (comparing within sex) are selected for use in subsequent analyses.

The measurement data have such a low power of resolution to separate geographic and species samples that inclusion of the multiple measurement data from single individuals would be superfluous and were not incorporated in any discriminant function analyses.

INTRASAMPLE VARIATION

Variation of characters within samples is necessary as background information to understanding species variation. Also, for the nonsexually dimorphic morphological features, the intrasample data are evaluated to determine whether the characters demonstrate age or sex variation. If the characters vary by either age or sex, the characters need to be analyzed separately by those categories. The available data for this study do not have large sample sizes for either intra- or intersample analyses. Given that fact, there is an advantage to combining data by either age or sex in order to increase sample sizes for analysis, when it is appropriate to do so.

Ideally, the intrasample analyses should be based on specimens from single localities from throughout the geographic distribution of the complex. However, there are not adequate data from some major geographic areas. Some intrasample data sets therefore comprise data pooled from nearby localities rather than single localities. The following samples are used for intrasample variation analyses (Figure 1): (1) *Costa Rica/Panama Border* region (8 localities, $n = 72$); (2) *Panama Canal* region (23 localities, $n = 46$); (3) *Lower Río Chucunaque, Panama* region (12 localities, $n = 58$); (4) *Ilha do Maracá, Brazil* (1 locality, $n = 36$); (5) *Boca do Acre, Brazil* (1 locality, $n = 36$); and (6) *Cuzco Amazónico, Peru* (1 locality, $n = 32$).

The basic approach to analyzing the data in terms of whether the data are combined or analyzed separately by age or sex is to be conservative in combining data. That is, if the data show any reasonable evidence for demonstrating variation by age or sex, that is sufficient for intersample analyses to be analyzed separately by the appropriate categories.

The dorsal pattern data from the *Lower Río Chucunaque, Panama* are used to explain how the data were analyzed. The raw data for this character are shown in Table 2, where five categories are used to summarize occurrence data for each character state: juvenile, juvenile male, juvenile female, male, and female. As not all nonadult specimens were dissected to verify sex, the categories juvenile versus juvenile male and juvenile female are not entirely discrete, but overall they represent a size difference with specimens categorized as juveniles being in most cases smaller than those categorized as juvenile males or juvenile females. The data from Table 2 were combined by either age or sex (Table 3). The observer error analysis for dorsal pattern indicated that there had to be a greater than 33% difference

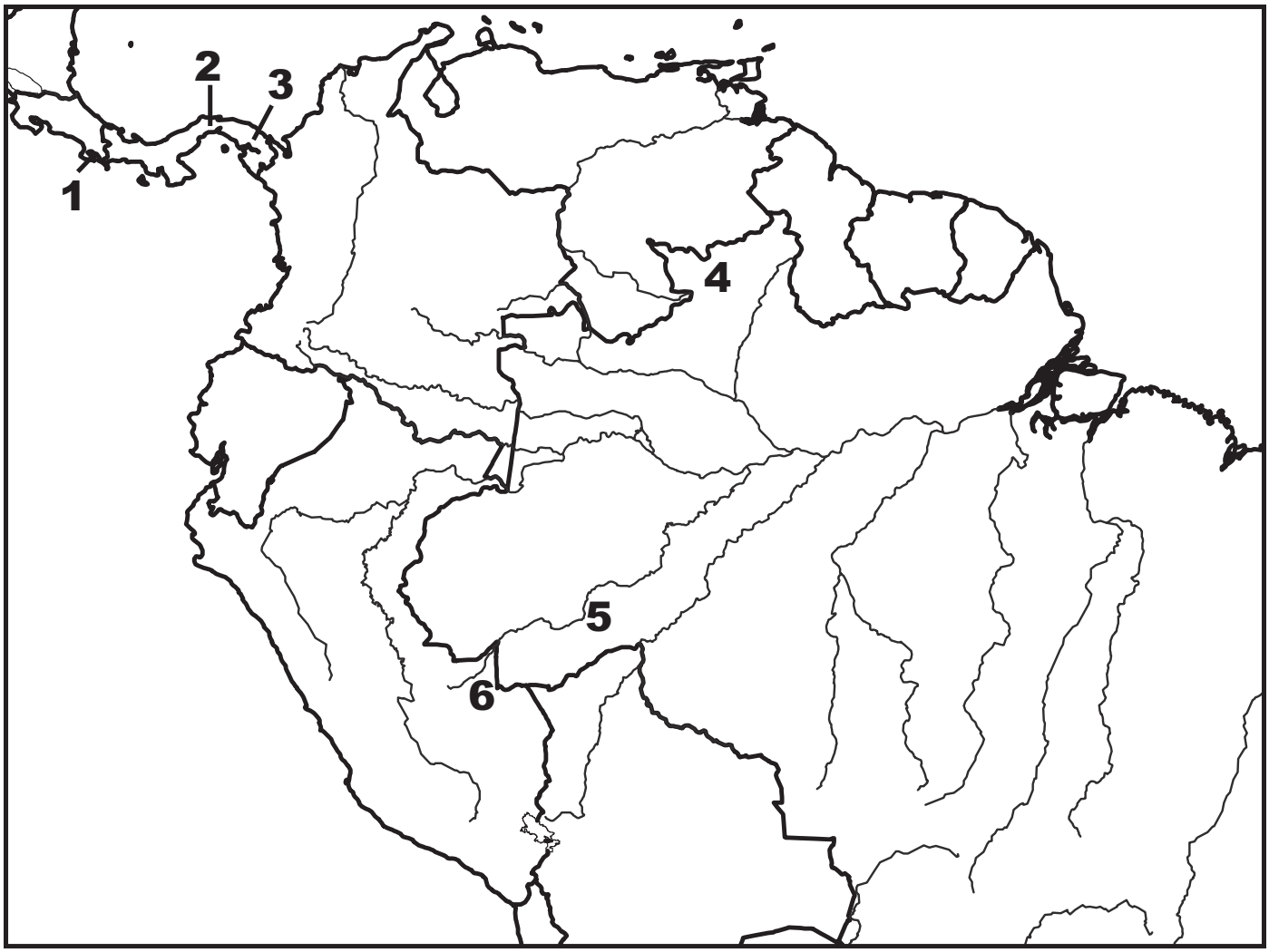

FIGURE 1. Map showing locations of specimens used for intrasample analyses: 1, *Costa Rica/Panama Border*; 2, *Panama Canal*; 3, *Lower Río Chucunaque*, *Panama*; 4, *Ilha do Maracá, Brazil*; 5, *Boca do Acre, Brazil*; and 6, *Cusco Amazónico, Peru*.

in state distributions to be meaningful. The comparison of juvenile with adult data are determined by subtracting the smaller percent value from the larger percent value for the States A, B, and C, as indicted in the formula ((37 – 5) + (13 – 0) + (82 – 63)) = 64%. Thus the dorsal pattern character state distribution for the juvenile/adult categorization exceeds the 33% threshold for observer error. For cases such as this, chi-square analyses were performed to determine whether the differences were statistically significant. In this case, only two states had expected values ≥5 (Table 4); accordingly, the chi-square analysis was performed only on the two character states with expected values ≥5. These analyses are thus conservative and need to be evaluated with that in mind. In cases where only one character state had expected values ≥5, the notation NA is used to indicate that the sample sizes are not appropriate (large enough) for statistical analysis. For the male–female data comparisons, the differences among state distributions are ((31 – 23) + (8 – 0) + (69 – 69)) = 16%, below the threshold for observer error and thus are not analyzed further. Such results are due to two causes, which cannot be accurately separated: (1) the variation reflects actual sample variation and is not meaningful, or (2) the variation recorded does not reflect the sample variation but is due to observer error.

Decisions on whether it is appropriate to combine categories of age or sex are made on evaluation of all of

TABLE 2. Dorsal pattern state occurrences (by age and sex) within the *Lower Río Chucunaque, Panama* sample State A, uniform between interocular bar (if present) and sacrum; state B, single chevron between interocular bar and sacrum without additional spots lateral to dorsal chevron; state C, pattern complex with two dorsal chevrons and spotting all over dorsum, chevrons often elongate and fused with each other and interorbital bar; J, juvenile. A dash (–) indicates no specimens demonstrated the pattern.

State	J	J♂	J♀	♂	♀
A	2	–	–	3	4
B	4	1	–	–	–
C	26	4	2	5	7

TABLE 3. Dorsal pattern state occurrences by age and sex, *Lower Río Chucunaque, Panama* sample. Numbers in parentheses are percentages by category (e.g., within juveniles). State A, uniform between interocular bar (if present) and sacrum; state B, single chevron between interocular bar and sacrum without additional spots lateral to dorsal chevron; state C, pattern complex with two dorsal chevrons and spotting all over dorsum, chevrons often elongate and fused with each other and interorbital bar.

	Age		Sex	
State	Juvenile	Adult	♂	♀
A	2 (5)	7 (37)	3 (23)	4 (31)
B	5 (13)	0 (0)	1 (8)	0 (0)
C	32 (82)	12 (63)	9 (69)	9 (69)

TABLE 4. Chi-square occurrence values for character states of the dorsal pattern, *Lower Río Chucunaque, Panama* sample. State A, uniform between interocular bar (if present) and sacrum; state B, single chevron between interocular bar and sacrum without additional spots lateral to dorsal chevron; state C, pattern complex with two dorsal chevrons and spotting all over dorsum, chevrons often elongate and fused with each other and interorbital bar.

State	Number of juveniles observed	Number of juveniles expected
A	2	6
B	5	3
C	32	30

the individual sample analyses results. In other words, an overall pattern among locality sample results is used to determine whether categories are appropriately combined or not for the intersample geographic variation analyses. This approach assumes that there is no geographic variation in whether characters vary by either age or sex (there are no data at the outset to support or refute this alternative). However, at a minimum, it is reasonable to assume that the three Middle American samples would likely demonstrate similar patterns of age or sex category variation as every herpetologist who has studied the Middle American members of the *L. bolivianus* complex in the last 50 years has thought they represent a single species. The following guidelines appear appropriate on the basis of examination of the overall results. If at least five of the samples have variation exceeding observer error and at least one of those samples is statistically significant, the categories being tested should be analyzed separately. If at least five of the samples do not exhibit variation exceeding observer error and the one that does is not statistically significant, the categories can be combined. All other instances require judgment based on whether chi-square results would likely be significant if sample sizes were increased (to realistic sample sizes).

Dorsal Pattern

The results of analyses by age and sex are shown in Table 5. The age results are equivalent in terms of the

TABLE 5. Age and sex intrasample analyses results for dorsal pattern. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	Yes	NS	No	–
Panama Canal	Yes	NS	No	–
Costa Rica/Panama Border	No	–	Yes	NS
Cuzco Amazónico, Peru	Yes	NS	Yes	NA
Boca do Acre, Brazil	Yes	NA	No	–
Ilha do Maracá, Brazil	No	–	Yes	NA

guidelines described above. The chi-square values for the three samples that exceed the observer error threshold are 0.158, 0.094, and 0.094. It is reasonable to expect that these values could reach a 5% significance level if sample sizes were increased. Therefore dorsal patterns are analyzed separately by age (juveniles versus adults) in subsequent intersample analyses. Male and female results are also equivalent. The one sample that is appropriate for statistical testing has a chi-square value of 0.371. The two samples that exceed the observer error threshold that are not appropriate for statistical analysis could easily have comparable character state distributions with changing only 2–4 state conditions. In this case, it is not probable that increasing sample sizes would lead to statistically significant results. Male and female dorsal pattern data are combined for the intrasample variation summary (Table 6) and in subsequent intersample analyses.

Subocular Spot

Application of the guidelines to the age and sex analysis results (Table 7) indicates that juvenile and adult subocular spot states should be analyzed separately, whereas the male–female dichotomy is equivocal. The three chi-square values comparing the male and female data are 0.612, 0.772, and 0.360. As it is improbable that realistically increasing sample size would result in statistical significance at the 5% level, the male and female data are combined for the intrasample variation summary (Table 6) and for subsequent intersample analyses.

Lip Stripe

The variation in results of the age and sex analyses (Table 8) mirrors that described for the subocular spot. Juvenile and adult data are best analyzed separately. The male and female category data are equivocal. The single chi-square test result for the *Boca do Acre, Brazil* data is 0.368. The distribution of occurrences of states from *Cuzco Amazónico, Peru* is almost identical and is rather similar for the *Panama Canal* sample. The male and female data are not differentiated for the intrasample summary (Table 6) or in subsequent intersample analyses.

Belly Pattern

The juvenile and adult analysis results (Table 9) are equivocal as to whether the data are best analyzed combined or separately. The three chi-square results involved are 0.348, 0.404, and 0.805. Realistically, increasing the sample sizes involved is unlikely to result in statistical significance. The juvenile and adult data are combined. Application of the guidelines for the male and female data analysis results (Table 9) yield combining of these categories as well for the intrasample summary (Table 6) and subsequent intersample analyses.

Thigh Pattern

Application of the guidelines to the age and sex analysis results (Table 10) indicates that both age and sex categories are best combined for the intrasample summary (Table 11) and in subsequent intersample analyses.

Shank Pattern

Application of the guidelines to the age and sex results indicated that both age and sex categories are best combined for the intrasample summary (Table 11) and in subsequent intersample analyses. None of the six samples analyzed met the observer error threshold for either age or sex analysis.

Dorsolateral Folds and Lateral Folds

Application of the guidelines to the age and sex analyses results for both characters (Tables 12, 13) indicate that age and sex categories are best combined for the intrasample summary (Table 11) and in subsequent intersample analyses.

Male Secondary Sexual Characters

The distributions of states among the samples are presented in Table 14.

Measurement Data

There is considerable variation in measurement value ranges within samples (Table 15). The intrasample variation in all variables far exceeds the measurement error variation.

There are unexpectedly large overlaps in body sizes (SVL) between specimens classified as juvenile and adult males and females. For the four samples with adequate data for males, the overlap in SVL between the smallest adult male and largest juvenile male ranges from 4.0 to 18.0 mm. For the two samples with adequate data for females, the overlaps range from 8.8 to 22.0 mm. The largest values in overlap between juveniles and adults in

TABLE 6. Occurrence (percent) of dorsal pattern, suborbital spot, lip pattern, and belly pattern states within samples by age and region. **Dorsal pattern states: A**, uniform between interocular bar (if present) and sacrum; **B**, single chevron between interocular bar and sacrum without additional spots lateral to dorsal chevron; **C**, pattern complex with two dorsal chevrons and spotting all over dorsum, chevrons often elongate and fused with each other and interorbital bar. **Suborbital spot states: A**, distinct; **B**, indistinct; **C**, absent. **Lip pattern states: A**, noticeable broad light stripe extending from front of snout to commissural gland, bordered below by continuous or broken markings on upper lip and either bordered above by dark line under eye or not; **B**, narrow light stripe from at least nostril to lower posterior end of eye; **C**, narrow light stripe from below front of eye through commissural gland; **D**, lip region uniform (about same intensity as background dorsal color) with or without a dark stripe under the eye and/or dark bars/mottling on the upper lip. **Belly pattern states: A**, belly speckled, spotted, or mottled anteriorly only or with an anterior-posterior gradient; **B**, belly boldly mottled anteriorly only or with an anterior-posterior gradient; **C**, belly speckled or mottled with same intensity over entire belly; **D**, belly boldly mottled with same intensity over entire belly; **E**, belly without a pattern.

Dorsal pattern			Suborbital spot			Lip pattern			Belly pattern	
State	Juveniles	Adults	State	Juveniles	Adults	State	Juveniles	Adults	State	Juvenile/Adult data combined
				Costa Rica/Panama Border						
	(*n* = 45)	(*n* = 27)		(*n* = 45)	(*n* = 27)		(*n* = 45)	(*n* = 27)		(*n* = 72)
A	2	15	A	84	30	A	93	26	A	56
B	18	3	B	16	59	B	0	0	B	4
C	80	81	C	0	11	C	0	0	C	19
						D	7	74	D	19
									E	1
				Panama Canal						
	(*n* = 29)	(*n* = 17)		(*n* = 29)	(*n* = 17)		(*n* = 29)	(*n* = 17)		(*n* = 45)
A	10	41	A	69	47	A	52	24	A	58
B	10	29	B	24	41	B	0	0	B	4
C	80	29	C	7	12	C	3	0	C	20
						D	45	76	D	7
									E	11
				Lower Río Chucunaque, Panama						
	(*n* = 39)	(*n* = 19)		(*n* = 39)	(*n* = 19)		(*n* = 39)	(*n* = 19)		(*n* = 58)
A	5	37	A	64	90	A	51	37	A	50
B	13	0	B	31	10	B	0	0	B	0
C	82	63	C	5	0	C	8	0	C	45
						C/D	5	0	D	2
						D	36	63	E	3
				Ilha do Maracá, Brazil						
	(*n* = 28	(*n* = 8)		(*n* = 28)	(*n* = 8)		(*n* = 28)	(*n* = 8)		(*n* = 36)
A	11	25	A	71	50	A	7	0	A	25
B	25	12	B	0	0	B	0	0	A/B	3
C	64	62	C	29	50	C	0	0	B	39
						C/D	7	12	C	14
						D	86	88	D	19
									E	0

(*continued*)

TABLE 6. (*Continued*)

Dorsal pattern			Suborbital spot			Lip pattern			Belly pattern	
State	Juveniles	Adults	State	Juveniles	Adults	State	Juveniles	Adults	State	Juvenile/Adult data combined
				Boca do Acre, Brazil						
	(*n* = 12)	(*n* = 24)		(*n* = 12)	(*n* = 24)		(*n* = 12)	(*n* = 24)		(*n* = 36)
A	8	4	A	42	83	A	17	42	A	83
B	33	71	B	0	0	B	0	0	B	0
C	58	25	C	58	17	C	58	17	C	0
						C/D	8	33	D	3
						D	17	8	E	14
				Cuzco Amazónico, Peru						
	(*n* = 16)	(*n* = 16)		(*n* = 16)	(*n* = 16)		(*n* = 16)	(*n* = 16)		(*n* = 31)
A	6	31	A	88	62	A	93	50	A	90
B	19	44	B	12	31	B	0	0	B	0
C	75	25	C	0	6	C	7	25	C	10
						D	0	25	D	0
									E	0

TABLE 7. Age and sex intrasample analyses results for subocular spot. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	Yes	NS	Yes	NS
Panama Canal	Yes	NS	No	–
Costa Rica/Panama Border	Yes	0.012	Yes	NS
Cuzco Amazónico, Peru	Yes	NA	Yes	NA
Boca do Acre, Brazil	Yes	NA	Yes	NA
Ilha do Maracá, Brazil	No	–	Yes	NS

TABLE 8. Age and sex intrasample analyses results for lip stripe pattern. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with; a dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	Yes	NS	No	–
Panama Canal	Yes	NS	Yes	NA
Costa Rica/Panama Border	Yes	0.0004	No	–
Cuzco Amazónico, Peru	Yes	NA	Yes	NA
Boca do Acre, Brazil	Yes	NS	Yes	NS
Ilha do Maracá, Brazil	No	–	No	–

this study noticeably exceed those from a study of the *Leptodactylus podicipinus–L. wagneri* complex (Heyer, 1994:4–9). The overlaps are comparable for unpublished data for large species of the *Leptodactylus pentadactylus* cluster (e.g., *L. knudseni* juvenile-adult male SVL overlap 26.6 mm, juvenile-adult female SVL overlap 11.0 mm; *L. labyrinthicus* juvenile-adult male SVL overlap 22.4 mm; juvenile-adult female SVL overlap 11.0 mm; *L. savagei* juvenile-adult male SVL overlap 29.0 mm.). These data suggest at least two nonexclusive explanations: (1) the morphological proxies used to differentiate adults from juveniles may not be consistently true and

TABLE 9. Age and sex intrasample analyses results for belly pattern. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed; c/w = compared with.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	No	–	No	–
Panama Canal	Yes	NS	Yes	NS
Costa Rica/Panama Border	Yes	–	No	–
Cuzco Amazónico, Peru	No	NS	No	–
Boca do Acre, Brazil	No	NS	No	–
Ilha do Maracá, Brazil	Yes	–	No	–

TABLE 10. Age and sex intrasample analyses results for thigh pattern. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	No	–	No	–
Panama Canal	No	–	No	–
Costa Rica/Panama Border	No	–	Yes	NA
Cuzco Amazónico, Peru	No	–	No	–
Boca do Acre, Brazil	No	–	No	–
Ilha do Maracá, Brazil	Yes	NA	No	–

(2) the variation observed in *Leptodactylus* is related to overall adult size.

INTERSAMPLE VARIATION

After mapping localities, 20 regional samples were chosen to analyze the nature of variation among them (Figure 2). The sampling strategy was to maximize both geographic coverage and sample sizes. This strategy was not possible in some areas of coverage, and smaller than desired samples are included because of their importance in geographic representation or potential nomenclatural impact.

Patterns and Folds

The approach used to analyze intersample variation for the morphological characters involving patterns and folds follows that used in the analysis of intrasample variation. Any comparisons between samples are first analyzed to determine whether the amount of variation exceeds observer error. For the comparisons that exceed observer error, chi-square tests were used when appropriate to determine statistical significance. Juvenile and adult data are analyzed separately for dorsal pattern, subocular spot, and lip stripe characters. Juvenile and adult data are combined for analysis of belly pattern, thigh pattern, shank pattern, dorsolateral fold, and lateral fold characters.

Rather than make comparisons between all pairs of samples for the characters involved, three networks of samples were used because the purpose of this evaluation is to determine whether the characters demonstrate any patterns of geographic variation. There are three major geographic areas occupied by the *Leptodactylus bolivianus* complex: (1) Middle America, for which three mainland samples and two island samples, one Atlantic and one Pacific were selected; (2) populations predominantly occurring in South American river basins draining to the Caribbean for which eight samples were selected (the *Northern Roraima* sample is geographically near the rest of the samples but is in an Amazonian drainage area); and (3) populations occurring in river basins draining to the Amazon River, for which six samples were selected. Geographically proximate samples are compared among these three major geographic areas to determine overall patterns of differentiation.

The full set of analytic results are shown for the first character analyzed as an example of the comparisons that exceed observer error, those for which χ^2 analyses are inappropriate (only one state with an expected sample size of 5 or greater), and the level and variation of χ^2 values when χ^2 values were appropriate. Results for the other characters are available from WRH on request and will be placed in the Smithsonian Institution Archives by 1 January 2012.

Dorsal Pattern

There are no significant differences among the three mainland Middle American samples (Table 16). The *Gulf of Panama* sample does not differ from the *Panama Canal*

TABLE 11. Occurrence (percent) of thigh pattern, shank pattern, dorsolateral fold, and lateral fold states within region samples. **Thigh pattern states: A**, posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for entire extent of posterior thigh; **B**, posterior thigh boldly mottled with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced. **Shank pattern states: A**, uniform; **B**, short dark transverse bars extending less than halfway across dorsal surface of shank; **C**, at least one dark transverse bar extending halfway or more across the dorsal surface of the shank. **Dorsolateral folds states: A**, dorsolateral fold distinct and continuous from supratympanic fold to end of body, or with a brief interruption of the fold, or the fold distinct only from the supratympanic fold to the sacrum; **B**, dorsolateral fold with several interruptions to indescernable (including lack of fold due to poor preservation). **Lateral folds states: A**, lateral fold distinct or slightly interrupted from supratympanic fold to leg; **B**, lateral fold distinct only in groin region, indistinct overall, or indiscernible (including due to poor preservation).

Thigh pattern		Shank pattern		Dorsolateral folds		Lateral folds	
State	Occurrence (%)	State	Occurrence (%)	State	Occurrence (%)	State	Occurrence (%)
			Costa Rica/Panama Border				
	(*n* = 72)		(*n* = 72)		(*n* = 72)		(*n* = 72)
A	17	A	7	A	71	A	29
A/B*	1	B	28	B	29	B	71
B	81	C	65				
			Panama Canal				
	(*n* = 46)		(*n* = 46)		(*n* = 45)		(*n* = 45)
A	0	A	2	A	67	A	29
A/B*	6	B	50	B	33	B	71
B	93	B/C*	2				
		C	46				
			Lower Río Chucunaque, Panama				
	(*n* = 58)		(*n* = 58)		(*n* = 58)		(*n* = 58)
A	0	A	0	A	81	A	57
A/B*	3	B	24	B	19	B	43
B	97	C	76				
			Ilha do Maracá, Brazil				
	(*n* = 36)		(*n* = 36)		(*n* = 36)		(*n* = 36)
A	8	A	0	A	100	A	0
A/B*	14	B	42	B	0	B	100
B	78	B/C*	3				
		C	56				
			Boca do Acre, Brazil				
	(*n* = 36)		(*n* = 36)		(*n* = 36)		(*n* = 33)
A	100	A	3	A	97	A	30
B	0	B	78	B	3	B	70
		C	19				
			Cuzco Amazónico, Peru				
	(*n* = 32)		(*n* = 32)		(*n* = 32)		(*n* = 32)
A	100	A	0	A	100	A	12
B	0	B	38	B	0	B	88
		C	62				

*Indicates a condition that is intermediate between the two states.

TABLE 12. Age and sex intrasample analyses results for dorsolateral folds. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	Yes	NS	No	–
Panama Canal	No	–	Yes	NS
Costa Rica/Panama Border	Yes	NS	No	–
Cuzco Amazónico, Peru	No	–	No	–
Boca do Acre, Brazil	No	–	No	–
Ilha do Maracá, Brazil	No	–	No	–

TABLE 13. Age and sex intrasample analyses results for lateral folds. Definitions: >OE, observer error threshold passed; NS, chi-square results not statistically significant at ≥0.05 level; NA, data inadequate for chi-square analysis; c/w = compared with. A dash (–) indicates that it would be inappropriate to perform a significance test as the observer error threshold was not passed.

	Juveniles c/w Adults		Females c/w Males	
Sample	>OE	Significance	>OE	Significance
Lower Río Chucunaque, Panama	Yes	NS	No	–
Panama Canal	No	–	Yes	NS
Costa Rica/Panama Border	No	–	No	–
Cuzco Amazónico, Peru	No	–	Yes	NA
Boca do Acre, Brazil	No	–	No	–
Ilha do Maracá, Brazil	No	–	No	–

sample but does differ from the other two mainland samples. The (Pacific) *Gulf of Panama* sample does not differ from the (Caribbean) *San Andrés/Providencia* sample. The *San Andrés/Providencia* sample additionally does not differ from the *Panama Canal* sample, but does differ from the *Southern Costa Rica/Eastern Panama* and *Darién* samples.

The geographically adjacent *Darién* and *Northern Magdalena Drainage* samples do not differ from each other (Table 16).

Among the primarily Caribbean drainage samples, there are only two statistically significant results: juvenile *Northern Guyana* data compared with juvenile *Suriname* data (Table 16 sample comparisons K:M) and juvenile *Northern Guyana* data compared with juvenile *Northern Roraima* data (Table 16 sample comparisons K:N). As there are 72 comparisons within this network of samples, having two statistically significant results is well within statistical expectations.

There are no statistically significant differences between the *Meta Drainage* and the Amazonian drainage samples (*Rio Solimões/Amazonas*, *Río Ucayali*, *Madre de Dios*, *Lower Rio Purús/Rio Acre*, *Middle/Lower Río Madeira*, *South Central Bolivia*) (Table 16). There is only one statistically significant difference in the juvenile data for the *Northern Roraima–South Central Bolivia* comparison (Table 16, sample comparisons N:T). There are no compelling data to favor the *Meta Drainage* sample or the *Northern Roraima* sample as showing a pattern in common with the rest of the Amazonian drainage samples.

There are no statistically significant differences among the Amazonian drainage network of samples (Table 16).

Subocular Spot

There are no demonstrable differences among juveniles in the Middle American network samples. The adult data show differences between the mainland samples *Southern Costa Rica/Eastern Panama* compared with the *Darién* data ($\chi^2 = 0.001$), and the *Panama Canal* sample compared with the *Darién* data ($\chi^2 = 0.020$). The adult data also differ between the *San Andrés/Providencia* sample and the *Southern Costa Rica/Eastern Panama* sample ($\chi^2 = 0.035$) and with the *Darién* sample ($\chi^2 = 0.001$).

The adult data differ between the samples that geographically link the *Darién* sample with the *Northern Magdalena Drainage* sample ($\chi^2 = 0.044$). Juvenile data are distinct for the following comparisons: *Middle-Southern Magdalena Drainage* sample compared with the *Northern Roraima* Sample ($\chi^2 = 0.033$); *Meta Drainage* sample compared with the *Northern Roraima* sample ($\chi^2 = 0.006$); *Southern Guyana* sample compared with the *Suriname* sample ($\chi^2 = 0.022$); and the *Southern Guyana* sample compared with the *Northern Roraima* sample ($\chi^2 = 0.004$). Adult data are distinct for the following comparisons: *Northern Magdalena Drainage* sample compared with the *Meta Drainage* sample ($\chi^2 = 0.042$), *Meta Drainage* sample with the *Southern Guyana* sample ($\chi^2 = 0.003$), *Meta Drainage* sample with the *Suriname* sample ($\chi^2 = 0.009$), and the *Meta Drainage* sample with the *Northern*

TABLE 14. Male secondary sexual character state occurrences (percent) within samples. **Arm states: A**, arm not hypertrophied; **B**, arm hypertrophied. **Lower jaw tubercle states: A**, absent; **B**, tuberculate. **Chest states: A**, no patch of tubercles; **B**, central patch of tubercles; **C**, lateral patches of tubercles in addition to central patch. **Thumb spine states: A**, one modest chisel-shaped spine; **B**, one hypertrophied chisel-shaped spine; **C**, two round spines. **Tympanum states: A**, annulus lacking tubercles; **B**, annulus tuberculate. A dash (–) indicates no state or no percent occurrence for samples with $n = 0$.

Arm hypertrophy		Jaw tubercles		Chest tubercles		Thumb spines		Tympanum tubercles	
State	Value	State	Value	State	Value	State	Value	State	Value
				Costa Rica–Panama Border					
	$n = 15$		$n = 12$		$n = 15$		$n = 15$		$n = 15$
A	33	A	67	A	53	A	0	A	0
B	67	B	33	B	47	B	0	B	100
				C	0	C	100		
				Panama Canal					
	$n = 11$		$n = 10$		$n = 11$		$n = 11$		$n = 11$
A	64	A	80	A	64	A	0	A	0
B	36	B	20	B	36	B	0	B	100
				C	0	C	100		
				Lower Río Chucunaque, Panama					
	$n = 8$		$n = 7$		$n = 8$		$n = 8$		$n = 8$
A	50	A	57	A	38	A	0	A	25
B	50	B	43	B	62	B	0	B	75
				C	0	C	100		
				Ilha do Maracá, Brazil					
	$n = 1$		$n = 0$		$n = 1$		$n = 1$		$n = 0$
A	100	–	–	A	100	A	100	–	–
B	0	–	–	B	0	B	0	–	–
				C	0	C	0		
				Boca do Acre, Brazil					
	$n = 4$		$n = 2$		$n = 4$		$n = 4$		$n = 4$
A	75	A	50	A	100	A	25	A	0
B	25	B	50	B	0	B	75	B	100
				C	0	C	0		
				Cuzco Amazónico, Peru					
	$n = 5$		$n = 4$		$n = 5$		$n = 5$		$n = 4$
A	0	A	25	A	20	A	0	A	0
B	100	B	75	B	20	B	100	B	100
				C	60	C	0		

Roraima sample ($\chi^2 = 0.027$). Overall, the *Meta Drainage* sample stands out as being distinctive within this network of samples.

The geographically network-linking *Meta Drainage* sample differs from both the *Rio Solimões/Amazonas* sample (adult data, $\chi^2 = 0.006$) and the *Madre de Dios* sample (adult data, $\chi^2 = 0.004$). The *Northern Roraima* sample differs from the *Madre de Dios* sample (juvenile data $\chi^2 = 0.011$), the *Lower Purús/Rio Amazonas* sample (adult data, $\chi^2 = 0.003$), and the *South Central Bolivia*

TABLE 15. Intrasample variation in measurement data. Definitions: *n*, sample size; A, *Lower Río Chucunaque, Panama* sample; B, *Panama Canal* sample; C, *Costa Rica–Panama Border* sample; D, *Cuzco Amazónico, Peru* sample; E, *Boca do Acre, Brazil* sample; F, *Ilha do Maracá, Brazil* sample; Min, minimum value (mm); Max, maximum value (mm); Range, range of values (mm); SVL, snout–vent length; HL, head length; HW, head width; EN, eye–nostril distance; TD, tympanum diameter. A dash (–) indicates there is only one individual in the sample.

		Males				Females			
Variable	**Sample**	***n***	**Min**	**Max**	**Range**	***n***	**Min**	**Max**	**Range**
SVL	A	9	78.9	97.9	19.0	16	75.5	89.3	13.8
	B	11	67.8	101.3	33.5	6	79.0	95.5	16.5
	C	15	76.0	92.8	16.8	12	74.5	86.8	12.3
	D	5	106.2	116.0	9.8	11	77.9	99.8	21.9
	E	4	79.0	97.4	18.4	20	63.0	86.3	23.3
	F	1	–	86.0	–	7	66.0	95.5	29.5
HL	A	9	29.1	36.2	7.1	16	28.4	32.0	3.6
	B	11	25.2	34.7	9.5	6	28.1	35.1	7.0
	C	15	28.4	33.3	4.9	11	26.4	32.4	6.0
	D	5	36.6	40.4	3.8	11	29.3	34.4	5.1
	E	4	28.7	37.3	8.6	20	24.0	32.7	8.7
	F	1	–	32.5	–	7	25.5	34.0	8.5
HW	A	9	27.1	36.9	9.8	16	25.0	29.2	4.2
	B	11	23.3	36.5	13.2	6	26.3	32.7	6.4
	C	15	26.0	33.1	7.1	12	25.0	28.6	3.6
	D	5	35.2	40.4	5.2	11	23.8	31.5	7.7
	E	4	24.2	31.0	6.8	20	20.0	28.1	8.1
	F	1	–	29.5	–	7	22.0	31.0	9.0
EN	A	9	8.5	9.5	1.0	16	7.7	9.4	1.7
	B	11	7.4	9.9	2.5	6	7.8	9.8	2.0
	C	15	8.0	9.8	1.8	12	7.5	9.0	1.5
	D	5	11.0	11.7	0.7	11	8.3	9.7	1.4
	E	4	7.8	9.8	2.0	20	6.5	8.7	2.2
	F	1	–	9.0	–	7	7.0	9.0	2.0
TD	A	9	5.5	7.1	1.6	16	5.2	6.2	1.0
	B	11	5.0	7.8	2.8	6	5.6	6.6	1.0
	C	15	5.2	6.4	1.2	12	5.2	6.3	1.1
	D	5	6.8	8.5	1.7	11	6.0	7.2	1.2
	E	4	5.8	7.6	1.8	20	4.5	6.5	2.0
	F	1	–	6.0	–	7	5.0	6.5	1.5
Thigh	A	9	37.3	43.0	5.7	15	32.3	41.0	8.7
	B	11	30.4	48.7	18.3	6	36.3	46.7	10.4
	C	15	33.8	42.0	8.2	12	30.9	39.2	8.3
	D	5	42.8	56.7	13.9	11	36.1	45.0	8.9
	E	4	37.8	47.1	9.3	20	30.0	39.8	9.8
	F	1	–	46.5	–	7	30.5	46.0	15.5
Shank	A	9	40.8	46.7	5.9	15	37.2	44.3	7.1
	B	11	34.8	49.0	14.2	6	41.3	48.9	7.6
	C	15	38.1	45.6	7.5	12	36.6	43.5	6.9
	D	5	51.6	58.5	6.9	11	40.6	48.2	7.6
	E	4	41.5	51.3	9.8	20	33.5	47.5	14.0
	F	1	–	46.5	–	7	36.5	50.0	13.5
Foot	A	9	39.8	46.6	6.8	16	38.1	44.4	6.3
	B	11	37.2	48.8	11.6	6	40.2	50.2	10.0
	C	15	37.8	45.5	7.7	12	37.6	44.7	7.1
	D	5	53.5	60.3	6.8	11	42.8	50.2	7.4
	E	4	43.5	52.4	8.9	20	35.0	46.6	11.6
	F	1	–	48.0	–	7	37.0	49.0	12.0

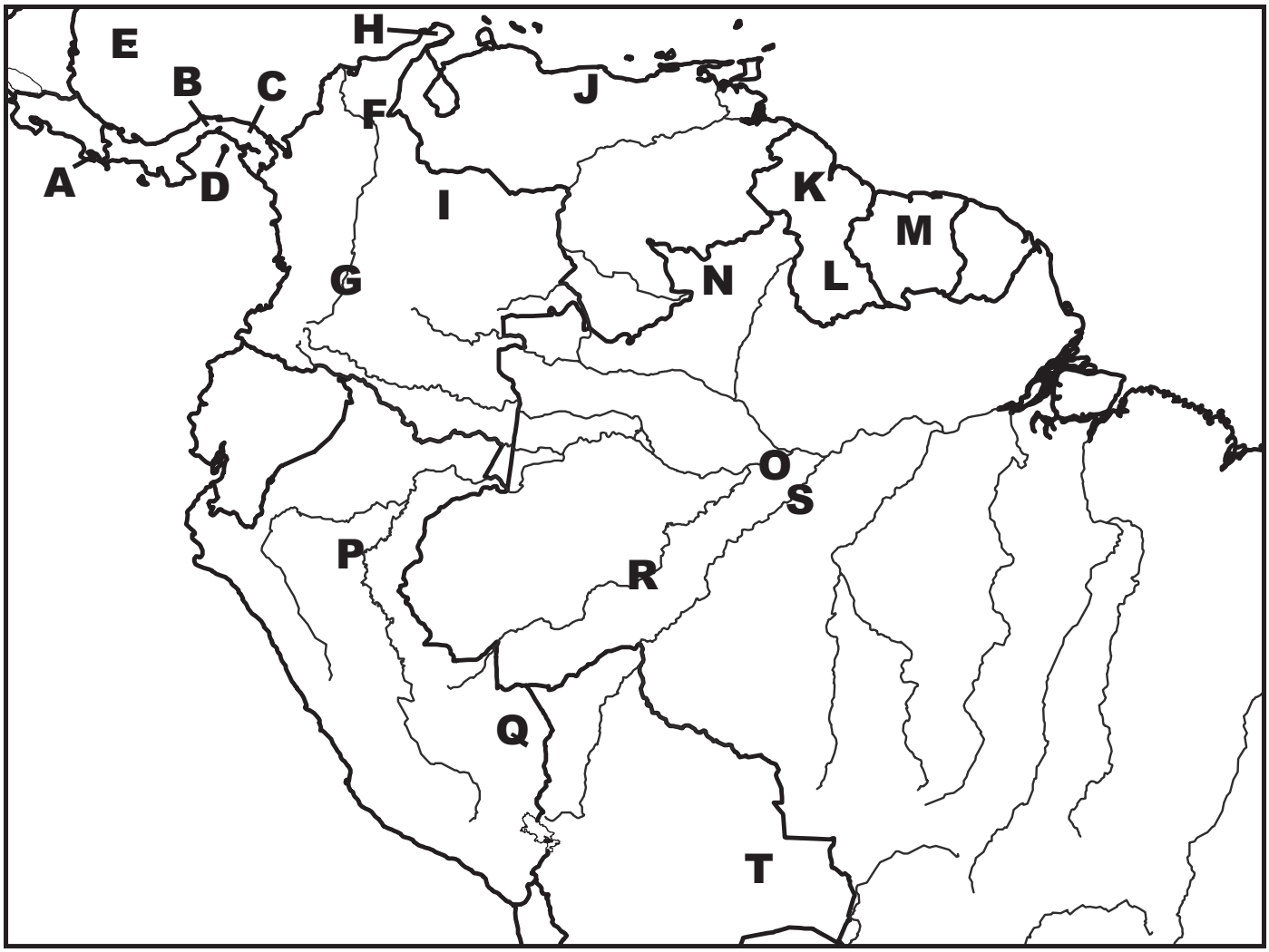

FIGURE 2. Map showing locations of 20 regional samples. A, *Costa Rica/Panama Border*; B, *Panama Canal*; C, *Darién*; D, *Gulf of Panama*; E, *San Andrés/Providencia*; F, *Northern Magdalena Drainage*; G, *Middle/Southern Magdalena Drainage*; H, *Santa Marta*; I, *Meta Drainage*; J, *Caracas*; K, *Northern Guyana*; L, *Southern Guyana*; M, *Suriname*; N, *Northern Roraima*; O, *Rio Solimões/Amazonas*; P, *Río Ucuyali*; Q, *Madre de Dios*; R, *Lower Rio Purus/Rio Acre*; S, *Middle/Lower Rio Madeira*; and T, *South Central Bolivia*.

sample (juvenile data, $\chi^2 = 0.001$). Both the *Meta Drainage* and *Northern Roraima* samples are weakly differentiated (at best) from the remaining Amazonian drainage samples.

Within the Amazonian drainage network exclusive of the *Northern Roraima* sample, the following comparisons are distinct: *Rio Solimões/Rio Amazonas* with the *Lower Rio Purús/Rio Acre* samples (adult data, $\chi^2 = 0.012$); *Madre de Dios* with the *South Central Bolivia* samples (juvenile data, $\chi^2 = 0.003$); and the *Lower Rio Purús/Rio Acre* with the *South Central Bolivia* samples (adult data, $\chi^2 = 0.032$).

Lip Stripe

There are only two statistically significant different comparisons within the Middle American network of samples: *Southern Costa Rica/Eastern Panama* with the *Panama Canal* samples (juvenile data, $\chi^2 = 0.016$) and the *Southern Costa Rica/Eastern Panama* with the *Darién* samples (juvenile data, $\chi^2 = 0.010$).

The data do not statistically differ between the network-linking *Darién* and *Northern Magdalena Drainage* samples.

TABLE 16. Intersample comparisons for dorsal pattern data. Significant chi-square (χ^2) values are in italic font and followed by an asterisk. Sample definitions: A, *Southern Costa Rica/Eastern Panama*; B, *Panama Canal*; C, *Darién*; D, *Gulf of Panama*; E, *San Andrés/Providencia*; F, *Northern Magdalena Drainage*; G, *Middle/Southern Magdalena Drainage*; H, *Santa Marta*; I, *Meta Drainage*; J, *Caracas*; K, *Northern Guyana*; L, *Southern Guyana*; M, *Suriname*; N, *Northern Roraima*; O, *Rio Solimões/Amazonas*; P, *Río Ucayali*; Q, *Madre de Dios*; R, *Lower Rio Purús/Rio Acre*; S, *Middle/Lower Rio Madeira*; T, *South Central Bolivia*. Other abbreviations: *n*, sample size; NA, samples not appropriate for chi-square analysis. A dash (–) indicates that it was inappropriate to perform a significance test as the observer error threshold was not passed.

	Juvenile data			Adult data				Juvenile data			Adult data		
Sample pair comparisons	*n*	Exceeds observer error	χ^2	*n*	Exceeds observer error	χ^2	Sample pair comparisons	*n*	Exceeds observer error	χ^2	*n*	Exceeds observer error	χ^2
A–B	49, 31	No	–	29, 21	Yes	0.111	J–K	30, 22	Yes	0.486	27, 14	Yes	0.235
A–C	49, 79	No	–	29, 49	Yes	0.102	J–L	30, 39	No	–	27, 18	Yes	0.214
B–C	31, 79	No	–	21, 49	Yes	0.343	J–M	30, 15	Yes	0.541	27, 13	Yes	0.628
A–D	49, 2	Yes	0.691	49, 21	Yes	***0.021****	J–N	30, 46	No	–	27, 23	Yes	0.614
B–D	31, 2	Yes	0.324	21, 21	Yes	0.296	K–L	22, 39	Yes	0.155	14, 18	Yes	0.434
C–D	79, 2	Yes	0.793	49, 21	Yes	***0.029****	K–M	22, 15	Yes	***0.042****	14, 13	Yes	0.248
D–E	2, 7	Yes	NA	21, 29	Yes	0.292	K–N	22, 46	Yes	***0.036****	14, 23	Yes	0.700
A–E	49, 7	Yes	NA	29, 29	Yes	***0.007****	L–M	39, 15	Yes	0.619	18, 13	No	–
B–E	31, 7	Yes	NA	21, 29	No	–	L–N	39, 46	No	–	18, 23	Yes	0.646
C–E	79, 7	Yes	NA	49, 29	Yes	***0.009****	M–N	15, 46	Yes	0.481	13, 23	Yes	0.754
C–F	79, 20	Yes	0.390	49, 23	No	–	I–O	6, 9	Yes	NA	12, 9	Yes	NA
F–G	20, 75	No	–	23, 85	No	–	I–P	6, 18	No	–	12, 8	Yes	NA
F–H	20, 64	No	–	23, 45	Yes	0.313	I–Q	6, 73	No	–	12, 19	Yes	0.857
F–I	20, 6	Yes	NA	23, 12	Yes	0.638	I–R	6, 13	No	–	12, 29	Yes	0.260
F–J	20, 30	No	–	23, 27	No	–	I–S	6, 2	Yes	NA	12, 8	Yes	NA
F–K	20, 22	Yes	0.338	23, 14	Yes	0.497	I–T	6, 51	Yes	NA	12, 7	Yes	NA
F–L	20, 39	No	–	23, 18	Yes	0.497	N–O	46, 9	Yes	0.582	23, 9	Yes	0.662
F–M	20, 15	Yes	0.222	23, 13	Yes	0.781	N–P	46, 18	No	–	23, 8	Yes	0.597
F–N	20, 46	No	–	23, 23	No	–	N–Q	46, 73	Yes	0.092	23, 19	No	–
G–H	75, 64	No	–	23, 45	Yes	0.411	N–R	46, 13	No	–	23, 29	Yes	0.098
G–I	75, 6	Yes	0.978	23, 12	Yes	0.792	N–S	46, 2	Yes	1.000	23, 8	Yes	0.814
G–J	75, 30	No	–	23, 27	No	–	N–T	46, 51	Yes	***0.033****	23, 7	Yes	0.875
G–K	75, 22	Yes	0.186	23, 14	Yes	0.663	O–P	9, 18	Yes	NA	9, 8	Yes	NA
G–L	75, 39	No	–	23, 18	Yes	0.507	O–Q	9, 73	Yes	0.356	9, 19	Yes	0.706
G–M	75, 15	No	–	23, 13	Yes	0.838	O–R	9, 13	Yes	0.545	9, 29	Yes	0.600
G–N	75, 46	No	–	23, 23	Yes	0.866	O–S	9, 2	No	–	9, 8	Yes	NA
H–I	20, 6	Yes	0.963	23, 12	Yes	0.524	O–T	9, 51	Yes	0.304	9, 7	Yes	NA
H–J	20, 30	No	–	23, 27	Yes	0.556	P–Q	18, 73	No	–	8, 19	Yes	0.414
H–K	20, 22	Yes	0.174	23, 14	Yes	0.505	P–R	18, 13	No	–	8, 29	Yes	0.226
H–L	20, 39	No	–	23, 18	Yes	0.152	P–S	18, 2	Yes	1.000	8, 8	Yes	NA
H–M	20, 15	No	–	23, 13	Yes	0.451	P–T	18, 51	Yes	0.221	8, 7	Yes	NA
H–N	20, 46	No	–	23, 23	Yes	0.630	Q–R	73, 13	Yes	0.483	19, 29	Yes	0.475
I–J	6, 30	Yes	0.915	12, 27	Yes	0.343	Q–S	73, 2	Yes	0.743	19, 8	No	–
I–K	6, 22	Yes	0.758	12, 14	No	–	Q–T	73, 51	No	–	19, 7	No	–
I–L	6, 39	No	–	12, 18	Yes	0.480	R–S	13, 2	Yes	NA	29, 8	No	–
I–M	6, 15	Yes	NA	12, 13	Yes	0.352	R–T	13, 51	Yes	0.304	29, 7	Yes	0.814
I–N	6, 46	No	–	12, 23	No	–	S–T	2, 51	Yes	0.637	8, 7	No	–

The data statistically differ between several of the primarily Caribbean draining network samples: *Northern Magdalena Drainage* with *Southern Guyana* (adult data, $\chi^2 = 0.018$); *Northern Magdalena Drainage* with *Suriname* (adult data, $\chi^2 = 0.018$); *Middle/Southern Magdalena Drainage* with *Northern Guyana* (adult data, $\chi^2 = 0.018$); *Santa Marta* with *Northern Guyana* (juvenile data, $\chi^2 < 0.001$, adult data, $\chi^2 = 0.001$); *Santa Marta* with *Southern Guyana* (juvenile data, $\chi^2 = 0.003$, adult data, $\chi^2 = 0.025$); *Santa Marta* with *Suriname* (adult data, $\chi^2 = 0.025$); *Meta Drainage* with *Northern Guyana* (adult data, $\chi^2 = 0.002$); *Meta Drainage* with *Southern Guyana* (adult data, $\chi^2 = 0.035$); *Caracas* with *Northern Guyana* (juvenile data, $\chi^2 = 0.009$; adult data, $\chi^2 = 0.012$); *Northern Guyana* with *Southern Guyana* (adult data, $\chi^2 = 0.035$); *Northern Guyana* with *Northern Roraima* (juvenile data, $\chi^2 < 0.001$; adult data, $\chi^2 = 0.001$); *Southern Guyana* with *Northern Roraima* (juvenile data, $\chi^2 < 0.001$), and *Suriname* with *Northern Roraima* (adult data, $\chi^2 = 0.053$). The amount of demonstrable variation in this network clearly exceeds any variation that could be accounted for by chance alone.

The network-linking *Meta Drainage* sample differs from the *Madre de Dios* sample (adult data, $\chi^2 = 0.052$) and the *Lower Rio Purús/Rio Acre* sample (juvenile data, $\chi^2 = 0.018$; adult data, $\chi^2 = 0.021$). The *Northern Roraima* sample differs from the *Rio Solimões/Rio Amazonas* sample (adult data, $\chi^2 = 0.031$), *Madre de Dios* (juvenile data, $\chi^2 < 0.001$; adult data, $\chi^2 = 0.035$), *Lower Rio Purús/Rio Acre* (juvenile data, $\chi^2 = 0.054$; adult data, $\chi^2 < 0.001$), and *South Central Bolivia* (juvenile data, $\chi^2 < 0.001$) samples. There seems to be more differentiation between the *Northern Roraima* sample with the other Amazonian drainage samples than between the *Meta Drainage* sample and the primarily Amazonian network samples.

Within the primarily Amazonian drainage sample network, there are only two statistically significant comparisons: the *Río Ucayali Drainage* sample with the *Lower Rio Purús/Rio Acre* sample (juvenile data, $\chi^2 = 0.010$), and the *Lower Rio Purús/Rio A*cre sample with the *South Central Bolivia* sample (juvenile data, $\chi^2 = 0.040$).

Belly Pattern

There are no significant differences among the Middle American samples or between the network-linking *Darién* with the *Northern Magdalena Drainage* samples.

There are several statistically significant different comparisons within the primarily Caribbean drainage network samples: *Northern Magdalena Drainage* with *Northern Guyana* ($\chi^2 = 0.002$), *Northern Magdalena Drainage* with *Southern Guyana* ($\chi^2 = 0.001$), *Northern Magdalena Drainage* with *Northern Roraima* ($\chi^2 < 0.001$), *Middle/Southern Magdalena Drainage* with *Northern Guyana* ($\chi^2 = 0.004$), *Middle/Southern Magdalena Drainage* with *Southern Guyana* ($\chi^2 < 0.001$), *Middle/Southern Magdalena Drainage* with *Northern Roraima* ($\chi^2 < 0.001$), *Santa Marta* with *Northern Guyana* ($\chi^2 = 0.010$), *Santa Marta* with *Southern Guyana* ($\chi^2 = 0.001$), *Santa Marta* with *Northern Roraima* ($\chi^2 < 0.001$), and *Caracas* with *Southern Guyana* ($\chi^2 = 0.030$). In general, there is a contrast between coastal and river valley samples from Colombia and Venezuela with the Guyana and Northern Roraima samples.

There are no statistically significant differences between the network-linking *Meta Drainage* sample with the Amazonian network samples. In contrast, there are three statistically significant differences among the *Northern Roraima* and other Amazonian network samples: *Madre de Dios* ($\chi^2 < 0.001$), Lower Rio Purús/Rio Acre ($\chi^2 = 0.001$), and *South Central Bolivia* ($\chi^2 < 0.001$).

There are no statistically significant differences among the primarily Amazonian network samples.

Thigh Pattern

There are no significant differences among the Middle American samples or between the network-linking *Darién* with the *Northern Magdalena Drainage* samples.

There are statistically significant differences between several of the primarily Caribbean network samples: *Northern Magdalena Drainage* with *Meta Drainage* ($\chi^2 = 0.003$), *Northern Magdalena Drainage* with *Southern Guyana* ($\chi^2 < 0.001$), *Middle/Southern Magdalena Drainage* with *Southern Guyana* ($\chi^2 < 0.001$), *Santa Marta* with *Meta Drainage* ($\chi^2 = 0.034$), *Santa Marta* with *Southern Guyana* ($\chi^2 < 0.001$), *Meta Drainage* with *Caracas* ($\chi^2 = 0.005$), *Meta Drainage* with *Northern Guyana* ($\chi^2 = 0.016$), *Meta Drainage* with *Suriname* ($\chi^2 = 0.015$), *Caracas* with *Southern Guyana* ($\chi^2 < 0.001$), *Northern Guyana* with *Southern Guyana* ($\chi^2 < 0.001$), *Southern Guyana* with *Suriname* ($\chi^2 < 0.001$), *Southern Guyana* with *Northern Roraima* ($\chi^2 < 0.001$).

Each comparison between the network linking *Meta Drainage* and *Northern Roraima* samples is statistically significant with the primarily Amazonian network samples: *Meta Drainage* with *Rio Solimões/Rio Amazonas* ($\chi^2 = 0.027$), *Río Ucayali* ($\chi^2 = 0.011$), *Madre de Dios* ($\chi^2 = 0.001$), *Lower Rio Purús/Rio Acre* ($\chi^2 = 0.001$), *Middle/*

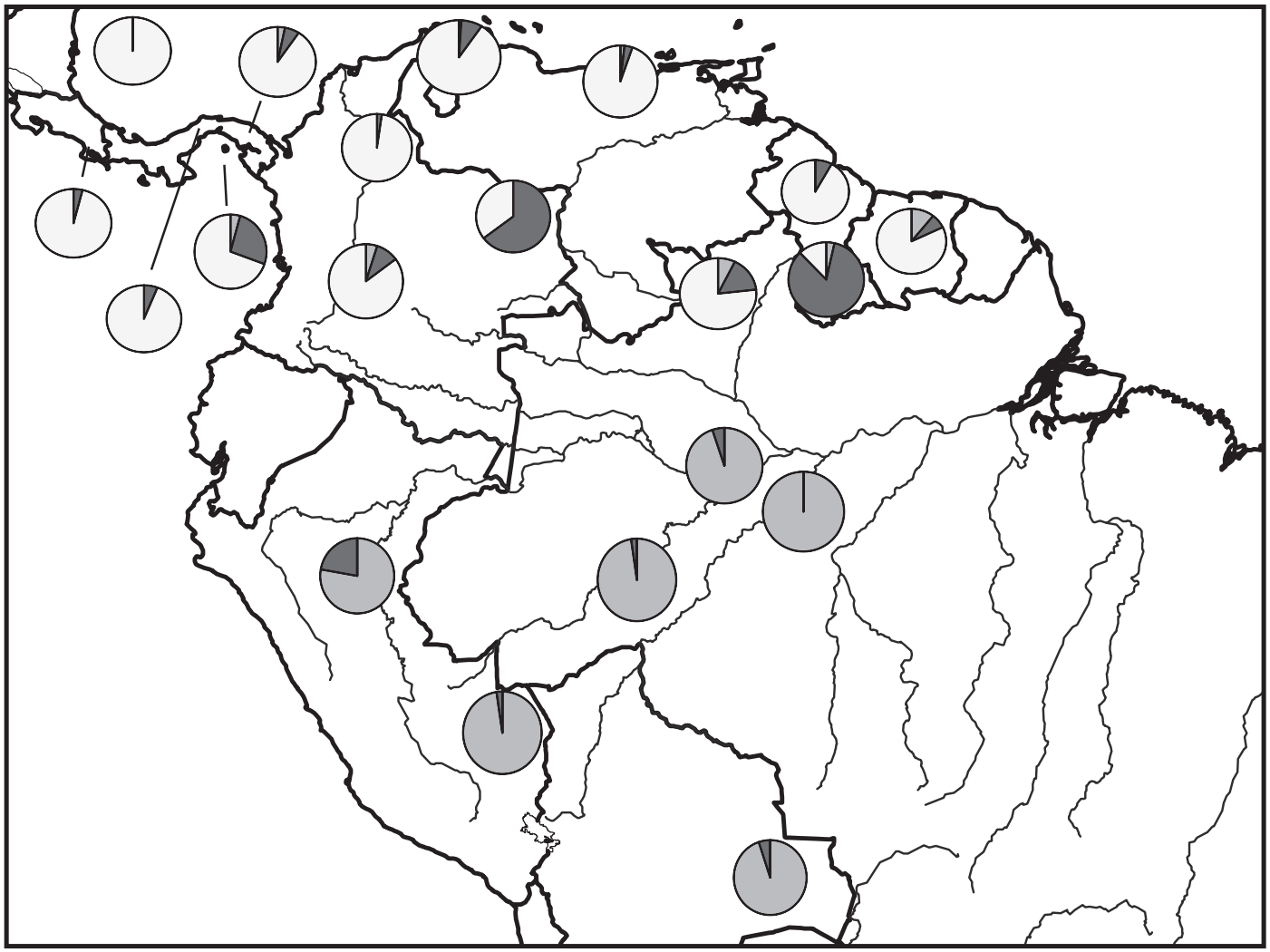

FIGURE 3. Variation in posterior thigh patterns among 20 regional samples. Pattern A, light gray; pattern B, white; intermediate pattern A/B, dark gray.

Lower Rio Madeira (χ^2 = 0.004), and *South Central Bolivia* (χ^2 = 0.001) samples; *Northern Roraima* with *Rio Solimões/Rio Amazonas* (χ^2 = 0.008), *Rio Ucayali* (χ^2 < 0.001), *Madre de Dios* (χ^2 < 0.001), *Lower Rio Purús/Rio Acre* (χ^2 < 0.001), *Middle/Lower Rio Madeira* (χ^2 = 0.048), and *South Central Bolivia* (χ^2 < 0.001) samples.

Within the primarily Amazonian network samples only the *Río Ucayali* and *Madre de Dios* samples differ significantly (χ^2 = 0.022).

The thigh pattern variation demonstrates the most geographically coherent pattern (Figure 3).

Shank Pattern

There are no statistically significant differences among the Middle American samples or between the network-linking *Darién* with the *Northern Magdalena Drainage* samples.

There are four statistically significant differences among the primarily Caribbean drainage network samples: *Northern Magdalena Drainage* with *Northern Guyana* (χ^2 = 0.009), *Meta Drainage* with *Northern Guyana* (χ^2 = 0.017), *Caracas* with *Northern Guyana* (χ^2 = 0.004), and

Northern Guyana with *Northern Roraima* ($\chi^2 = 0.003$). The *Northern Guyana* sample stands out within this network.

There is one statistically significant difference between the network-linking *Meta Drainage* sample with the *Lower Rio Purús/Rio Acre* sample ($\chi^2 = 0.011$). There are no statistically significant differences between the network-linking *Roraima* sample with the other Amazonian samples.

There are two statistically significant differences among the primarily Amazonian drainage network samples: *Madre de Dios* with *Lower Rio Purús/Rio Acre* ($\chi^2 = 0.005$) and *Lower Rio Purús/Rio Acre* with *South Central Bolivia* ($\chi^2 = 0.016$).

Dorsolateral Folds

There are no statistically significant differences among the Middle American samples or between the network-linking *Darién* with the *Northern Magdalena Drainage* samples.

There are several statistically significant differences among the primarily Caribbean drainage network samples: *Northern Magdalena Drainage* with *Santa Marta* ($\chi^2 = 0.010$), *Northern Magdalena Drainage* with *Caracas* ($\chi^2 < 0.001$), *Northern Magdalena Drainage* with *Southern Guyana* ($\chi^2 = 0.002$), *Northern Magdalena Drainage* with *Suriname* ($\chi^2 = 0.023$), *Northern Magdalena Drainage* with *Northern Roraima* ($\chi^2 < 0.001$), *Middle/Southern Magdalena Drainage* with *Northern Roraima* ($\chi^2 = 0.013$), and *Northern Guyana* with *Northern Roraima* ($\chi^2 = 0.033$). The distinctiveness of the *Northern Magdalena Drainage* sample is due to more occurrences of the dorsolateral folds with several interruptions to indiscernible conditions: it is possible that this is due to poor preservation rather than a real sample difference.

There are no statistically significant differences between the network-linking *Meta Drainage* and *Northern Roraima* samples with the rest of the Amazonian drainage network samples.

There are no statistically significant differences between any of the Amazonian network samples.

Lateral Folds

There are no statistically significant differences among the Middle American samples or between the network-linking *Darién* with the *Northern Magdalena Drainage* samples.

The following primarily Caribbean draining network samples differ statistically significantly: *Middle/Southern Magdalena Drainage* with *Northern Roraima* ($\chi^2 = 0.013$), *Santa Marta* with *Northern Guyana* ($\chi^2 = 0.023$), *Santa Marta* with *Northern Roraima* ($\chi^2 < 0.001$), *Caracas* with *Northern Guyana* ($\chi^2 = 0.009$), and *Caracas* with *Northern Roraima* ($\chi^2 = 0.003$).

The network-linking *Meta Drainage* sample statistically differs from the *Río Ucayali* sample ($\chi^2 = 0.025$). The network-linking *Northern Roraima* sample statistically differs from the *Río Ucayali* ($\chi^2 = 0.007$), *Lower Rio Purús/Rio Acre* ($\chi^2 = 0.048$), and *South Central Bolivia* ($\chi^2 = 0.013$) samples.

There are three sample comparisons that statistically differ significantly within the Amazonian network: *Río Ucayali* with *Madre de Dios* ($\chi^2 = 0.005$), *Madre de Dios* with *Lower Rio Purús/Rio Acre* ($\chi^2 = 0.030$), and *Madre de Dios* with *South Central Bolivia* ($\chi^2 = 0.005$).

Male Secondary Sexual Characters

Sample sizes are inadequate to perform statistical tests for many of the sample comparisons. In addition, the problem of seasonal development of sexual characters has to be addressed. Comparisons among samples should be made on males that are sexually active and have developed their full extent of secondary sexual characteristics. For this purpose, the presence of vocal slits is inappropriate, as once the slits are developed, they are not lost and regained on a seasonal basis. For comparative purposes, any noticeable arm hypertrophy is used as a proxy for full development of secondary sexual characters, realizing that this proxy is approximate and not definitive.

The data are best analyzed visually with the raw numbers of state occurrences for all 20 geographic samples (Table 17).

There are two obvious features in the data. First, there is no coherent pattern to the state occurrences for the jaw tubercle and tympanic annulus tubercle characters. The most reasonable interpretations for these two characters are that (1) tympanic annulus tubercles, once developed, are not shed seasonally; and (2) jaw tubercles are shed seasonally and are redeveloped each year by all adult males in all populations. Second, there is geographically coherent variation among the states of the chest tubercles and thumb spine characters. As can be seen from the raw data (Table 17), several of the samples have individuals with only a central patch of chest tubercles (state A) as well as individuals with central and lateral patches of chest tubercles (state B). This variation is interpreted to be ontogenetic; that is, for individuals that have both central and lateral tubercle patches, the central patch develops before

TABLE 17. Number of males exhibiting secondary sexual character states. Number of occurrences may not be equal among characters due to missing observations. Samples are defined in Table 16. **Jaw tubercle states:** A, absent; B, present. **Chest tubercle states:** A, absent; B, central patch; C, central and lateral patches. **Thumb spine states:** A, 1 slightly chisel-shaped; B, 1 robustly chisel-shaped; C, 2 round. **Tympanic tubercle states:** A, absent; B, present. A dash (–) indicates no specimens had the character state involved.

	Jaw tubercles		Chest tubercles			Thumb spines				Tympanic tubercles	
Sample	A	B	A	B	C	A	A/B*	B	C	A	B
A	3	5	2	8	0	0	0	0	10	0	10
B	1	5	1	6	0	0	0	0	7	0	7
C	0	5	0	10	0	0	0	0	10	0	10
D	2	3	1	6	0	0	0	0	7	0	7
E	2	10	4	13	0	0	0	0	16	0	14
F	3	1	3	6	0	0	0	0	9	1	8
G	6	26	2	36	0	0	0	0	42	1	41
H	1	3	4	9	0	0	0	0	13	0	13
I	–	–	1	3	0	0	0	0	3	0	3
J	2	3	2	6	0	0	0	0	8	0	8
K	4	3	4	1	2	4	3	0	0	0	7
L	1	0	1	0	1	2	0	0	0	0	2
M	1	3	3	3	0	4	2	0	0	1	5
N	–	–	0	1	3	4	0	0	0	0	4
O	0	1	0	0	1	0	0	1	0	0	1
P	–	–	–	–	–	–	–	–	–	–	–
Q	1	3	3	0	2	0	0	6	0	0	5
R	–	–	1	0	0	0	0	1	0	0	1
S	–	–	2	0	0	0	0	2	0	0	2
T	0	2	1	0	1	0	0	2	0	0	2

*In the case of thumb spines, there were some individuals that exhibited intermediate states between state A and state B.

the lateral patches develop on an annual basis. This interpretation is supported by comparison of the jaw tubercle and chest tubercle data, assuming that both jaw and chest tubercles start developing simultaneously each season in a given male. Thus the critical aspect for this character is whether any individuals in a population exhibit both central and lateral tubercle patches. It is assumed that if any individuals in a population have both central and lateral patches, all individuals in that population will develop both. The data appear to demonstrate a geographic break between the *Caracas* and *Northern Guyana* samples (Table 17). Samples for which all males lack chest tubercles provide no information on whether males develop lateral patches (*Rio Purús/Rio Acre* and *Lower/Middle Madeira* samples). The only adult male in the *Río Ucayali* sample did not have noticeably hypertrophied arms. The geographically most incongruous sample is the *Suriname* sample, where all three males with chest tubercles have only central patches.

Variation in thumb spines indicates three groupings of the geographic samples. The first is the contrast of two versus one spine, with the *Costa Rica/Eastern Panama* through *Caracas* samples having two spines (see Figure 13A) and all other samples characterized by having one spine. There is also variation among the one spine samples whether the spine is slightly chisel shaped (see Figure 13C) or obviously chisel shaped (see Figure 13B). The *Río Ucayali* sample male lacking arm hypertrophy has a medium-sized slightly chisel-shaped spine, suggesting that the spine is not fully developed.

Measurement Data

Discriminant function analyses were performed on the 20 geographic samples separately for the female and male data. The data sets were run with SYSTAT 11 software (Engelman et al., 2004) using the complete, forward, and backward stepwise models. The results for the complete, forward, and backward stepwise models are very similar; only the complete model results are discussed.

The first canonical vector accounts for 63% of the cumulative dispersion for the female data, the first two canonical vectors account for 81%, and 90% is reached with the third canonical vector. None of the 20 samples are completely correctly identified in the posterior classification matrix using all data nor in the jackknife classification matrix. The poor discrimination of the data set is reflected in the plot of the first two canonical vectors (Figure 4). Three distinctive sample groups can be distinguished from each other and the rest of the samples: (1) the *San Andrés/Providencia* and *Northern Magdalena Drainage* samples, (2) the *Gulf of Panama* sample, and (3) the *Southern Guyana* sample (Figure 4).

The first canonical vector accounts for 55% of the cumulative dispersion for the male data, the first two canonical vectors account for 75%, and 90% is reached between the fourth and fifth canonical vectors. All individuals in the *Río Ucayali* and *South Central Bolivia* samples were posteriorly correctly identified in the full data classification matrix, but all individuals from any sample were not correctly posteriorly classified in the jackknife classification matrix. The plot of the first two canonical vectors shows the same level of poor discrimination of the samples

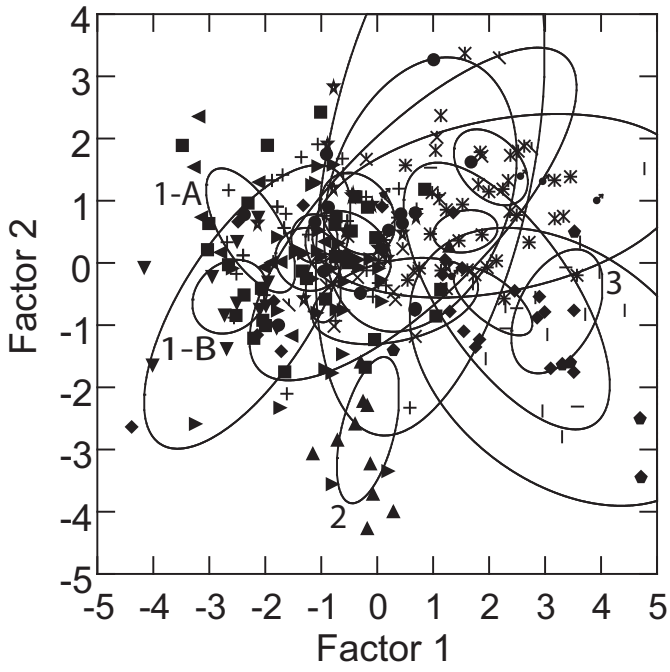

FIGURE 4. Discriminant function analysis results of first and second canonical vectors for female measurement data of 20 geographic samples (denoted by various symbols). The *Northern Magdalena Drainage* (1-A, triangle with apex at left)/*San Andrés/Providencia* (1-B, triangle with apex at bottom), *Gulf of Panama* (2, triangle with apex at top), and *Southern Guyana* (3, narrow vertical bar) samples are distinctive from each other.

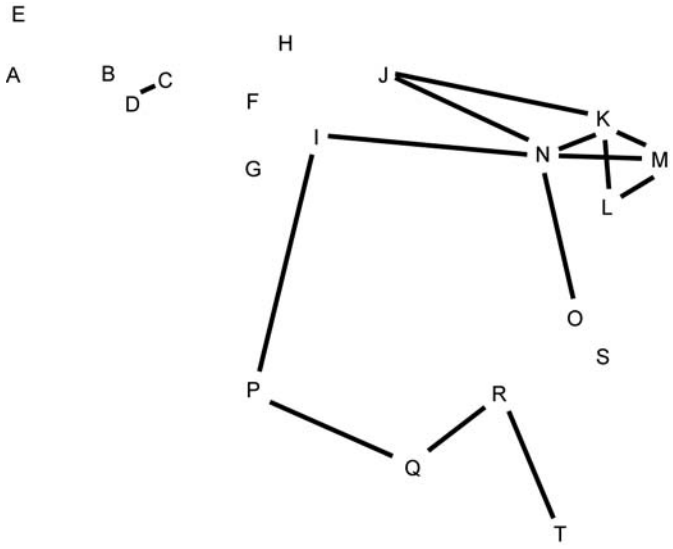

FIGURE 5. Moderate (3 characters) to high (4 or 5 characters) levels of differentiation between geographically adjacent samples.

as the female data (results not shown). There are three samples that are distinct from each other and the rest of the samples: (1) *Gulf of Panama*, (2) *Northern Guyana*, and (3) *Madre de Dios*.

The measurement data indicate that the *Gulf of Panama* sample is the most distinctive as its distinctiveness appears in both the female and male data analyses.

GEOGRAPHIC VARIATION AMONG THE SAMPLES

Adult Form Morphological Characters

For the characters analyzed statistically (including discriminant function analyses results), within the three networks, there is a moderate trend of greater differentiation between geographically distant sample pairs, as would be expected. In terms of evaluating geographic variation, the most insightful patterns of differentiation are those between adjacent geographic samples. The patterns are best visualized by comparing moderate to high (differing by three to five characters) levels of differentiation between geographically adjacent samples (Figure 5). Overall, there is considerable differentiation among adjacent sample pairs that are geographically distant from each other.

Taking all the differentiation data together (Figure 5), the most significant break between geographically adjacent samples is between the *Caracas* and *Northern Guyana* samples. These two samples differ from each other by four statistically meaningful characters, and the males of the *Caracas* sample lack lateral patches of chest tubercles and have two rounded thumb spines whereas the *Northern Guyana* sample males have lateral patches of chest spines and have one slightly chisel-shaped thumb spine. This *Caracas* sample break is discrete from the *Northern Guyana* sample and is most consistent with species level differentiation. The *Caracas* sample character distribution pattern continues along coastal Venezuela to the Estado de Sucre and the Island of Trinidad. There were few locality samples analyzed between southern Anzoategui State in Venezuela and the Guyana and Venezuelan border along the Estado de Bolívar (Venezuela), most of which lacked sexually active adult males. The closest Venezuelan locality sample with an adult male with noticeably hypertrophied arms with two round thumb spines is from Bolívar State, Santo Tomé de Guayana, San Felix (AMNH 81456) and an adult male with a single slightly chisel-shaped thumb spine is from

Bolívar State, 13 km south, 1 km east Puente Cuyuni (KU 166408), over 250 km distant from each other. Additional data are needed from the Venezuelan states of Amazonas and Bolívar to determine the precise distribution of the two species involved and whether they occur in sympatry.

For the northeast set of samples (*Southern Costa Rica/ Eastern Panama* through *Caracas*) the level of variation is consistent with intraspecific variation, although there is considerable variation between the *Darien* and *Gulf of Panama* samples (Figure 5C, D).

In contrast, there is much more variation among the remaining samples (Figure 5). A clear geographic pattern is demonstrated by whether the male thumb spine is strongly chisel shaped and the posterior thigh predominantly uniform or moderately mottled (*Rio Solimões/ Amazonas* through the *Río Ucayali* and *South Central Bolivia* samples) or a weakly chisel-shaped thumb spine and a predominantly boldly mottled posterior thigh pattern (*Northern Roraima* through *Suriname* samples). The morphological data are equivocal in terms of whether the break between this clustering of samples represents intra- or interspecific variation.

Larval Morphology

Given the problems of tadpole identification described in Methods and Materials, justification concerning the identification of the larval samples described below is called for. Five species of *Leptodactylus* occur in Costa Rica: *L. insularum, L. fragilis, L. melanonotus, L. savagei,* and *L. poecilochilus*. All five Costa Rican species have published larval descriptions and figures (*L. insularum* as *L. bolivianus, L. savagei* as *L. pentadactylus, L. fragilis* as *L. labialis*; Heyer 1970), and the tadpoles of the five species can readily be identified based on morphological features. Only *L. melanonotus* larvae might be confused with *L. insularum* larvae. The dorsal oral papilla gap of *L. insularum* is narrower (21–31% of oral disk) than the gap in *L. melanonotus* from Costa Rica (40–60%). The *L. insularum* larval samples from Isla San Andrés (Colombia) do not have any noticeable differences with respect to the *L. insularum* from Costa Rica.

Four larval samples are available in addition to the description by Heyer (1970) for this species. All materials share the following features: sinistral spiracle, median vent, entire oral disk, a single or double row of oral disk marginal papillae, a tooth row formula of 2/3, and dorsal fin origin at the tail-body juncture. The larval materials are from the following localities, referred to henceforth by the specific localities only: USNM 241340: Isla San Andrés, San Andrés y Providencia, Colombia; USNM 330406: near Cañas, Guanacaste, Costa Rica; KU 68365: Puerto Cortes, Puntarenas, Costa Rica; and USNM 330837: Rincón de Osa, Puntarenas, Costa Rica.

The two specimens from Isla San Andrés and the sample from Puerto Cortés are faded. The dorsal bodies of the Cañas tadpoles are relatively uniform brown; the guts are very visible from the lateral view with the portion anterior to the guts relatively uniform tan/brown; the ventral aspect of the body is lighter than the dorsum, but melanophores are present on the portion anterior to the guts; the tail musculature is uniform brown; the tail fins are much lighter than the musculature but either with scattered melanophores or a relatively uniform tan pattern. The Puerto Cortés tadpole dorsal aspects of the head and bodies are mottled brown; from the side, the head-bodies are mottled; in ventral view, the area of the oral disk to the guts has scattered melanophores, with the area over the guts without melanophores; the tail fins and musculature are dark, almost uniform, brown.

There is variation among larval quantitative character data (Table 18).

TABLE 18. Quantitative larval data. Number in parentheses under Source is number of specimens upon which the data are based. Definitions: ED, eye diameter; HBL, head–body length; ODW, oral disk width; TL, total length; TN, number of A2 teeth in 0.3 mm. A dash (–) indicates the values were not available in the data source.

Locality	Source	Gosner stages	TL, mm	HBL/TL, %	ED/HBL, %	ODW/BL, %	TN
Colombia, Isla San Andrés	USNM 241340 (2)	37	32.6–35.2	32–34	11–12	27–28	17–19
Costa Rica	Heyer, 1970	34	32.0	32–44	5–10	20–28	–
Costa Rica, Cañas	USNM 330406 (5)	28–31	24.4–28.5	38–42	6–7	23–27	18–22
Costa Rica, Puerto Cortés	KU 68365 (5)	32–34	29.3–32.4	37–39	6–7	21–23	18–20
Costa Rica, Rincón de Osa	USNM 330837 (5)	25–30	17.4–18.4	44–47	8–10	20–23	25–28

The variation of the data described above is difficult to evaluate in terms of discerning intraspecific from interspecific differentiation. At least some of the quantitative variation (Table 18) should be correlated with ontogeny as represented by Gosner developmental stages. Head and body lengths were measured differently for the Heyer (1970) data than for the data presented here, which compromises comparability. If one makes the reasonable assumption that all of the Costa Rican data represent a single species, those data pretty much encompass all of the variation observed among all the larval data.

Advertisement Calls

There are only three relatively noisy recordings on hand for members of the *L. bolivianus-insularum* complex suitable for analysis in addition to the information published by Fouquette (1960) (Table 19).

Costa Rica Recording

Figure 6

Recording KU 801, as indicated previously, has a noticeably erratic tape speed, which adds an error factor to the quantitative data. The call consists of a single note composed of 1 or 2 pulses. The recording has two call rates, in the beginning 19 s the call rate is 1.2 calls/s, in the last 39 s, the call rate is 2.5 calls/s. The first half of the call has a sharper rising frequency modulation than the last half of the call. The highest frequency occurs at the end of the call or shortly before the end of the call (at about 85% total call duration). There is considerable variation in dominant frequencies among the calls analyzed doubtless due to tape speed variation; the most consistent values are those reported in Table 19. Harmonic structure is present, but not strongly developed.

Panama Recording

Figure 7

The Barro Colorado Nature Monument recording has calls consisting of single notes. There is sufficient other sound in the broadcast channel that the amplitude intensity of the call is not definitively clear; most calls have evidence of two primary pulses per note. The first portion of the note (approximately 30%) has a faster rise of frequency than the next 50% of the note. The final approximate 20% of the note has a noticeable falling frequency. Harmonic structure is present and stronger than in the KU 801 recording.

Peru Recording

Figure 8

Recording USNM 268 cut 11 has calls consisting of 1 or 2 notes. The quality of the recording is not sufficient to evaluate detailed temporal structure. The analyses suggest that the second note for two note calls or the single note of one note calls have at least two pulses. The first 20% (approximately) of the call has a sharply rising frequency, with a decreased rate of rising frequency for the remainder of the call. The highest frequency is consistently at the end of the call. There is no visible harmonic structure in the audiospectrogram analyses of the calls; there is too much noise in the wave form analyses to determine with certainty whether there is harmonic structure or not.

TABLE 19. Advertisement call data. Definitions: ID, identification; SVL, snout–vent length. A dash (–) indicates values not available from the data source.

Country	Recording ID	Temperature, °C	Voucher SVL, mm	No. notes/ call	Calls/s	Call duration, s	Beginning frequency, Hz	Highest frequency, Hz	Ending frequency, Hz	Dominant frequency, Hz	Harmonics
Costa Rica	KU 801	26	–	1	1.2–2.5	0.08	220–300	1,080–1,200	1,060–1,200	920–1,130	+
Panama	BCI CD	–	–	1	1.5	0.10–0.12	110–180	890–1,100	510–600	740–870	+
Panama	Fouquette, 1960	24	–	–	1.9	0.10	200	900	750	–	+
Peru	USNM 268, cut 11	23.5	112	1–2	0.5	0.12–0.16	130–200	730–780	730–780	600–690	?

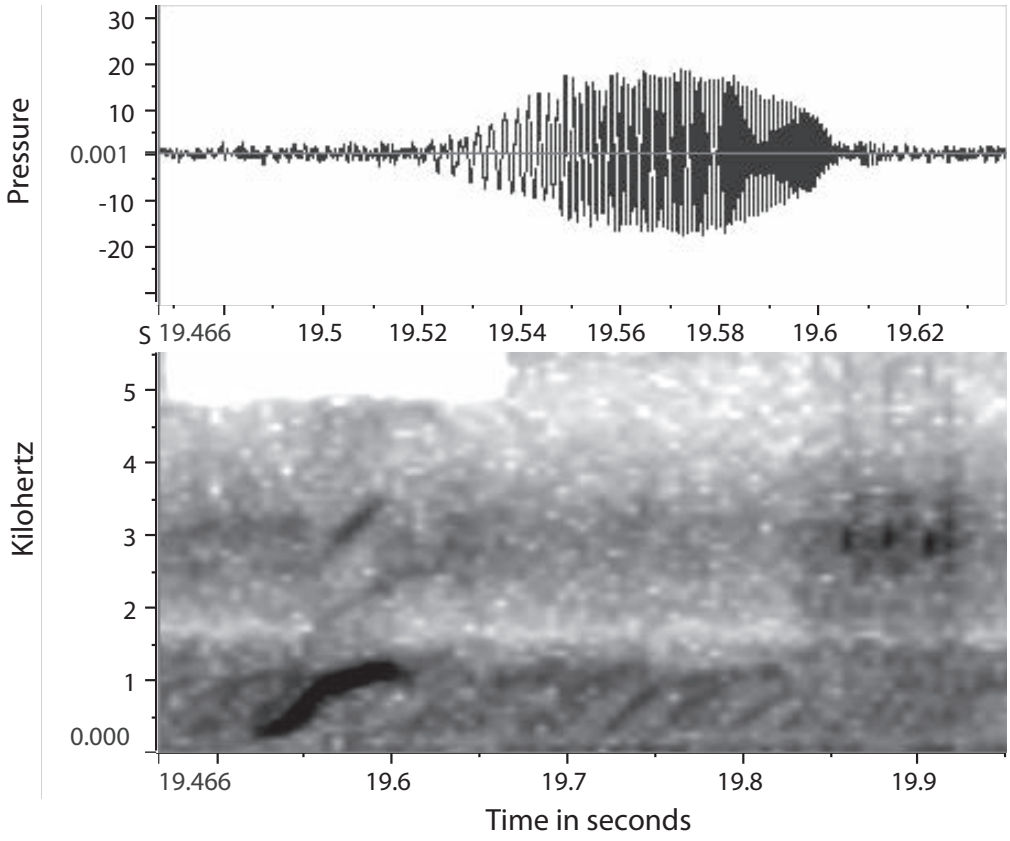

FIGURE 6. Advertisement call of *Leptodactylus*, KU recording 801 from just west of Piedras Blancas, Puntarenas, Costa Rica, 8 April 1966, water temperature 78°F.

The available advertisement call data (Table 19) are most consistent with two call types representing distinct species: (1) the recordings from Costa Rica and Panama; and (2) the recording from Peru.

MOLECULAR RELATIONSHIPS

A total of 800 aligned base pairs were included in the analyses, of them 540 were constant and 260 variable (172 parsimony informative). Base composition consisted of A = 34%, C = 23%, G = 18%, and T = 25%. Thirty-one (3.87%) positions included an indel position in at least one taxon.

All analyses recovered a clade that included all representative samples of the *L. latrans* species group (Figure 9). Within this clade, a subclade consisting of *L. latrans* and *L. chaquensis* is recovered and is the sister group to the *L. insularum-bolivianus* complex clade. Within the *L. insularum-bolivianus* complex, three subclades are recovered: subclade A, including samples from Panama, Venezuela, and Colombia, corresponds to the taxon known as *L. insularum*; subclade B, found in Peru and Bolivia, represents the taxon known as *L. bolivianus*; and subclade C is recognized herein as a new species in this complex occurring in the Guiana Shield region.

The genetic divergences among the four subclades recovered within the *L. latrans* species complex range from 9.5 to 14.1% (see Figure 9 for range of divergence values followed by the mean value below corresponding branches). Mean value divergences among the three species recognized in the *L. bolivianus–insularum* complex from other species of the *L. latrans* species group range from 4.2 to 7.2%. Divergences among the three species recognized in the *L. bolivianus–insularum* complex are *bolivianus–insularum* 4.9%; *insularum–sp. nov.* 6.2%; and *bolivianus–sp. nov.* 5.1%. In addition, mean divergences among the sampled species of the *latrans* species group are *insularum–latrans* = 12.2%; *insularum–chaquensis* = 12.1%; *bolivianus–latrans* = 10.1%; *bolivianus–chaquensis* =

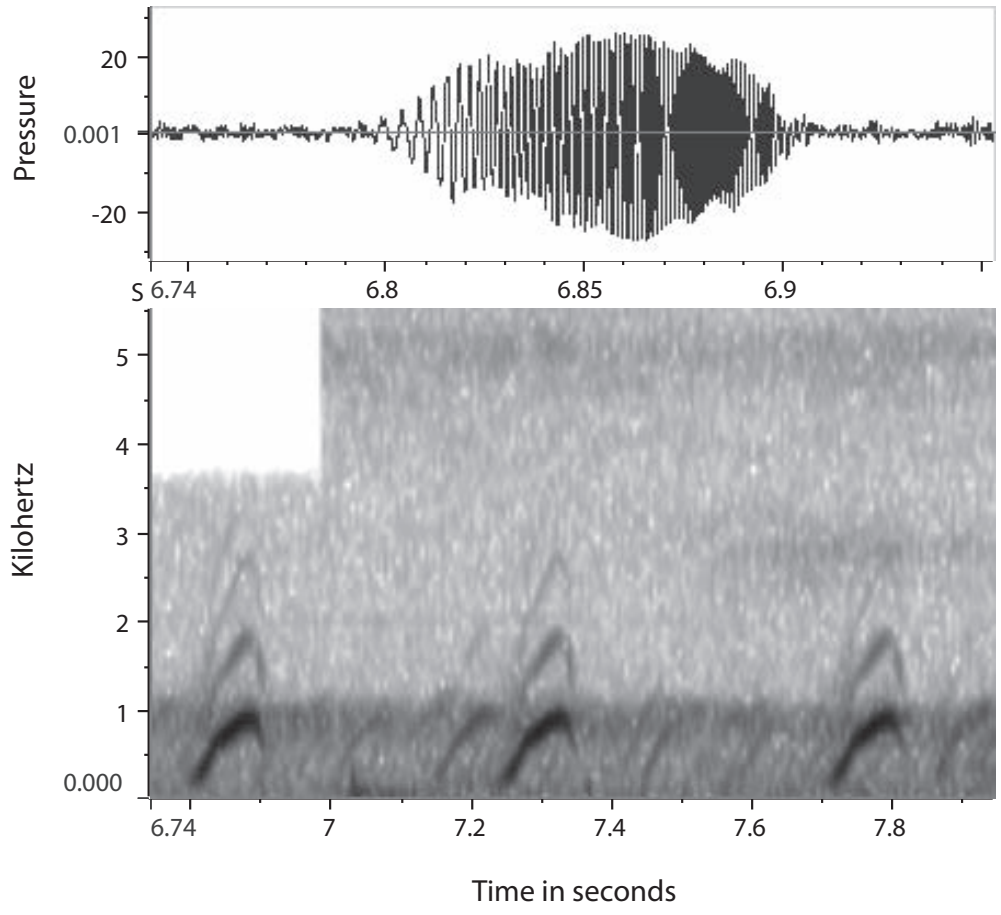

FIGURE 7. Advertisement call of *Leptodactylus* from Barro Colorado Nature Monument CD recording, track 46, Panama.

10.8%; *latrans–sp. nov.* =12.9%; *chaquensis–sp. nov.* = 12. 6%; and *latrans–chaquensis* = 6.8%. The existence of an undescribed taxon demonstrated by the genetic diversification recovered in this analysis in combination with the subsequent identification of diagnostic morphological characters supports the recognition of the new species. The genetic diversification among this complex of species is consistent with the level of diversification among the other two species in the *L. bolivianus* complex as well as level of diversification between species in the *L. latrans* and those in the *L. bolivianus* complex.

INTERSPECIFIC VARIATION

Three outlying localities for which there are only juvenile specimens available were considered problematic as to species identification in the preceding analyses. As previously indicated, Marinus Hoogmoed (pers. comm.) informed WRH at the VI World Congress of Herpetology in 2008 that he had reexamined frogs from the state of Pará, near the state of Amapá and found them to be members of the *Leptodactylus bolivianus* complex. These specimens belong to the new species recognized herein. Thus the single specimen from Amapá examined in this study is considered to represent the new species as well.

There are also a number of localities between the distributions of *L. bolivianus* and the new species for which only juveniles and/or females are available and rather arbitrary criteria were used to assign species identifications. The following localities are considered to represent *L. bolivianus*. **COLOMBIA. Vaupes:** Río Querari near junction of Río Querari and Río Vaupés. **BRAZIL. Amazonas:** Parque Nacional Pico da Neblina, São Gabriel da Cachoeira; Rio Cuieiras, entering Rio Negro on left bank; Rio Demeni, Barcelos; São Gabriel da Cachoeira; São

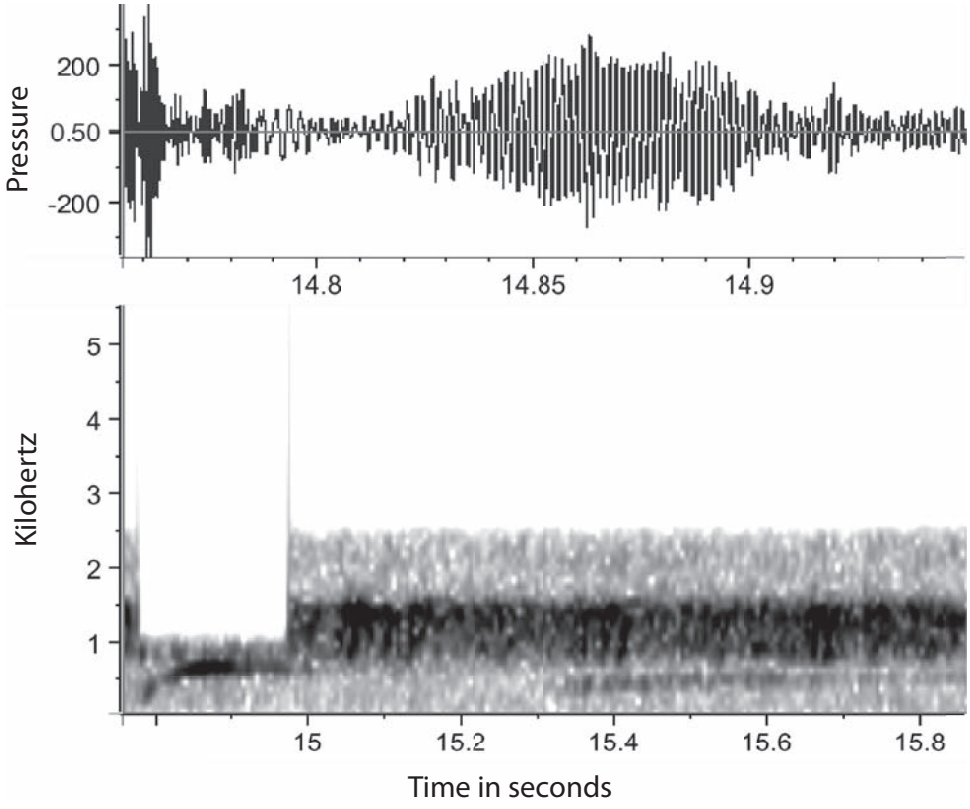

FIGURE 8. Advertisement call of *Leptodactylus*, USNM recording 268 cut 11 from Tambopata, Madre de Dios, Peru.

João, near Tapurucuara. **VENEZUELA. Amazonas:** Catarata de Huá (Salto de Huá); Río Casiquiare, 8 miles [12.9 km] below Orinoco (Canal Casiquiare); Río Mavaca, 108 km SSE Esmeralda. Presumed localities for the new species follow. **BRAZIL. Roraima:** Uiacás, Rio Urariquera; Serra dos Surucucus; Missão Catrimani; Cachoeira do Cujubim, Rio Catrimani; Parque Nacional Viruá, próximo a Caracaraí; Praia do Guariúba; Santa Maria do Buiaçu. The comparisons that follow include the preceding locality/specimen data, and even if most presumed identifications are incorrect, the overall results would not be meaningfully impacted.

Measurement Data

Complete model discriminant function analyses of the measurement data for females and males indicate incomplete separation of the three species, with the male data (Figure 10) showing better discrimination than the female data (Figure 11).

For the male analysis, the first canonical vector accounts for 84% of the cumulative dispersion and the first two canonical vectors account for 100% of the cumulative dispersion. Eighty-three percent of *L. bolivianus*, 92% of *L. insularum*, and 85% of the new species are correctly identified using all data in the posterior classification matrix. The male data jackknifed classification matrix for *L. bolivianus*, *L. insularum*, and *Leptodactylus*, new species are 78, 92, and 79% respectively.

For the female data discriminant analysis, the cumulative proportions of total dispersion are identical to the male data (84 and 100%). Seventy-one percent of *L. bolivianus*, 87% of *L. insularum*, and 80% of *Leptodactylus*, new species are correctly identified using all data in the posterior classification matrix. The female data jackknifed classification matrix is 67, 84, and 78% respectively.

The measurement data indicate that there is differentiation among the three species, but the differentiation is not complete enough to be useful as diagnostic information to correctly identify all specimens to species.

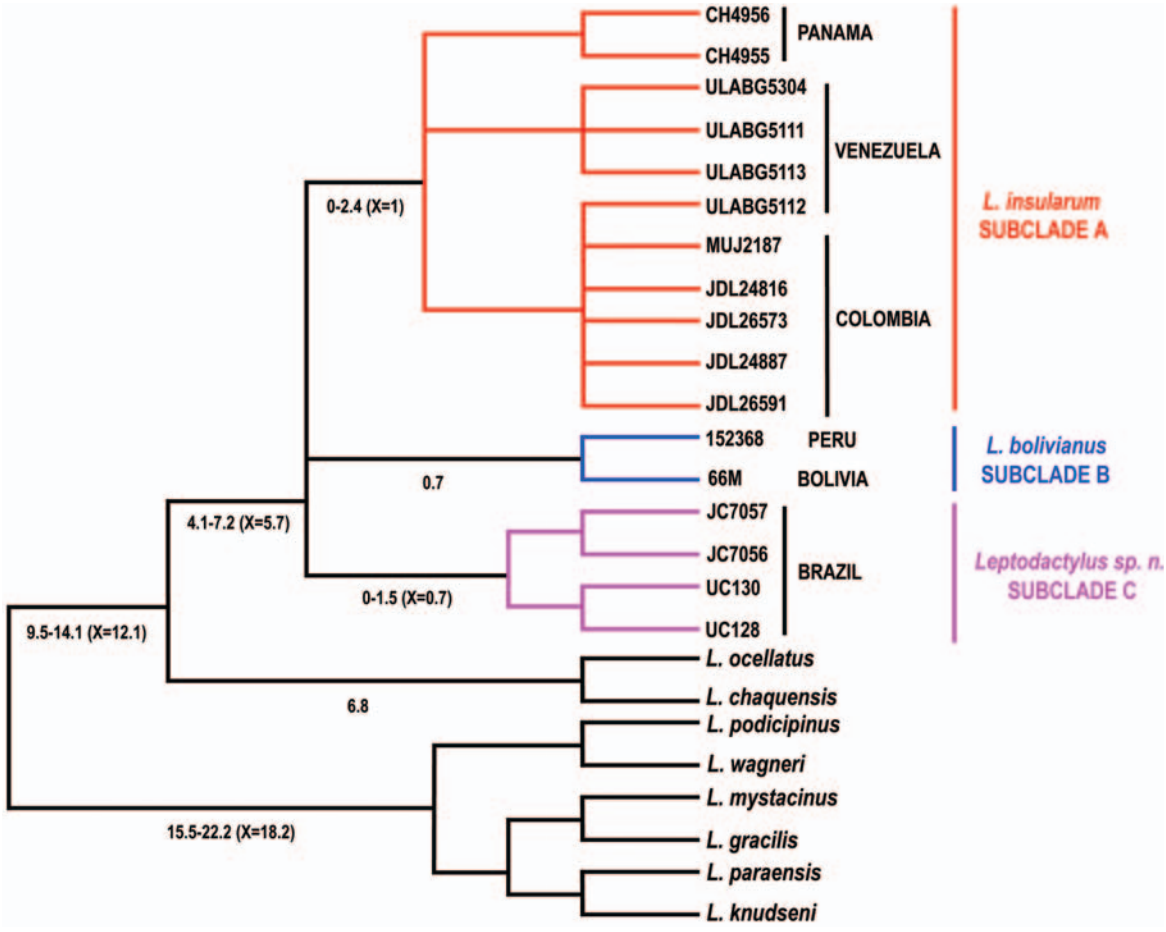

FIGURE 9. Maximum likelihood cladogram of the *Leptodactylus latrans* species group with other representatives of *Leptodactylus* species lineages.

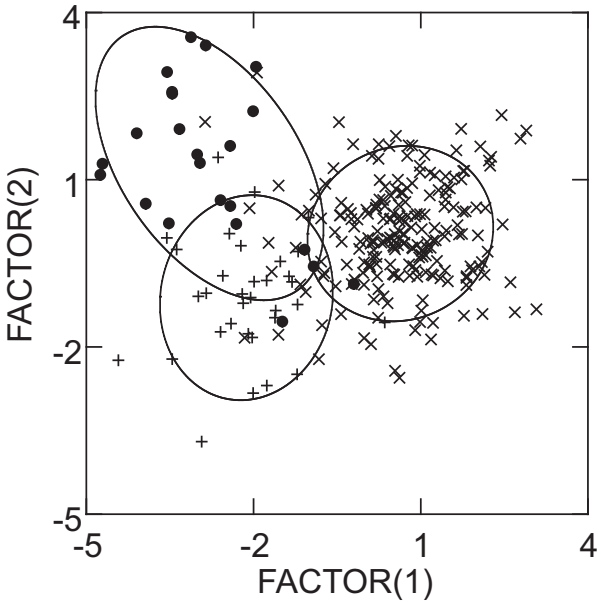

FIGURE 10. Plot of first two canonical vectors for male measurement data for the three species of the *Leptodactylus bolivianus* cluster. Dot (•), *L. bolivianus*; ×, *L. insularum*; +, new species. Centroid confidence ellipses, $p = 0.95$.

Morphological Data

Forty-four percent of the morphological character comparisons among species pairs are compromised by high observer error (Table 20). The high observer error is accounted for, in part, by the fact that the three species are rather morphologically similar to each other.

For the statistically significant character comparisons, the most differentiated character states are listed as follows.

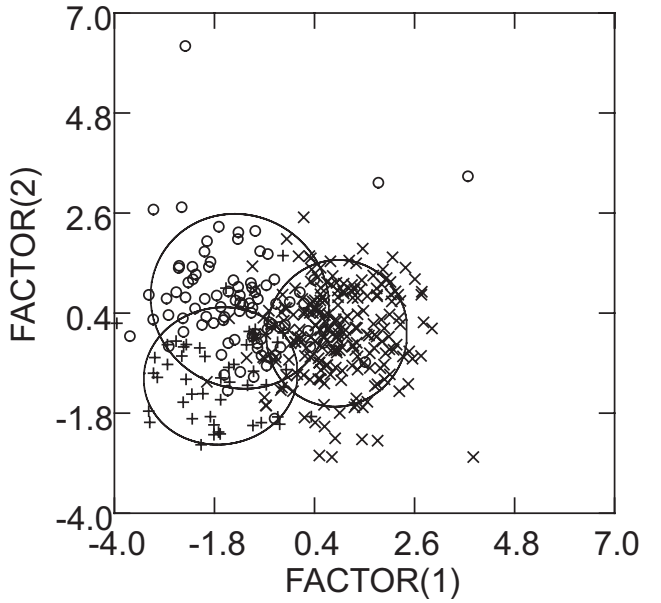

FIGURE 11. Plot of first two canonical vectors for female measurement data for the three species of the *Leptodactylus bolivianus* cluster. Circle, *L. bolivianus*; ×, *L. insularum*; +, new species. Centroid confidence ellipses, $p = 0.95$.

Leptodactylus bolivianus and L. insularum Comparisons

Dorsal pattern A: uniform between interocular bar [if present] and sacrum; *L. insularum* with greater occurrence. Lip stripe pattern D: lip region uniform; *L. bolivianus* with greater occurrence. Posterior thigh patterns A and B: both species differing by 86% for each state. Belly pattern C: belly speckled or mottled with same intensity over entire belly; *L. insularum* with greater occurrence. Thumb spines state C: two round spines; *L. insularum* with 100% occurrence.

Leptodactylus bolivianus: New Species Comparisons

Posterior thigh pattern A: uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for entire extent of posterior thigh; *L. bolivianus* with greater occurrence. Belly pattern A: speckled, spotted, or mottled anteriorly only or with an anterior-posterior gradient; *L. bolivianus* with greater occurrence. Chin tubercles: both states equally differentiated in the two species. Chest tubercles state B: central patch only; new species with greater occurrence. Thumb spines, state B: one hypertrophied chisel-shaped spine; *L. bolivianus* with exclusive occurrence.

Leptodactylus insularum: New Species Comparisons

Belly pattern B: boldly mottled anteriorly only or with an anterior-posterior gradient; new species with greater occurrence. Lateral folds, both states equally differentiated in both species. Chest tubercles state B: central patch of tubercles only; new species with greater occurrence. Thumb spines, state A: one modest chisel-shaped spine on each thumb; new species with greater occurrence. The male secondary sexual characteristics demonstrate the greatest diagnostic differentiation among the three species.

SPECIES ACCOUNTS

There are several characters examined in this study that demonstrate negligible differences among the three species. All three species exhibit the following character states, which are not repeated in the individual species accounts.

Dorsal patterns are uniform between the interocular bar (if present) and the sacrum or with a single chevron between the interocular bar and sacrum without additional spots lateral to the dorsal chevrons or a complex pattern with two dorsal chevrons and spotting all over the dorsum, chevrons often elongate and fused with each other and the interorbital bar. Light subocular patches vary from very distinct to not discernible. The dorsal surface of the shank patterns are uniform or speckled or with short dark transverse bars extending less than halfway across the shank or with at least one dark transverse bar extending halfway or more across the shank or with large dark spots in the middle of the upper shank. Dorsolateral folds are distinct and continuous from the supratympanic fold to the end of the body, or with a brief interruption of the fold, or the fold is distinct only from the supratympanic fold to the sacrum, or the fold has several interruptions, or the fold is indiscernible. The toes are fringed. Adult males have either keratinous tubercles on the tympanum or lack tubercles.

Specimens from two localities cannot be identified to species: USNJM 162697, 216724–216725, Venezuela, Bolívar, Los Patos, 25–28 km SE El Manteco, 350 m, 7°11′N, 62°22′W; and UMMZ 195839, Venezuela, Amazonas, San Fernando de Atabapo, 4°3′N, 67°28′W. The specimens involved are metamorphs, juveniles, or females.

TABLE 20. Statistical comparison of morphological character states for species pairs of the *Leptodactylus bolivianus* complex; df = degrees of freedom. Significant probability (p) results are in bold italic font.

Characters	*Leptodactylus bolivianus* and *L. insularum*	*Leptodactylus bolivianus* and *L.* new species	*Leptodactylus insularum* and *L.* new species
Dorsal pattern	$\chi^2 = 25.186$ df = 4 ***p = 0.000***	Character state occurrence < observer error	Character state occurrence < observer error
Lip stripe	$\chi^2 = 33.945$ df = 3 ***p = 0.000***	Character state occurrence < observer error	Character state occurrence < observer error
Subocular spot	$\chi^2 = 2.443$ df = 2 $p > 0.1$	$\chi^2 = 1.306$ df = 2 $p > 0.5$	$\chi^2 = 3.711$ df = 2 $p > 0.1$
Tibia pattern	Character state occurrence < observer error	Character state occurrence < observer error	Character state occurrence < observer error
Posterior thigh pattern	$\chi^2 = 238.297$ df = 2 ***p = 0.000***	$\chi^2 = 152.059$ df = 2 ***p = 0.000***	Character state occurrence < observer error
Belly pattern	$\chi^2 = 25.186$ df = 4 ***p = 0.000***	$\chi^2 = 76.760$ df = 4 ***p = 0.000***	$\chi^2 = 55.100$ df = 4 ***p = 0.000***
Dorsolateral folds	Character state occurrence = observer error	Character state occurrence < observer error	Character state occurrence < observer error
Lateral folds	Character state occurrence = observer error	Character state occurrence < observer error	$\chi^2 = 10.281$ df = 1 ***p = <0.005***
Chin tubercles	$\chi^2 = 0.035$ df = 1 $p > 0.5$	$\chi^2 = 8.607$ df = 1 ***p = 0.000***	$\chi^2 = 0.489$ df = 1 $p > 0.5$
Chest tubercles	$\chi^2 = 0.096$ df = 1 $p > 0.1$	$\chi^2 = 7.747$ df = 2 ***p < 0.025***	$\chi^2 = 7.281$ df = 2 ***p < 0.05***
Thumb spines	$\chi^2 = 18.040$ df = 1 ***p = 0.000***	$\chi^2 = 16.333$ df = 1 ***p = 0.000***	$\chi^2 = 20.5$ df = 1 ***p = 0.000***
Tympanum tubercles	Character state occurrence < observer error	Character state occurrence < observer error	Character state occurrence < observer error

There are additional localities for which the morphological data are ambiguous as to which species is involved for samples geographically between the new species and *L. insularum*. The identifications for these localities are based on river drainage distributions, and new data are needed to verify which of the two species occur at the localities involved. These latter localities are distinguished on the distribution map (see Figure 14) and in the distribution sections of the following pertinent accounts.

Leptodactylus bolivianus Boulenger, 1898

FIGURE 12

Leptodactylus bolivianus Boulenger, 1898:131. Lectotype: Museo Civico di Storia Naturale de Genova 28875, male, designated by Capocaccia (1957:214). Lectotype locality: Barraca (Río Madidi, Bolivia).

Leptodactylus romani Melin, 1941:54. Lectotype Göteborgs Naturhistoriska Museum (GNM) 499, juvenile, designated by Heyer (1969:6). Lectotype locality: Taracuá, Rio Uaupés, Brazil.

FIGURE 12. Photo of *Leptodactylus bolivianus* from Tambopata, Peru (courtesy of Reginald B. Cocroft).

ETYMOLOGY. There is no stated etymology in the original description. The species is named for the country of Bolivia from which the specimens were collected.

DIAGNOSIS. Most specimens of *L. bolivianus* have the posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for the entire extent of the posterior thigh; most specimens of *L. insularum* and *L.* n. sp. have boldly mottled posterior thighs with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced. *Leptodactylus bolivianus* sexually active adult males have lateral patches of chest tubercles in addition to a central patch; *L. insularum* only have the central patch of tubercles. The only entirely consistent character diagnosing the three species is the adult male thumb spine. The single spine on each thumb in *L. bolivianus* is extremely chisel shaped (Figure 13B); *L. insularum* has two rounded, pointed spines per thumb (Figure 13A); *L.* n. sp. has a single, modestly chisel-shaped spine per thumb (Figure 13C).

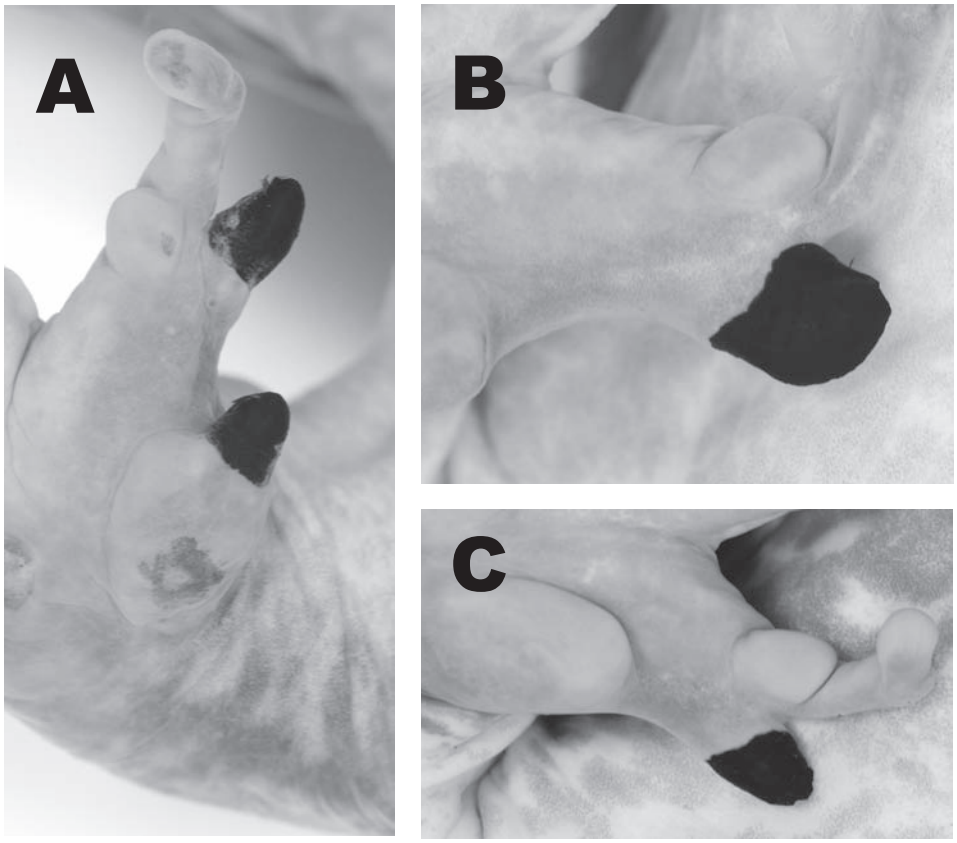

FIGURE 13. Male thumb spines. A. *Leptodactylus insularum*, USNM 565028; B. *Leptodactylus bolivianus*, USNM 280211; C. *Leptodactylus* new species, USNM 566172.

CHARACTER SUMMARY. The most frequent lip stripe pattern consists of a noticeable broad light stripe extending from the front of the snout to the commissural gland, bordered below by continuous or broken markings on the upper lip and either bordered above by a dark line under the eye or not. The most frequent posterior thigh pattern is a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for the entire extent of the posterior thigh. The most frequent lateral fold conditions are distinct folds only in the groin region, or indistinct overall, or indiscernible. The most frequent adult male chin tubercle condition is presence of the tubercles.

Female (n = 91) SVL 61.2–107.7 mm (m [mean] = 85.3), male (n = 25) 79.0–121.5 mm (m = 104.6). Female head length/SVL ratio 0.33–0.40 (m = 0.365), male 0.34–0.41 (m = 0.365). Female head width/SVL ratio 0.29–0.37 (m = 0.323), male 0.31–0.39 (m = 0.345). Female eye-nostril distance/SVL ratio 0.09–0.12 (m = 0.102), male 0.09–0.11 (m = 0.102). Female tympanum distance/SVL ratio 0.06–0.10 (m = 0.074), male 0.06–0.08 (m = 0.071). Female thigh length/SVL ratio 0.40–0.53 (m = 0.462), male 0.39–0.51 (m = 0.458). Female shank length/SVL ratio 0.44–0.56 (m = 0.516), male 0.46–0.56 (m = 0.502). Female foot length/SVL ratio 0.47–0.60 (m = 0.528), male 0.48–0.57 (m = 0.526).

TADPOLE. Unknown.

ADVERTISEMENT CALL. Calls of 1–2 notes at a rate of 0.5/s; call duration 0.12–0.16 s; call amplitude modulated, each note with loudest portion in middle of note; call frequency modulated starting at a low frequency (130–200 Hz) followed by a long portion of higher frequency (730–780 Hz) (Figure 8). Recording USNM 268 cut 11.

DISTRIBUTION. Central and western portions of the Amazon basin in Bolivia, Brazil, Colombia, Peru, and Venezuela (Figure 14).

SPECIMENS EXAMINED (n = 347). Localities enclosed by square brackets require validation that the species is correctly identified. **BOLIVIA. Beni:** Alejandria, Río Mamoré, 12°4′S, 65° 9′W, AMNH 79053–79066; Beni Biosphere Reserve, El Trapiche/Palm camp, 300 m, 14°30′S, 66°0′W, USNM 306617; Ivon, 11°7′S, 66°9′W, UMMZ 58837; Puerto Almacen (= Mayor Pedro Vaca Diez), 260 m, 14°47′S, 64°51′W, AMNH 72381; Río Grande, 5 km NW of mouth, 15°51′S, 64°39′W (coordinates for mouth of Río Grande), AMNH 79042, 79052; Río Mamoré, Río Ibarre (= Ibare), 14°37′S, 64°57′W, AMNH 79033–79041, 79044–79055; Río Mapiri, mouth, below upper Beni, UMMZ 58834 (3), 58835 (2); Rurrenabaque, 220 m, 14°28′S, 67°34′W, MCZ-A 10091, UMMZ 58836 (3), 58838 (4); Santa Rosa, Río Mamoré, 15°38′S, 64°37′W, AMNH 72382–72395; Tumi Chucua, 170 m, 10°8′S, 66°10′W, USNM 280211. **Cochabamba:** Puerto Chipiriri, 300 m, 16°32′S, 65°16′W (coordinates for mouth of Río Chipiriri), AMNH 72238–72241; Puerto San Francisco, 36 km N Chipiriri, 16°30′S, 65°20′W, KU 135508; Villa Tunari, 10.5 km E, 500 m, 16°59′S, 65°20′W, KU 183010–183011. **Pando:** Nacebe, 11°0′S, 67°25′W, NMP6V 72179/3, NMP6V 72179/4. **Santa Cruz:** Buenavista (= Buena Vista), 400 m, 17°27′S, 63°40′W, UMMZ 66482, 66485, 66535, 83101 (3); Buena Vista, 5 km W, USNM 146513–146514, 146522; Río Chaparé, 5 km N boca, 15°55′S, 64°38′W, AMNH 79046–79047; Río Ichilo, 34 km S boca Río Chaparé, 16°07′S, 64°49′W, AMNH 79043; Río Ichilo, ~54 km S boca Río Chaparé, 16°10′S, 64°40′W, AMNH 79048–79051.

BRAZIL. ACRE: Feijó, 8°9′S, 70°21′W, MZUSP 106665–106667; Igarapé de Nico, Rio Acre, 9°49′S, 67°36′W, USNM 202431; Reserva Florestal Humaitá, 9°45′S, 67°40′W, MZUSP 70866; Rio Branco, 60 m, 9°58′S, 67°48′W, MZUSP 6487; Rio Muru, 8°09′S, 70°45′W, MZUSP 106673–106675; Tarauacá, 190 m, 8°10′S, 70°46′W, FMNH 83238, MZUSP 64730,106668–106672. **AMAZONAS:** Lago do Miuá, Anavilhanas, 3°42′S, 62°8′W, INPA 1232; [Barcelos, Rio Demeni, 1°0′S, 62°58′W, INPA 9613]; Boca do Acre, 8°45′S, 67°24′W, MZUSP 50230–50248, USNM 202432–202448; Borba, 25 m, 4°25′S, 59°35′W, MNRJ 3169, 13522, MZUSP 56499–56501, USNM 202451–202453; Fortaleza, médio Purus, 6°26′S, 64°17′W, MZUSP 4474, 4480, 8141; Humaitá, San Miguel, 70 m, 7°31′S, 63°2′W (coordinates for Humaitá), MNRJ 19295–19296; Igarapé Belém, Rio Solimões, 3°55′S, 69°37′W, AMNH 97077–97079; [Rio Cuieiras, 2°50′S, 60°30′W, MZUSP 65391, 65394 (metamorphs)]; [São Gabriel da Cachoeira, Parque Nacional Pico da Neblina, 0°5′S, 65°55′W, AL 2015, INPA 15634, 15637]; [São João, próximo Tapurucuara, 0°33′S, 64°57′W, MZUSP 37509]; Xerua, lado direito, Rio Juruá, 6°8′S, 67°50′W, INPA 2248. **RONDÔNIA:** Abunã, Rio Madeira, 9°42′S, 65°23′W, AL 122; Calama, 50 m, 8°4′S, 62°52′W, USNM 202455; Foz do Jamari, 8°28′S, 63°30′W, MZUSP 56504–56505; USNM 202449–202450; Porto Velho, 98 m, 8°46′S 63°55′W, INPA 15458, MZUSP 16657; São Carlos, 100 m, 8°28′S, 63°30′W, USNM 202454.

COLOMBIA. AMAZONAS: Leticia, ~100 m, 4°9′S, 69°57′W, AMNH 81859–81862, KU 124742, USNM 140289, 147029–147030, ICNMHN 46855; Leticia, Parque Nacional Natural Amacayacó, Cabaña Matamata, 3°58′S, 70°10′W, IND-AN 2100; Puerto Nariño, 3°45′S, 70°22′W, KU 153296–153297. **VAUPÉS:** [Río Querari near

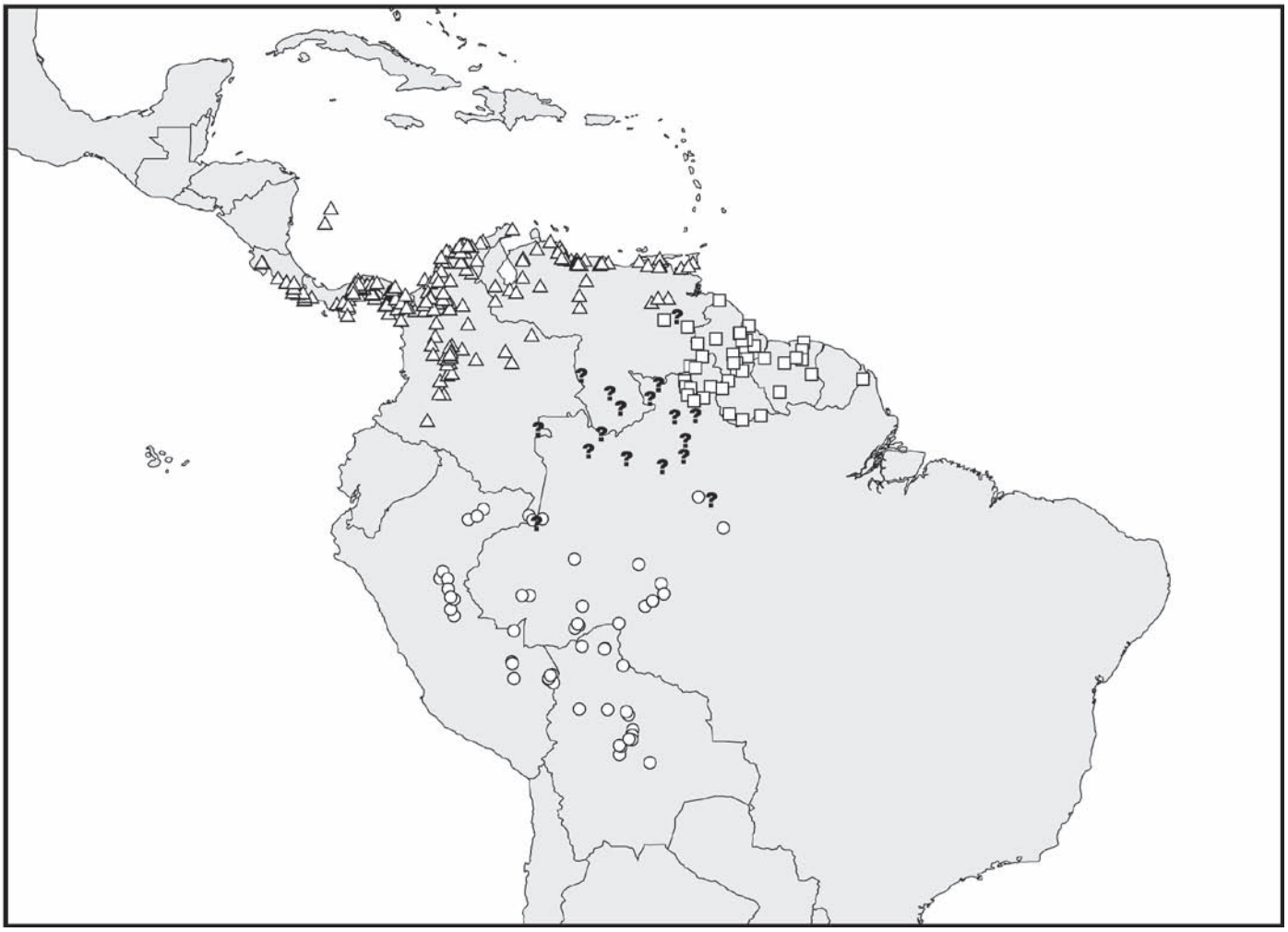

FIGURE 14. Distribution of *Leptodactylus bolivianus* (circles), *L. guianensis* (squares), and *L. insularum* (triangles). Question marks indicate localities where additional data are required to confirm the species identities.

junction of Río Querari and Río Vaupés, 1°4′N, 69°51′W, USNM 193874 (21.4 mm juvenile)].

PERU. Locality uncertain, possible Iquitos, AMNH 42890. **Huánuco:** Tournavista, 1200 m, 8°55′56″S, 74°42′19″W, MCZ-A 75025. **Loreto:** near Contamana, Rean Rean, Lago Suhuayo, Río Ucuyali, 7°15′S, 74°54′W, AMNH 42899, 43416; Domo Santa Clara, Orellana, ~100 m, 6°54′S, 75°4′W, USNM 127192–127194; Explorama Lodge, junction Río Yanomono and Río Amazonas, KU 220363; Iquitos, 100 m, 3°46′S, 73°15′W, AMNH 42570, MCZ-A 100080; Pampas Hermosas (= Pampa Hermosa), Río Cushabatay, 7°12′S, 75°18′W, AMNH 42141, 42147, 42831, 43120; Roaboya, ~100 m, 7°48′S, 74°52′W, AMNH 42603–42604, 42920, 45532; "San Antonio," Río Itaya, 3°56′S, 73°43′W, AMNH 43214. **Madre de Dios:** Cocha Cashu, Río Manú between Río Panagua and Río Cachira, ~400 m, 11°51′S, 71°19′W, KU 154880–154885; Cuzco (= Cusco) Amazonico, 200 m, 12°35′S, 69°5′W, KU 194911, 204508, 205143–205168, 207732–207734, 209182; Itahuanía, 450 m, 12°47′S, 71°13′W, FMNH 81533; Las Pampas del Río Heath, 160 m, 13°0′S, 69°0′W, USNM 314902; Pakitza, 350 m, 11°57′S, 71°17′W, USNM 342635–342640; Puerto Maldonado, 189 m, 12°36′S, 69°11′W, FMNH 83306; Tambopata Reserve, Explorer's Inn, 30 km (airline) SSW Puerto Maldonado, 280 m, 12°50′S, 69°17′W, USNM 222277–222280, 247337–247355, 247634–247644, 268961–268967, 342998, 343243; Tres Chimbadas, ~20 min downstream from Explorer's Inn, 12°47′S, 69°17 W, USNM 222275–222276, 222281. **Ucayali:** Balta, Río

Curanja, ~300 m, 10°8′S, 71°13′W, KU 196540–196554; Iparía, TNHC 37407; Pucallpa, 150 m, 8°23′S, 74°32′W, FMNH 56306–56307, 56446; Yarinacocha, 100–170 m, 8°15′S, 74°43′W, FMNH 45402–45405, 56292, 56295–56298, 56301.

VENEZUELA. Amazonas: [Río Casiquiare, 8 miles below Orinoco, 3°5′N, 65°53′W, AMNH 23167]; [Río Mavaca, 108 km SSE Esmeralda, 140 m, 2°15′N, 65°17′W, USNM 216705–216723]; [Río Maturacá, Catarata de Huá (= Salto de Huá), 100 m, 0°48′N, 66°21′W, USNM 83575].

Leptodactylus guianensis, new species

Figure 15

Holotype. USNM 531509, an adult male from Guyana; Rupunini, Iwokrama Forest Reserve, Sipuruni River, Pakatau Camp, 4°45′17′′N, 59°01′28′′W, 85 m. Collected by Maureen A. Donnelly and Megan Chen on 15 July 1997.

Paratopotyes. USNM 531508 (juvenile female), 531510–531511 (adult females), same locality and collectors; collected on 15 July 1997, 16 July 1997, and 21 July 1997 respectively.

Paratypes. AMNH 145130 and 145131 (adult males, tissue sampled), Guyana: East Berbice–Corontyne Region, Dubulay Ranch on the Berbice River, 60 m, 5°40′55″N, 57°51′32″W; collected by Charles J. Cole and Carol R. Townsend on 30 August 1995.

All other specimens examined are referred specimens, not paratypes.

FIGURE 15. Color photo of *Leptodactylus guianensis* from Coeroeni Island, Suriname (courtesy of Marinus S. Hoogmoed).

Etymology. The species is named for its distribution coinciding in large part with the Guiana Shield.

Diagnosis. Most specimens of *L. guianensis* have boldly mottled posterior thighs with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced; most specimens of *L. bolivianus* have the posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for the entire extent of the posterior thigh. The only consistent character diagnosing the three species is the adult male thumb spine. The single spine on each thumb in *L. guianensis* is modestly chisel shaped (Figure 13C), the single thumb spine of *L. bolivianus* is extremely chisel shaped (Figure 13B), and there are two rounded and pointed spines on each thumb in *L. insularum* (Figure 13A).

Description of Holotype. Figure 16. Snout rounded in dorsal and profile views. Canthus rostralis indistinct. Loreal concave-obtuse. Tympanum distinct, greatest diameter about 9/10 eye diameter. Tympanic annulus and adjacent area with numerous small keratinized tubercles. Vomerine teeth in two strongly arched series, narrowly separated from each other medially, positioned posteriorly to choanae. Each elliptical choana with anterior shelf extending over anterior edge of choana. Vocal slits elongate, parallel to jaw. Vocal sac not visible externally. Finger lengths in increasing order V < III < II < IV. Fingers with lateral fleshy ridges, clusters of small keratinized tubercles on upper side of finger II joints and rows of similar tubercles on the inner ridges of fingers III and IV. Finger subarticular tubercles pungent. Inner palmar tubercle large, oblong, pungent; outer palmar tubercle large, flat, elliptical, bifid distally, narrowly separated from inner tubercle. Smaller, rather indistinct palmar tubercles proximal to each subarticular tubercle and one indistinct palmar tubercle between metacarpal tubercles. Arms hypertrophied. Each thumb with one conical dark keratinized spine with a slightly developed chisel-shaped tip, tip on right thumb spine with three small cusps, tip on left with two small cusps. No large spines on chest. Upper and lower lips with numerous small keratinized tubercles. One central patch of numerous small keratinized tubercles on chest and a pair of tubercle patches on the lateral portions of the chest. Ulnar ridges weakly developed. Dorsal texture smooth anteriorly, with small scattered keratinized tubercles on posterior 2/3 of dorsum. A pair of well-developed dorsolateral folds extending from just posterior to the eye to the upper leg. A pair of slightly interrupted lateral folds from above the arm to groin. A pair of elongate glands above arm insertion. Belly disk fold distinct posteriorly

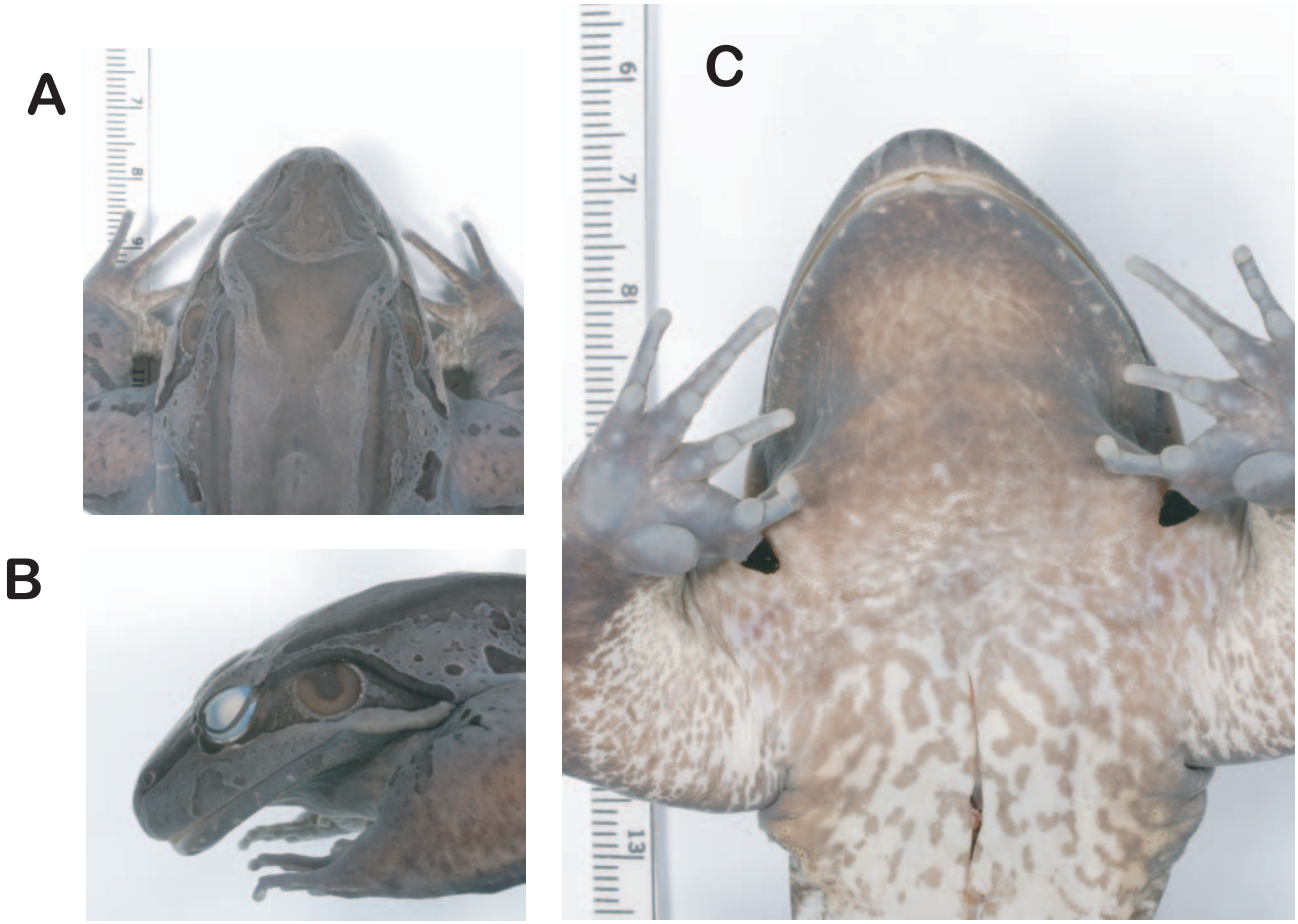

FIGURE 16. Dorsal view of (A) head, lateral view of head (B), and ventral view of the head, hands, and chest (C) of *Leptodactylus guianensis*, new species, USNM 531509.

only. Scattered keratinized tubercles ventrally in arm insertion area. Toe tips rounded. Toes with well-developed fringes and basal webbing. Subarticular tubercles moderately pungent. Weak metatarsal ridge. Outer metatarsal tubercle almost rounded, about 1/3 size of prominent, elongately oval inner metatarsal tubercle. Tarsal fold well developed, straight except for most proximal end, extending about 9/10 length of tarsus. Tarsal fold with scattered small keratinized tubercles. Tarsal fold abutting inner metatarsal tubercle. Upper shank surface shagreened with scattered small keratinized tubercles. Scattered keratinized tubercles in arm insertion area. Outer tarsus and sole of foot with scattered small keratinized tubercles.

Upper lip with light outlined dark triangular mark, apex ending just short of eye. Light stripe from below eye becoming very distinct under the tympanum and extending to end of commissural gland. Dark stripe from tip of snout through nostril to anterior edge of eye. Dark posteriorly directed interorbital triangle continuous with chevron in scapular region, rest of dorsum nondescript. Pair of dark oval spots above arm insertion. Posterior portion of lateral fold distinctly lighter, some lateral warts light highlighted as well. Chin and anterior chest darkly mottled. Central chest and anterior belly with light and dark labyrinthine pattern, grading posteriorly to almost completely lacking melanophores except for a few small scattered dots. Posterior thigh mottled, overall dark appearance, with sizeable irregular lighter vermiculations.

Measurements (mm): SVL 100.5, head length 34.4, head width 35.5, eye–midnostril distance 10.4, greatest tympanum diameter (including annulus) 7.9, thigh length 47.2, shank length 50.8, foot length 51.8.

Character Summary. The most frequent lip stripe patterns consist of a noticeable broad light

stripe extending from the front of the snout to the commissural gland, bordered below by continuous or broken markings on the upper lip and either bordered above by a dark line under the eye or not; or lip region uniform (about same intensity as background dorsal color) with or without a dark stripe under the eye and/or dark bars/mottling on the upper lip. The most frequent posterior thigh pattern is boldly mottled with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced. The most frequent lateral fold conditions are distinct folds only in the groin region, or indistinct overall, or indiscernible. The most frequent adult male chin tubercle condition is absence of tubercles.

Female (n = 52) SVL 66.0–109.2 mm (m [mean] = 88.2), male (n = 34) 79.5–109.5 mm (m = 94.8). Female head length/SVL ratio 0.33–0.40 (m = 0.359), male 0.33–0.39 (m = 0.365). Female head width/SVL ratio 0.31–0.37 (m = 0.327), male 0.32–0.39 (m = 0.352). Female eye–nostril distance/SVL ratio 0.09–0.11 (m = 0.099), male 0.09–0.12 (m = 0.101). Female tympanum diameter/SVL ratio 0.06–0.08 (m = 0.069), male 0.06–0.08 (m = 0.072). Female thigh length/SVL 0.42–0.52 (m = 0.476), male 0.44–0.53 (m = 0.484). Female shank length/SVL ratio 0.48–0.57 (m = 0.529), male 0.50–0.56 (m = 0.525). Female foot length/SVL ratio 0.48–0.58 (m = 0.527), male 0.46–0.57 (m = 0.527).

Tadpole. Unknown.

Advertisement Call. Unknown.

Distribution. Lowland portions of Guiana Shield regions of Guyana, Suriname, Venezuela, and adjacent Brazil (Figure 14).

Specimens Examined (n = 226). Localities enclosed by brackets require validation that the species is correctly identified. **BRAZIL. Roraima:** Boa Vista, 90 m, 2°49′N, 60°40′W, MZUSP 62482, 65508–65510, 66053; BR-174 at km 110 (Rio Surumu), 3°25′N, 60°18′W, MZUSP 60616; Marco da Fronteira BV-8, 4°28′N, 61°8′W, INPA 1306, MZUSP 65760–65761, 65783–65786, USNM 302445; [Cachoeira do Cujubim, Rio Catrimani, 1°45′N, 62°17′W, MZUSP 58533]; Cachoeira do Paredão, 2°57′N, 61°35′W, MZUSP 70634; Colonia Apiaú, 150 m, 2°38′N, 61°12′W, MZUSP 65923, 65948, 65953, 66317–66319, 68632–68633, USNM 302268–302270; Fazenda Salvamento, 3°20′N, 61°24′W, MZUSP 66031; Igarapé Cocal, near Tepequém, 3°45′N, 61°44′W, MZUSP 66008, 67004; Ilha do Maracá, 3°25′N, 61°40′W, MZUSP 60628, 62420–62426, 65548–65558, 65573, 65613, 65670–65672, 65698–65700, 65703–65705, 65735–65736, 65743–65744, 70649–70650, 70724; [Missão Catrimani, 0°27′N, 61°41′W, MZUSP 68734–68736]; [Parque Nacional Viruá, Roraima, próximo a Caracaraí, Igarapé Viruá, 1°0′N, 61°15′W, AL 12241–12242]; [Praia do Guariúba, 0°20′N, 61°44′W, MZUSP 67237–67240, USNM 302471–302473]; [Santa Maria da Boiaçu, MZUSP 67330, 67332–67335, 68296]; [Serra do Surucucus, 2°47′N, 63°40′W, MZUSP 65817]; [Uiaca, Rio Uraricoera (= Uaicás, Rio Urariquera), 3°33′N, 63°11′W, AMNH 100009–100010].

GUYANA. Region unknown: Anowini Creek, Essequibo River, UMMZ 79474; Essequibo River, AMNH 49257. **Barima–Waini Region:** Maburama, 100 m, 8°12′N, 59°47′W, UTA 55504. **Cuyuni–Mazaruni Region:** Blackwater Creek, 6°5′N, 59°59′W, AMNH 43670; Bartica, 3 m, 6°24′N, 58°37′W, MCZ-A 50704; Kalacoon, Mayarum River (= Mazaruni River), AMNH 3990; Kartabo, 100 m, 6°21′N, 58°41′W, AMNH 10397, 10423, 11709; Paruima (= Paruima Mission, Kamarang River), 614 m, 5°48′N, 61°1′W, UMMZ 85151, 58152(2). **Demerara–Mahaica Region:** Georgetown, sea level, 6°48′N, 58°10′W, AMNH 5064. **East Berbice–Corontyne Region:** Dubulay Ranch on the Berbice River, 60 m, 5°40′55″N, 57°51′32″W, AMNH 145130–145131, 145133–145134. **Potaro–Siparuni Region:** Magdalen's Creek Camp, near (~300 yards) [275 m] NW bank of Konawaruk River (~25 miles [40 km] linear) WSW Mabura Hill, 120 m, 5°13′7″N, 59°2′43″W, AMNH 166359–166366. **Upper Demerara–Berbice Region:** Berbice River camp at ~18 miles [30 km] (linear) SW Kwakwani (~2 miles [3.2 km] downriver from Kurundini River confluence), 60 m, 5°5′6″N, 58°14′14″W, AMNH 166341–166349, 166356–166358; Malali (= Malali rapids), 100 m, 5°37′N, 58°22′W, AMNH 45773; Penal Settlement, 6°24′N, 58°39′W, AMNH 10422; Rockstone, 100 m, 5°59′N, 58°33′W, FMNH 26633–26636, UMMZ 80442; Wismar, ~100 m, 6°0′N, 58°18′W, UMMZ 76681(2). **Upper Takutu–Upper Essequibo Region:** North of Acarahy Mountains, west of New River, 1°50′N, 57°30′W, KU 69667–69686; Iwokrama Forest Preserve, Burro-Burro river, Burro-Burro Camp, 83 m, 4°43′52″N, 58°51′2″W, AMNH 164217–164218, USNM 531502; Iwokrama Forest Preserve, Kabocalli Camp, 101 m, AMNH 164219–164221; Iwokrama Forest Preserve, ~3 miles [4.8 km] S Kurupukari Base Camp on Georgetown-Lethem road, Three Mile Camp, 102 m, 4°37′59″N, 58°42′52″W, USNM 531501; Iwokrama Forest Preserve, 5 hours (downstream) Kurupukari Base Camp on Essequibo River, Kabocali Camp, 101 m, 4°17′6″N, 58°30′34″W, USNM 531503–531507; Iwokrama Forest Preserve, Sipuruni River, Pakatau Camp, 85 m, 4°45′17″N, 59°1′28″W,

USNM 531508 (Paratype, juvenile female), 531509 (Holotype, male), 531510–531511 (Paratypes, females); Iwokrama Forest Preserve, Pakatau Creek, 85 m, 4°45′N, 59°1′W, AMNH 164223–164227; northern Rupununi Savanna, Karanambo (on Rupununi River), McTurk Ranch, 110 m, AMNH 136033–136034; Karanambo Ranch, Maricuba Lake, ~100 m, 3°45′8″N, 59°18′36″W, USNM 497730; Kuyuwini, 1°55′N, 59°15′W, AMNH 43439, 43684, 46278–46279, 46286; Moco-Moco (= Mocomoco), 29 km SE Lethem, base of Kanuku Mountains, 100 m, 3°18′12″N, 59°39′0″W, USNM 497731; Mocho Mocho (= Moco Moco, = Mocomoco), USNM 146369–146374; Onora River (= Unorowo River), tributary of Essequibo River, 1°35′N, 58°30′W, AMNH 49331–49335, 53440–53445.

SURINAME. Brokopondo: Brownsberg Nature Park, near Mazaroni top, ~450 m, 4°55′N, 55°12′W, AMNH 87757; Loksietattie, Saramacca River, 5°2′N, 55°32′W, FMNH 134739. **Commewijne:** Plantation Ma Retraite, sea level, 5°52′N, 55°8′W, AMNH 125820. **Nickerie:** Kaiserberg airstrip, Zuid River, FMNH 128810–128818, 128820–128825, 128919, 128926, 128929, 128944. **Para:** near Zanderij, sea level, 5°27′N, 55°12′W (coordinates for Zanderij), USNM 159513–159514. **Saramacca:** Foengoe Island Airstrip, Raleigh cataracts, Coppename River, 50 m, 4°44′N, 56°12′W, AMNH 87758. **Sipaliwini:** Drietabbetje, Tapahony River, 4°7′N, 54°40′W, MCZ-A 97282; Marapi LLB Station on Covantyn River, 5°N, 57°W, MCZ-A 110622. **Wanica:** Pad (Path) van Wanica, 5°47′N, 55°20′W, KU 204508.

VENEZUELA. Bolívar: Arabupo (= Arabopo), 1,200–1,300 m, 5°6′N, 60°44′W, UMMZ 85198; ~35 km S El Manteco, 350 m, 5°6′N, 62°37′S, TCWC 60158; 13 km S, 1 km E Puente Cuyuni, 140 m, 6°43′N, 61°35′W (coordinates for Puente Cuyuni), KU 166405–166408; Santa Lucia de Surukun, 45 km NE Icabaru, 851 m, 4°33′N, 61°25′W, USNM 216726.

Leptodactylus insularum Barbour, 1906

Figure 17

Leptodactylus insularum Barbour, 1906:228. Type locality: Saboga Island, Panama.

Barbour (1906) described *Leptodactylus insularum* sp. nov. based on a lot of 12 specimens with the single MCZ number 2424 from Saboga Island in the Gulf of Panama. The type description is not based on a single specimen but is a composite description including male and female data. Subsequently, 11 of the 12 original syntypes were exchanged or had new MCZ catalogue numbers assigned to them. There remains one specimen with MCZ number 2424. As the specimen bearing number 2424 is the only specimen currently with that number in the MCZ collection, we hereby designate MCZ 2424 as the lectotype of *Leptodactylus insularum* Barbour, 1906.

FIGURE 17. Photo of *Leptodactylus insularum* from San Andrés, Colombia (courtesy of Roy W. McDiarmid).

Lectotype Description. Adult female (Figure 18) with pigmented ova. Snout nearly rounded in dorsal view, rounded in profile view. Canthus rostralis indistinct. Loreal concave-obtuse. Tympanum distinct, greatest diameter about 7/10 eye diameter. Tympanic annulus smooth. Vomerine teeth in arched series, apices anterior, narrowly separated from each other medially, posterior to choanae. Choanae damaged, shape uncertain. Finger lengths II = IV > III ≈ V. Fingers with weak lateral ridges or smooth. Finger subarticular tubercles rounded, not pungent. Inner palmar tubercle large, ovate, prominent; outer palmar tubercle present, somewhat indistinct, right hand tubercle weakly heart-shaped, left present, shape unclear. Dorsum smooth. A pair of distinct dark dorsal folds from just behind eye to sacrum, folds indistinct from sacrum to juncture of leg and body. Supratympanic fold dark brown from eye to behind jaw. Short dark lateral folds extending from supratympanic fold at level of posterior tympanum to ending shortly after posterior arm insertion. No distinct glands. Belly disk fold indistinguishable. Arms and body lacking tubercles. Toe tips round, swollen, just larger than toes immediately behind tips. Toes with lateral fringes and weak basal webbing between toes I–IV. Toe subarticular tubercles large, rounded.

FIGURE 18. Dorsal and ventral views of the lectotype of *Leptodactylus insularum*, MCZ 2424.

Weak outer metatarsal ridge continuous with outer ridge on toe V. Outer round metatarsal tubercle about ¼ size of oval inner metatarsal tubercle. Distinct tarsal fold lacking tubercles, extending from and continuous with inner metatarsal tubercle to about 9/10 length of tarsus. Upper thigh surfaces smooth. Upper shank surfaces with numerous small white tubercles. Outer surface of tarsus with small white tubercles, a few of which with brown keratinized tips. Sole of foot smooth.

Face with indistinct markings, a slightly darker indistinct interrupted stripe from nostril to eye, postocular area darker brown than upper lip area. Dorsum brown with indistinct darker marks scattered between dorsolateral folds. Flanks with irregular dark brown spots on tan to brown background. Belly with small tan dots visible under magnification, overall belly very light tan, throat slightly darker. Posterior thigh surfaces with a pattern of large darker and lighter brown mottling.

Measurements (mm): SVL 83.8, head length 29.2, head width 29.1, eye–nostril distance 7.8, tympanum diameter (including annulus) 5.6, thigh length 38.8, shank length 42.3, foot length 24.6.

ETYMOLOGY. Barbour did not specifically indicate the derivation of the species name. All of the specimens indicated in the original description are from islands in the Gulf of Panama, hence the specific name.

DIAGNOSIS. Most specimens of *L. insularum* have boldly mottled posterior thighs with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced; most specimens of *L. bolivianus* have the posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually for the entire extent of the thigh. *Leptodactylus insularum* adult males only have central chest patches of tubercles; *L. bolivianus* males have lateral

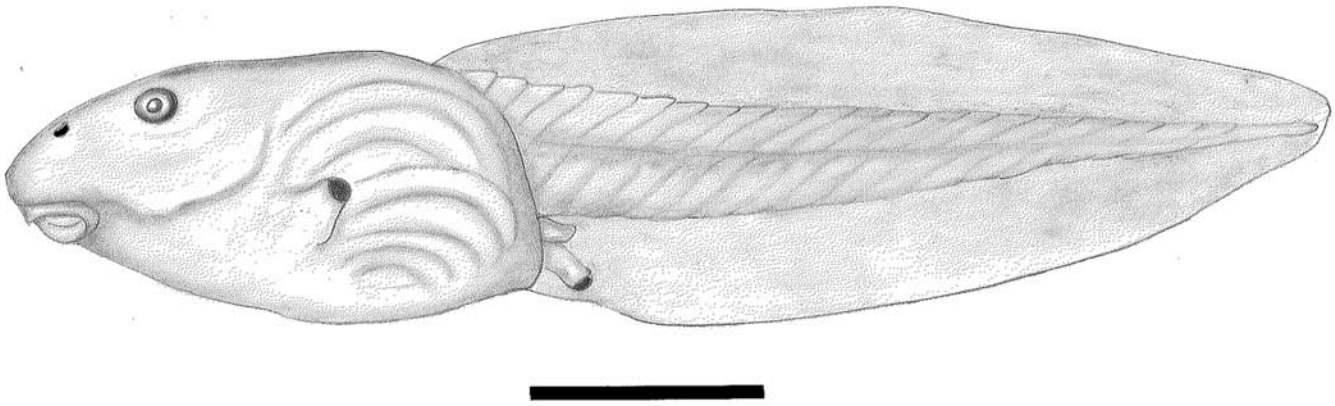

FIGURE 19. Illustration of lateral view of *Leptodactylus insularum* tadpole, USNM 576264, Costa Rica, Guanacaste, ~12 km (airline) SSW Cañas, scale bar = 5 mm.

patches of chest tubercles in addition to the central patch. The one entirely consistent character diagnosing the three species is the adult male thumb spine. The two spines on each thumb of *L. insularum* are rounded and pointed (Figure 13A); the single spine on each thumb of *L. bolivianus* and *L. guianensis* is modestly to extensively chisel shaped (Figure 13B, C).

Character Summary. The most frequent lip stripe pattern consists of lip region uniform (about same intensity as background dorsal color) with or without a dark stripe under the eye and/or dark bars/mottling on the upper lip. The most frequent posterior thigh pattern is boldly mottled with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced. The most frequent lateral fold condition is fold distinct or slightly interrupted from supratympanic fold to leg. The most frequent adult male chin tubercle condition is presence of tubercles.

Female (n = 224) SVL 60.4–99.1 mm (m [mean] = 81.3), male (n = 216) 66.0–104.6 mm (m = 86.8). Female head length/SVL ratio 0.33–0.43 (m = 0.365), male 0.32–0.41 (m = 0.364). Female head width/SVL ratio 0.30–0.38 (m = 0.334), male 0.32–0.40 (m = 0.353). Female eye–nostril distance/SVL ratio 0.08–0.12 (m = 0.102), male 0.09–0.12 (m = 0.102). Female tympanum diameter/SVL ratio 0.05–0.08 (m = 0.070), male 0.06–0.08 (m = 0.070). Female thigh length/SVL ratio 0.36–0.58 (m = 0.452), male 0.39–0.51 (m = 0.451). Female shank length/SVL ratio 0.44–0.58 (m = 0.495), male 0.43–0.58 (m = 0.489). Female foot length/SVL ratio 0.44–0.57 (m = 0.506), male 0.42–0.59 (m = 0.503).

Tadpole. Figures 19, 20. Exotrophic, lentic, benthic guild member (McDiarmid and Altig, 1999, guild IIA1); oral disk anteroventrally positioned, entire (not emarginated), moderate anterior papilla gap (~31% oral disc width), single and/or double row of marginal papillae; tooth row formula 2/3; spiracle sinistral; vent median; dorsal fin origin at tail-body junction; dorsum with a relatively uniform profusion of melanophores, venter with light to moderate profusion of melanophores, venter with light to moderate profusion of melanophores from oral disk to guts, scattered melanophores over guts, tail musculature uniform moderate to dark brown, tail fins ranging

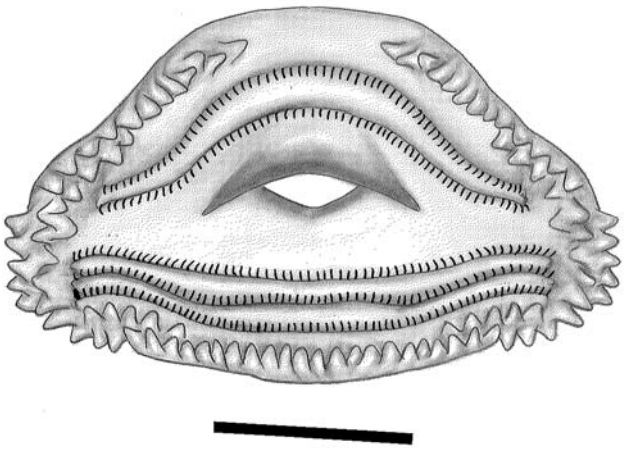

FIGURE 20. Illustration of oral disk of *Leptodactylus insularum* tadpole, USNM 576264, Costa Rica, Guanacaste, ~12 km (airline) SSW Cañas, scale bar = 1 mm.

from same pattern as tail musculature to ventral fin with a gradient of no melanophores next to body to uniform brown around midfin; maximum total length stage 37, 35.2 mm (based on voucher specimens KU 68365, USNM 241340, 330406, 330837, 576264).

This description overall agrees with the previous one reported by Heyer (1970).

Advertisement Call. Calls of single notes at rates of 1.2–2.5/s; call duration 0.08–0.12 s; call amplitude modulated, loudest portion from about midcall to 4/5 call duration; call frequency modulated beginning at 110–220 Hz with the highest frequency starting about at 1/3 call length of 890–1,200 Hz (Figures 6, 7) (based on voucher recordings from Fouquette, 1960; Ibáñez et al., 1999; and KU recording 801).

Distribution. Mainland Pacific versant of Costa Rica, throughout lowland Panama, Caribbean drainages of Colombia and Venezuela, Trinidad (Figure 14).

Specimens Examined (n = 1,018). **COLOMBIA. Antioquia:** Chigorodó, ~100 m, 7°41′N, 76°42′W, AMNH 76186, USNM 151887; El Cinco, 7°45′35.4”N, 74°46′49.1″W, MPUJ 2073–2074; Jericó, 1967 m, 5°47′N, 75°47′W, MCZ-A 24886; Medellin, 1500 m, 6°15′N, 75°35′W, AMNH 38828; San Luis, corregimiento La Danta, near Puerto Carreño, ICNMHN 17225; Nechí, 100 m, 8°7′N, 74°46′W, FMNH 54568, 54571; 20 km abajo del [below the] Río Nechí, frente al caserio [in front of the village] Bijagual, 7°50′13″N, 74°47′43.7″W, MPUJ 2840; Urabá, Río Currulao, 50 m, 7°0′N, 75°30′W, FMNH 63847. **Arauca:** Estación Cravo Norte region, ICNMHN 26833–26841, 27226–27231, 27680–27684, 27890. **Atlántico:** Barranquilla, Airport Soledad, sea level, 10°55′N, 74°46′W, USNM 152641; Municipio Los Pendales, 10°37′N, 75°13′W, ICNMHN 42275; Estación Piscicultura de Soplaviento, 10°23′36″N, 75°8′28″W, IND-AN 567, 569, 572, 576, 579–581; Puerto Colombia, 10°59′28″N, 74°57′43″W, ICNMHN 1020; Santa Lucia, 10°21′N, 74°58′W, ICNMHN 46136; Tubará, corregimiento Cuatrobocas, 10°55′N, 73°57′W, ICNMHN 49010. **Bolívar:** no other locality data, MPUJ 2187–2190; Isla Fuerte, 9°25′N, 76°10′W, FMNH 152371; San Cristóbal, Canal del Dique, ~100 m, 9°53′N, 75°15′W (coordinates for San Cristóbal), AMNH 75742–75746, 75748; Hacienda El Ceibal, 80 m, 10°37′N, 75°14′W, ICNMHN 44117–44135; Hacienda La Aguada, 22 km from San Onofre, USNM 152653; Santa Rosa, 600 m, 7°58′N, 74°3′W, ICNMHN 2102, 2105–2106, 2108, 2245–2246, 2262–2287, 2289. **Boyacá:** Las Mercedes, Hacienda Los Balcones, 380 m, ICNMHN 38433–38437; Muzo, 1240 m, 5°33′N, 74°6′W, IND-AN 2739, 2742; Puerto Boyacá-Puerto Romero carretera, km 29–35, 300–320 m, ICNMHN 38438–38440; Puerto Boyacá, Insp. Puerto Romero, ICNMHN 44726–44730; Puerto Boyacá, Vereda El Okal, Quebrada La Fiebie, km 29–30 Otanche road, 280 m, ICNMHN 45427–45437, 45433, 45438. **Caldas:** Finca Tintiná, 5°14′29.5″N, 75°41′11.1″W, MPUJ 1827; Guarinocito, 280 m, 5°23′N, 74°43′W, AMNH 81855–81858, CMB 548–549, MPUJ 374; Reserva Riomanso, 5°40′N, 74°46′W, MPUJ 3016; Sitio La Turqueza, 5°1′9.9″N, 75°42′5.5″W, MPUJ 1825–1826, 2075; 15.6 km carretera de la Victoria a la Central, ICNMHN 34607–34608. **Casanare:** Orocué, 200 m, 4°48′N, 71°20′W, ICNMHN 34683; San Luis de Palenque, 5°25′N, 71°40′W, ICNMHN 2664. **César:** Finca El Diamante, 15 km south of Bosconia, 9°58′N, 73°51′W (coordinates for Bosconia), USNM 200350–200353; Casacara, Municipio Codazzi, 9°20′N, 74°45′W, ICNMHN 39935–39937; Finca Mundo Nuevo, 9°45′N, 73°33′W, MPUJ 1388–1390; Valle Dupar (= Valledupar), Santa Marta mountains, 202 m, 10°29′N, 73°15′W, UMMZ 54597–54598. **Chocó:** Acandí, corregimento Gilgal, 2000 m, ICNMHN 18112–18114; Parque Nacional Natural Katios, 7°50′N, 77°7′W, ICNMHN 3224, 47167–47174, 47177–47186, IND-AN 2000–2002; Río Sucio Hacienda Sautatá, 20 m, 7°50′0″N, 77°4′5″W, ICNMHN 187–192; Río Sucio, Vereda Las Teresitas, 7°9′25″N, 77°28′15″W, ICNMHN 47188. **Córdoba:** Ayapel, casco Urbano, 8°18′58″N, 75°8′30″W, ICNMHN 48165; Ayapel, corregimiento Nariño, caserío Playa Blanca, 8°17′47″N, 74°58′46″W, ICNMHN 48166; Ayapel, Hacienda La Balsa, 8°18′5″N, 75°2′24″W, ICNMHN 48167; Ayapel, Hacienda Quibrache, 8°17′47″N, 74°58′46″W, ICNMHN 48163; Lórica, Bosque Ita, 20 m, 9°16′N, 75°49′W, ICNMHN 48172; Lórica, Estación Piscicola, CVS, 20 m, 9°13′N, 75°50′W, ICNMHN 48168–48170, 48320; Lórica, La Montaña del Mono, 20 m, 9°13′N, 75°50′W, ICNMHN 48171; Monte Libano (= Montelíbano), 8°5′N, 75°29′W, ICNMHN 42755–42756, MZUSP 106090–106091; Montería, Hacienda El Diluvio, 64 m, 8°43′18″N, 75°59′26″W, ICNMHN 48159–48161; Municipio de Montería, ICNMHN 19550–19551; Montería, Maracayo, 8°24′32″, 75°53′26″W, ICNMHN 48157–48158; Montería, Quebrada Betancí, 8°22′44″N, 75°49′55″W, ICNMHN 48156; Pueblonuevo, Hacienda Toronto, 8°24′N, 75°17′W, ICNMHN 48173; Represa de Urrá, 70–150 m, 8°1′4″N, 76°12′53″W, ICNMHN 41313; Río Manso, tributary upper Río Sinu, 7°41′N, 76°10′W, USNM 151031–151033; Tierra Alta, 70 m, 8°11′N, 76°4′W, FMNH 61807–61812, KU 144967–144977, UMMZ 132466(4). **Cundinamarca:** Beltrán, 230 m,

4°47′N, 74°47′W, ICNMHN 2339–2343, 2603, USNM 145708–145742, 147254; Girardot, ~300 m, 4°18′N, 74°48′W, AMNH 71575–71577, 75158, USNM 146216–146218, 146393, 146467; Girardot, Isla El Sol, al Este de la confluencia del Río Bogotá, 310–320 m, ICNMHN 273–275, 277–279, 281–293. **Guajira:** Municipio Patomino, 11°14′8.1″N, 73°33′7.3″W, MPUJ 2897; Nazareth (= Nazaret), 200 m, 12°11′N, 71°17′W, USNM 115112–115114, 194735; near Piojo, USNM 152676–152677; Río Barbacoas, Arroyo de Arenas, 11°25′N, 73°5′W, UMMZ 54601; Riohacha, corregimiento Calabacito, 130 m, 11°33′N, 72°55′W (coordinates for Riohacha), ICNMHN 11859–11865. **Huila:** Campoalegre, 530 m, ICNMHN 9365; Finca La Tribuna, 3°4′4″N, 75°22′3″W, MPUJ 3573–3576; Finca San Rafael, 3°3′15″N, 75°6′25.5″W, MPUJ 1786–1787; Parque Nacional Natural Cueva de los Guacharos, Municipio Acevedo, 1200 m, 1°35′N, 76°1′W, USNM 148859. **Magdalena:** Balneario El Rodadero de Guaira, 11°12′N, 74°13′W, KU 169075–169089; Bolívar, Santa Marta mountains, 10°21′N, 74°9′W, UMMZ 54596; Ciénaga, sea level, 11°1′N, 74°15′W, USNM 144157–144578; Don Diego, sea level, 11°15′N, 73°42′W, UMMZ 48118, 48119(3), 48488(10); Fundación, 60 m, 10°26′N, 74°7′W, MCZ-A 8966–8967, UMMZ 48106–48108, 48110–48111, 48113–48117; Isla Salamanca–Los Cocos, sea level, 10°59′N, 74°27′W, IND-AN 622–626, 6629; Mamatoco (= Manatoca, Mamatoca), 11°15′N, 74°9′W, UMMZ 48120; Minca, on road to San Lorenzo, 11°12′N, 74°4′W, USNM 150926; Parque Nacional Natural Tayrona (= Tairona, Tayruna), IND-AN 2462, 2523; Parque Nacional Natural Tayrona, Arrecifes, 11°19′28.5″N, 73°57′43.7″W, IND-AN 2483; Parque Nacional Natural Tayrona, Cañaveral, sea level, 11°19′N, 73°56′W, IND-AN 2441–2442, 2488–2490; Parque Nacional Natural Tayrona, 2–3 km cabaña Cañaverales, ICNMHN 13611–13613, 13659; Parque Nacional Natural Tayrona, Cinto, 11°20′N, 74°4′W, IND-AN 2443, 2446; Parque Nacional Natural Tayrona, trail between Cañaveral and El Pueblito, USNM 200348–200349; Parque Nacional Natural Tayrona, El Cedro, 11°19′54.67″N, 74°0′54.31″W, IND-AN 2463; Parque Nacional Natural Tayrona, within 2 km of El Cedro station, USNM 200354; Parque Nacional Natural Tayrona, near La Gayraca, USNM 200356; Parque Nacional Natural Tayrona, Los Naranjos, 150 m, 11°18′N, 73°54′W, IND-AN 2482; Río Buritaca, 11°16′N, 73°47′W (coordinates for mouth of river at Atlantic Ocean), USNM 150992–150994; Río Frio, 30 m, 10°55′N, 74°10′W, MCZ-A 16067–16068; between Santa Marta and Mamatoco (Manatoca, Mamatoca), USNM 150893–150924. **San Andrés y Providencia:** Isla de Providencia, 13°21′N, 81°22′W, MCZ-A 19261–19266, USNM 76956–76957, 146214–146215; Isla de Providencia, Agua Mansa, IND-AN 1096–1102; Isla de Providencia, reservoir, 80 m, ICNMHN 33421–33427; Isla de San Andrés, 12°32′N, 81°42′W, AMNH 71578–71580, KU 265811–265812, MCZ-A 17370–17375; Isla San Andrés, near Punta Paraiso, UMMZ 127882. **Santander:** El Centro, 100–200 m, 6°57′N, 73°46′W, FMNH 81759, 81761, USNM 144832–144837. **Sucre:** Hacienda La Estanzuela, 4 km east of Tolú, 9°31′N, 75°32′W, USNM 200343–200347; Municipio San Juan de Onofre, corregimiento de Berrugas, Finca Los Morros, 5 m, ICNMHN 43006; Sincelejo to Tolú, USNM 150743–150749. **Tolima:** El Cardonal, 5°5′13″N, 74°41′53″W, MPUJ 1402, 1405, 1409, 1416, 1418–1419, 1421; Espinal, Magdalena Valley, 200 m, 4°7′N, 74°53′W, MCZ-A 15067, 15071; Finca El Antonio, 3°47′37.8″N, 75°18′7″W, MPUJ 1788; Guayabal, 4°58′N, 74°54′W, MPUJ 1391–1394, 1398; Honda, 230 m, 5°12′N, 74°45′W, AMNH 22604, UMMZ 76073; 4 km northwest of Honda, 5°14′N, 74°46′W, AMNH 57549–75751; Municipio Margarita, ICNMHN 42765–42766; Melgar, orillas Río Sumapáz, 400 m, 4°12′N, 74°39′W, ICNMHN 1422, 18143; Municipio Venadillo, 4°43′17″N, 74°55′54″W, ICNMHN 43172.

COSTA RICA. Guanacaste: Estación Experimental Enrique Jiménez Nunez, 13.6 miles SW Cañas, 20 m, 10°20′N, 85°9′W, USNM 219762–219763; Río Bebedero, 5 km S Bebedero, 5 m, KU 65653–65656. **Puntarenas:** Dominical, ~6.4 km N, 9°17′N, 83°51′W, KU 204367; Golfito, 10 m, 8°38′N, 83°11′W, KU 32294–32304, 65651–65652, 86292; Palmar (= Palmar Sur), 8°58′N, 83°27′W, KU 32293, 65648; Palmar Sur, 5.6 km SE, 8°56′N, 83°25′W, KU 65646; Palmar Sur, 7 km SE, 8°56′N, 83°25′W, KU 65647; Parrita, 9°30′N, 84°19′W, MZUSP 106680; Rincón de Osa, 8°42′N, 83°29′W, KU 102098, USNM 227621–227624; San Isidro del General, 15–20 miles [24–32 km] W on Dominical road, 9°16′N, 83°50′W, KU 32392; Villa Neilly, 1.6 km WNW, 75 m, 8°40′N, 82°55′W, KU 65649–65650; Volcan Buenos Aires, 1 mile [1.6 km] E, Cone Finca, 9°13′N, 83°25′W, UMMZ 117577.

PANAMA. Province unknown: Between Campana and La Venta, USNM 103481; Canal Zone, KU 67947; Istmo do Panamá, MZUSP 5454; Río Morte Arnade,USNM 53928; Tabernilla, FMNH 16683. **Chiriquí:** David, 0.7 miles [1.1 km] north, 0.2 miles [0.3 km] west, 8°26′N, 82°26′W, AMNH 69714; Progreso, 8°27′N, 82°50′W, UMMZ 58153–58165; Puerto Armuelles, 8°17′N, 82°52′W, MCZ-A 24236–24238; Puerto Armuelles, 7.5

km N, 8°19′N, 82°49′W, KU 107163–107197. **Coclé:** Penonomé, 6 km SSW, 30–70 m, 8°31′N, 80°22′W, KU 107203; Río Chorrera, 8°18′N, 80°23′W, USNM 53885; Valle de Anton, 560 m, 8°37′N, 80°8′W, USNM 139703. **Colón:** Achiote, 9°13′22″N, 80°1′9″W, KU 76514–76516; France Field, 9°22′N, 79°52′W, MCZ-A 10143–10144; Frijoles, lake at, 9°10′N, 79°50′W, FMNH 6003–6004; Gatun, 9°16′N, 79°55′W, FMNH 16658. **Darién:** Camp Townsend, Camp Creek, 8°10′N, 77°42′W, AMNH 40931, 40998–40999, 41027–41031, 41033–41037, 41063, 41727; Camp Townsend, below Yavisa, Río Chucunaque on Río Darién, 8°10′N, 77°42′W, AMNH 41715; Cana, 460 m, 7°45′55″N, 77°41′6″W, USNM 50222; Canclones, 8°20′N, 77°46′W, UMMZ 132664, 132667(5), 132668–132669; Canglon, camp site below, Río Chucunaque, AMNH 41128, 41130–41131; Chucunaque, Río Chucunaque, AMNH 41121–41122; Camp Chucunaque, ~0.5 miles [0.8 km] S, AMNH 40614–40615, 40643–40644, 40699, 40713–40715, 40929, 41088–41089, 41734, 41766, FMNH 170431, KU 80380–80384, 107209–107222, UMMZ 125006, 137749–137750, USNM 140575–140583; Jaqué, 7°31′N, 78°10′W, KU 115280; Mount Sapo, 7°58′31″N, 78°21′43″W, MCZ-A 9179–9180; Ortiga Camp, 8°45′N, 78°0′W, FMNH 170432, 170464; Río Abobi, Río Chucunaque, AMNH 40897; mouth of Río Canglon, Río Chucunaque, 8°20′N, 77°46′W, AMNH 40585–40587, UMMZ 123281(2), 124998–125002, 125003(2), 125004–125005, 132670; Río Chico, near Avelinos, 8°12′N, 77°36′W, AMNH 39787, 40979–40984; near Río Membrillo, Río Chucunaque, 8°39′N, 77°47′W, KU 116816–116821; ~7 km above Río Mortí, Río Chucunaque, 8°52′N, 77°59′W, KU 107223–107227, 107906; just below mouth of Río Mortí, Río Chucunaque, 8°50′N, 77°59′W, UMMZ 132663; intersection of Río Chucunaque and Río Ucurganti, 8°25′N, 77°48′W, USNM 140616–140619; Río Chucurti (= Subcurtí), near camp, 8°45′N, 77°57′W, AMNH 40622, 40627; near mouth of Río Clarita, 8°6′N, 77°28′W, UMMZ 132665–132666; Río Jesusito, Sapo Mountains, 8°1′N, 78°16′W, MCZ-A 9167; mouth of Río Membrillo, 8°39′N, 77°47′W, AMNH 40551–40554, Río Pita, 10 m, FMNH 67884, 67886–67892; camp above Río Sansan (= Río Sansón), Río Chucunaque, 8°24′N, 77°47′W, AMNH 40646–40647; Río Suchuti (= Río Sucutí = Río Subcurtí), 8°45′N, 77°57′W, AMNH 40798; mouth of Río Tuquesa on Río Chucunaque, 8°23′N, 77°47′W, AMNH 40761–40765; Río Ucurganti, ~7 km above mouth, 30 m, 8°28′N, 77°48′W, KU 10725, 116822–116823; Santa Fe camp, FMNH 170434–170435; Three Falls Creek (= Camp Creek, Camp Townsend), 8°10′N, 77°42′W, AMNH 41711; Yavisa, across stream from, 8°11′N, 77°41′W, AMNH 41145. **Herrera:** Parita, 8°0′N, 80°31′W, USNM 127262–127286; 5 km northwest Santa María, near Río Santa María, 8°8′N, 80°41′W, KU 107202. **Los Santos:** 6 km (road) southwest Los Santos, 7°56′N, 80°25′W (coordinates for Los Santos), USNM 203649; Tonosí, 40 m, 7°24′N, 80°27′W, KU 108616. **Panamá:** Ancon, 8°58′N, 79°33′W, MCZ-A 10142; Balboa, 8°57′N, 79°34′W, KU 107204; Barro Colorado Island, 9°11′N, 79°57′W, FMNH 173206, MCZ-A 19207; Briya Point near Palo Seco, near Balboa, 8°55′N, 79°34′W (coordinates for Palo Seco), MCZ-A 10145; Bruja Point, MCZ-A 10669, 11750; Cocoli, 8°59′N, 79°35′W, USNM 143347; Fort Clayton, 9°0′N, 79°45′W, MCZ-A 10141; Fort Kobbe, 8°55′N, 79°35′W, USNM 193348; Howard Air Force Base, 8°55′N, 79°36′W, USNM 193337, 193339; Isla Chapera, 10 m, 8°35′N, 79°2′W, AMNH 87337–87339; Isla Cantadora, 8°38′N, 70°02′W, AMNH 87334–87336; Isla Saboga, 10 m, 8°37′N, 79°4′W, AMNH 87340–87344; Juan Mina, 25–30 m, 9°10′N, 79°39′W, FMNH 57537–57539, 60488, 67883, KU 107206, UMMZ 135375; Los Overos, on Río Indio, road to Las Minas, 593785 0988063 UTM, USNM 565027–565028; Miraflores, 9°0′N, 79°36′W, USNM 53841; Nueva Gorgona, 8°33′N, 79°52′W, AMNH 69715–69719; Panama City (= Old Panama = Panama Viejo), 8°58′N, 79°32′W, AMNH 41068–41070, 52742, FMNH 60487, 170433, KU 116814–116815; Pipeline Road, 9°7′N, 79°47′W to 9°15′N, 79°49′W, UMMZ 152934; Puerto La Chorrera, near, 8°52′N, 79°43′W; Río Mamone (= Río Mamoní), El Capitán (= La Capitana), 9°9′N, 79°4′W, FMNH 16657, USNM 53982; Río Siluganti (= Río Chuluganti), 9°13′13″N, 78°48′9″W, UMMZ 137745–137748; Río Tocumen, 9°1′N, 79°23′W, MCZ-A 10140; Rousseau, 8°58′N, 79°35′W, KU 67959; Saboga Island, 10 m, 8°37′N, 79°4′W, MCZ-A 2444, 99314–99319; San Miguel (= Isla San Miguel), 8°22′N, 78°55′W, AMNH 6669, MCZ-A 2424, 6901–6902, UMMZ 48076, USNM 58062; Summit Gardens, 9°4′N, 79°39′W, KU 107904–107905, UMMZ 167314; Tapia, 9°4′N, 79°25′W, AMNH 18915–18917, 18920; Tocumen, 9°5′N, 79°23′W, USNM 147504; 8 km NE Tocumen, 9°6′N, 79°20′W, KU 107207–107208; near Venado Beach, 8°54′N, 79°36′W, USNM 19336. **San Blas:** Camp Sasardí, 12 m, 9°0′N, 77°48′W, KU 115275–115279. **Veraguas:** 3.4–5 km N Montijo, 7°59′N, 81°4′W, KU 107907–107908, 107198–107201.

TRINIDAD. Nariva: Brickfield, 10°20′N, 61°16′W, FMNH 49661–49663. **St. Patrick:** Icacos Peninsula, USNM 306180; Icacos Point, 10°2′N, 61°55′W, USNM 287010; Laurier Pond near Siparia, 10°8′N, 61°30′W (coordinates for Siparia), FMNH 218782–218783.

VENEZUELA. Anzoategui: Carapá, near, 8°22′N, 63°13′W, USNM 80613–80614. **Apuré:** Hato La Guanota, 4 km W San Fernando de Apuré, 7°52′N, 67°32′W, TCWC 45153, 45167. **Aragua:** Cuyagua, 10°29′N, 67°42′W, USNM 258124; El Limón, above Maracay, 400 m, 10°18′N, 67°38′W, UMMZ 157138; Hacienda La Trinidad, near Maracay, 455 m, 10°17′N, 67°37′W, FMNH 35988–35989; Lake Valencia, northern shore, 415 m, 10°11′N, 67°45′W, UMMZ 157102–157103; Maracay, 455 m, 10°15′N, 67°36′W, FMNH 190403, MCZ-A 50703; south of highway between Maracay and Valencia, 13 km W Maracay, 420 m, 10°16′N, 67°38′W, UMMZ 157104–157106; near Ocumare, 25 m, 10°28′N, 67°46′W, UMMZ 122371(2), 171686–171689; road between Ocumare (inland village) and Rancho Grande, 10°22′N, 67°42′W, UMMZ 157100; Parque Nacional Henri Pittier (Estación Biologica Rancho Grande), 910–1,170 m, 10°22′N, 67°41′W, AMNH 70653, KU 132782, MZUSP 8083, UMMZ 122372, 157101; near Turmero, east of Maracay, 466 m, 10°14′N, 67°29′W (coordinates for Turmero), FMNH 27349. **Bolívar:** Ciudad Guayana, Parque Loefling, 100 m, 8°8′N, 63°33′W, USNM 229778; Santo Tomé de Guayana (= San Felix de Guayana), 50 m, 8°21′12″N, 62°39′10″W, AMNH 81456–81457. **Carabobo:** El Central, 10 km NW, 25 m, 10°32′N, 68°23′W, USNM 216759; Río San Esteban, 10°28′41″N, 68°1′15″W, UMMZ 55580. **Distrito Federal:** La Guaira, sea level, 10°36′N, 66°56′W, USNM 22539, 27793. **Falcón:** near La Pastora, 12 km ENE Mirimiri, 220 m, 11°12′N, 68°38′W, USNM 216729–216730; Municipio Mauroa, 22 km SW Guajiro (= Goajiro) by car, AMU 3290; Pauji, Acosta district, 11°1′N, 68°34′W, MCZ-A 25985–25988; Riecito, Acosta district, MCZ-A 26143; Río Socopito, 80 km NW Carora, 470 m, 10°28′N, 70°44′W, USNM 216731; entre Municipios Sucre y Petit, Sector La Ceiba, Río Mitare, a la altura del pueblo de El Paso, 11°6′N, 69°55′W, AMU 3234. **Guárico:** 8 km N, 2 km W Corozo Pando, ~8°30′N, 67°35′W, TCWC 45852; Sosa, 9°20′N, 67°12′W, FMNH 125399, 176330. **Mérida:** Río Limones, 8°51′N, 71°27′W, ULABG 5090, 5111–5113, 5170, 5304. **Miranda:** Araguita, 1 km S, 10°13′N, 66°28′W, KU 166409–166410; Campo Grande de Caucagua, 10°17′N, 66°20′W, MZUSP 58708–58711; Hacienda Bejuquero, 1 km south Río Chico, 1 m, 10°19′N, 65°58′W, USNM 216750–216753. **Monagas:** Caripito, Río Caripe, ~100 m, 10°8′N, 63°6′W, AMNH 70650–70652; Escuela Granja Parcela, 8 km west-southwest Caripito, KU 117127. **Portuguesa:** Urbanización Andrés Eloy Blanco, Guanare, 180 m, 9°3′N, 69°45′W, ELM 1167. **Sucre:** Río Cocollar, 10°10′N, 63°47′W, FMNH 17769–17771; Guaraúnos, ~100 m, 10°33′N, 63°7′W, KU 166404; Tocal, 11 km SSW Cumuna, 10°24′N, 64°14′W, KU 117122. **Táchira:** La Fria Pueblo Nuevo, 8°13′N, 72°15′W, UMMZ 55582. **Trujillo:** near El Divide, 28–30 km NW Valera, 90 m, 9°31′N, 70°44′W, USNM 216754–216758. **Yaracuy:** El Central, 10 km NW Urama, 25 m, 10°32′N, 68°23′W, USNM 216760; Palma Sola, 5 km SE, 10°36′N, 68°34′W, UMMZ 55581; 19 km NW of Urama, km 40, 25 m, 10°37′N, 68°24′W, USNM 216727–216728, 216732–216749. **Zulia:** Encontrados, 10 m, 9°30′N, 72°14′W, FMNH 2605; Setor Vera de Agua, km 12 on road El Vigía–Santa Barbara, 115 m, 8°42′2″N, 71°4′37″W, ULABG 5313.

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Appendix 1: Observer Error Assessment for Morphological Data

UPPER SHANK, OUTER TARSUS, AND SOLE OF FOOT TEXTURES

After recording data on 1,002 specimens, it seemed that the amount of variation within population samples was about the same as that among samples for the surface textures of the dorsal surface of the shank, outer surface of the tarsus, and sole of foot. An analysis was undertaken to determine whether there was meaningful intersample variation in the characters involved.

Data were summarized for eight samples throughout the geographic distribution of the species complex: (1) 12 juveniles, 20 females, 4 males from Boca do Acre, Amazonas, Brazil; (2) 28 juveniles, 7 females, 1 male from Ilha do Maracá, Roraima, Brazil; (3) 10 juveniles, 4 females, 5 males from Hacienda El Ceibal, Bolívar, Colombia; (4) 30 juveniles, 3 females, 2 males from Beltrán, Cundinamarca, Colombia; (5) 23 juveniles, 4 females, 5 males from between Santa Marta and Mamatoco, Magdalena, Colombia; (6) 40 juveniles, 5 females, 3 males from a few localities along the Río Chucunaque, Darién, Panama; (7) 46 juveniles, 1 female, 1 male from Pakitza and Tambopata, Madre de Dios, Peru; (8) 10 juveniles, 5 females, 3 males from 19 km northwest Urama, Falcón, Venezuela. Data were summarized for juveniles, females, and males for the following states of a surface shagreen: none, weak shagreen, and shagreen. These states were scored separately for the shank, tarsus, and foot. The shagreen texture is sandpaper-like, discernable only under magnification, with the skin surface just moist so the light reflects off the small protuberances comprising the shagreen. Shagreened surfaces seem to be sloughed off in specimens that are not well preserved. Data were also summarized for juveniles, females, and males for the following tubercle states: none, few, scattered, and many. These states were summarized separately for the shank, tarsus, and foot. The tubercles involved in well-preserved specimens are small, black keratinized protuberances. In less than well-preserved specimens, the outer keratinized covering may be lost leaving (smaller) white tubercles. These structures are consistently interpretable only under magnification.

The character states form a continuum (e.g., for shagreen, three states were coded as none, weak shagreen, and shagreen). If a given specimen was scored as

"none" in the first data-taking session and "none" in the second data-taking session, there is no difference in the scoring between the two sessions for shagreen for the specimen involved. If a given specimen was scored as "none" in the first data-taking session and "weak shagreen" in the second data-taking session, that is considered to be a one-step difference; if the specimen was scored as "none" in the first data-taking session and "shagreen" in the second session, that is considered to be a two-step difference. The two-step difference represents a larger observer error than a one-step difference. The term step does not have any cladistic connotation as used in the following analyses.

Examination of the distribution of occurrences of character states among the eight samples indicates that there are no occurrence differences among juveniles, females, and males within each locality. The distributions of states are so obvious that statistical analyses are not required (data available from WRH upon request). The data appeared as though there might be meaningful variation among the localities for both the shagreen and tubercle characters for the three different surfaces involved. As the character states are defined qualitatively, subjective interpretation could well vary from data-taking session to data-taking session by the same observer. As there were large time intervals between bouts of data taking for this project, the interpretations could well be quite different. To evaluate the impact of observer error, USNM specimens for two of the samples that were involved in the first bout of data taking (Amazonas, Brazil, and Cundinamarca, Colombia) were rescored for the characters involved. Thus each specimen had two character states recorded. For example, the upper shank surface on the first observation might have been scored as "none" and when the second observation was made on the same specimen, it could have scored as "shagreened." Alternatively, the first and second evaluations of the same specimen could have been "none" and "none." The results are reported separately for the two characters.

Shagreen

For the upper shank surface, 33% of the individuals were scored as having the same state, 10% were scored as having a 1-step difference (all of the differences involved were those between the Weak Shagreen and Shagreen states), and 57% of the individuals were scored as having a 2-step difference (all involved No Shagreen and Shagreen states). For the outer tarsal surface, 15% were scored as having the same state, 33% with a 1-step difference (all involved No Shagreen and Weak Shagreen states), and 52% a 2-step difference (all involved No Shagreen and Shagreen states). For the sole of the foot surface, 92% were scored as having the same state (No Shagreen) and 8% as having a 1-step difference (all involved No Shagreen and Weak Shagreen states). These results indicate that observer error masks any differences that occur among individuals for the states involved. The only conclusion that can be supported with the large observer error is that the sole of the foot generally lacks a shagreen in members of this species complex and that most individuals have shagreened dorsal shank and outer tarsal surfaces.

Tubercles

For the upper shank surface, 49% of the individuals were scored as having the same state, 47% were scored as having a one-step difference (all involving the scattered tubercles and many tubercles states), and 4% as having a two-step difference (all involving the no tubercles and scattered tubercles states). For the outer tarsal surface, 54% of the individuals were scored as having the same state, 39% with a one-step difference (mostly involving the few tubercles and scattered tubercles states), and 6% of the individuals with two-step differences (all involving the no tubercles and scattered tubercles states). For the sole of the foot surface, 37% of the individuals were scored as having the same state, 60% were scored as having a one-step difference (mostly involving the no tubercles and few tubercles states), and 2% with a two-step difference (involving the no tubercles and scattered tubercles states). Observer error for the tubercle states has less of an impact than for the shagreen states but is still considerable. Ninety-four to 98% of all error falls within a one-step difference of the four-state continuum of categories. All of the differences observed in distribution of states among the eight geographic samples occurred between adjacent states, however, indicating that the variation is not meaningful when observer error is accounted for.

Owing to observer error and limited variation observed in the eight geographic samples, it is clear that geographic variation cannot be demonstrated convincingly for the texture characters involved. Consequently, data for these characters were not taken after February 2005.

The following characterization can be made for the *Leptodactylus bolivianus* complex. Shagreened surfaces are most commonly observed on the entire dorsal shank and outer tarsal surfaces. The sole of the foot usually does not have any shagreen; however, in the few cases where shagreen is present, it is limited to the proximal half of the fifth metatarsal region of the sole of the foot. Almost all

individuals have small black keratinized (or white) tubercles rather evenly distributed on the dorsal surface of the shank and outer surface of the tarsus. The sole of the foot may lack tubercles entirely, although most specimens have them. When black (or white) tubercles are present, they are typically smaller than those found on the dorsal shank and outer tarsal surfaces and characteristically occur only on the outer half of the sole of the foot.

METHODOLOGY FOR REMAINING CHARACTERS EXCLUSIVE OF MALE SECONDARY SEXUAL CHARACTERS

The same series of 36 specimens from Beltrán, Cundinamarca, Colombia evaluated for observer error in texture characters (preceding section) were used to evaluate observer error for sex, belly pattern, dorsal pattern, lip stripe pattern, posterior thigh pattern, upper shank pattern, longitudinal body folds, and male secondary sexual characteristics. Data were originally recorded for these specimens at the beginning of the study prior to 27 August 2004. Character states were defined as written statements (e.g., for upper shank textures) or, where written statements were not as clear as sketches, drawings were rendered. As data taking proceeded, if a character was similar but not identical to a sketch, a brief statement characterizing the difference was added (Figure A1.1). Some pattern states were added after 27 August 2004. In theory, as any new pattern was encountered during the study, a new standard was prepared for it. Thus any specimen that was examined early in the study should not be scored as having a standard subsequently added if the specimen were to be reexamined. In order to test this aspect along with observer error in general, the Beltran series was rescored on 29–30 August 2005. In order to determine how repeatable the character states were scored over a short period of time, the Beltrán series was scored a third time on 13–14 September 2005.

Sex

The scoring for sex was anticipated to be invariant among the observation recording sessions with the exception of a few of the larger juveniles that were not examined internally the first session and scored simply as juveniles but were examined internally the second session and were recorded as either juvenile females or juvenile males.

Six specimens were scored differently between the first and second and first and third data-recording sessions on the basis of dissections made during the second session. These differences are not considered to be observer errors.

Specimen USNM 145715 (76 mm SVL) was scored as an adult female in sessions 1 and 2 but a juvenile female in session 3. Reexamination of the specimen indicates that with the dissection view available during sessions 1, 2, and 3, only the ovary was visible. Further dissection was required to expose an oviduct, which is curly; thus the specimen is an adult female following the criteria used in this study.

Specimen USNM 145716 (71 mm SVL) was scored as a juvenile female in sessions 1 and 3 and an adult female in session 2. Reexamination of the specimen indicates that is has a straight oviduct and the ovaries are expanded containing small white ova. The straight oviduct condition is used to define the juvenile female condition in this study.

Specimen USNM 145721 (63 mm SVL) was scored as juvenile in session 1, a juvenile female in session 2, and a juvenile male in session 3. A fourth examination to determine which of the results in sessions 1, 2, or 3 was correct clearly indicated that the specimen has a straight oviduct with expanded ovaries with small white ova upon reexamination. The specimen is unquestionably a juvenile female; there had to be a transcription recording error in session 3, where the condition observed was not the condition recorded during session 3.

Specimen USNM 145724 (59 mm SVL) was recorded as a juvenile in session 1, a juvenile female in session 2, and an adult female in session 3. Reexamination of the specimen indicates that the oviduct is curly and the ovaries are expanded with moderate sized white ova, an adult female according to the criteria in this study.

In conclusion, there are two kinds of observer error detected in the reexamination process for determination of sex. The first is making different conclusions about whether near-adult sized females are adults or juveniles (5% scoring error). The second kind of error is a data transcription error (1% error).

Belly Pattern

A total of 15 belly pattern standards were recorded for the three Beltrán sample data-taking sessions. Two pattern standards, added after the initial session, were scored with a 24% frequency in sessions 2 and 3. This indicates that not all variation thought to be important was recognized at the beginning of the study.

Only 14% of the standards were scored as the same between sessions 1 and 2, and 17% between session 1 and 3; whereas 39% of the standards were identically scored

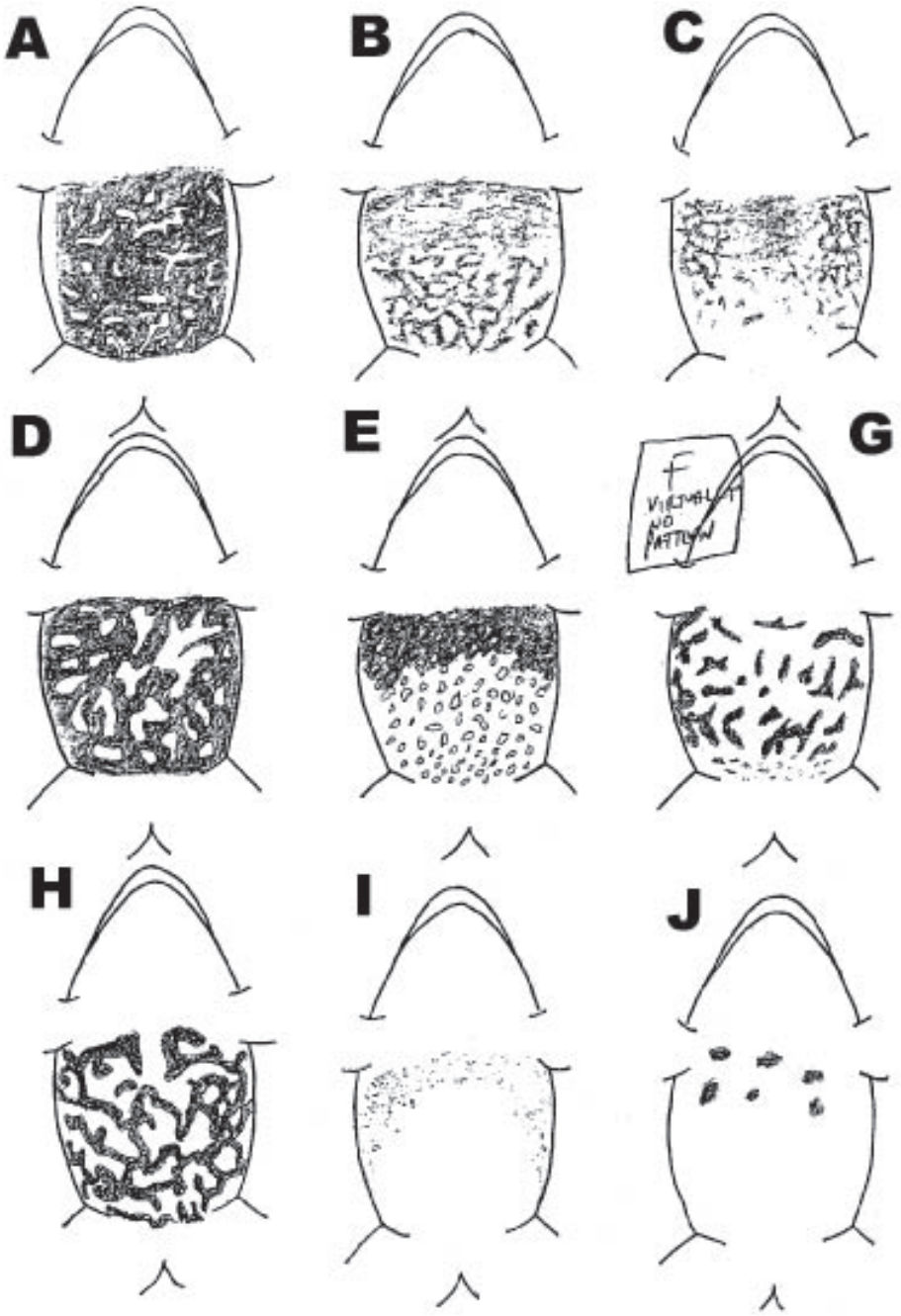

FIGURE A1.1. Example of design pattern. First page of belly pattern standards used in study to score character states. The original sheet had written annotations defining additional standards in relation to the pattern involved: A-1, dark and light areas about equal; A-2, mostly dark with few light spots, more light spots posteriorly; A-3 as A-1, except darker to lighter gradient anteriorly-posteriorly; B-1, mottling on posterior, almost uniform dark anteriorly; B-2, uniformly mottled as on posterior; B-3, gradient of anterior pattern posteriorly; B-4, posterior pattern with anterior-posterior gradient; B-5, anterior pattern on entire belly; C-1, anterior mottling continuous across middle; C-2, almost lacking pattern; C-3, uniformly darkly mottled (fine); C-4, uniformly lightly mottled (fine); D-1, mostly light; D-2, indistinct anteriorly; E-1 (only anterior portion filled in), fewer spots; E-2, very few spots; F, virtually no pattern; F-1, just a few small blotches lateral-most; F-2, uniformly dark; G-1, very little in middle; G-2, groups of melanophores instead of discrete marks; G-3, pattern anteriorly only; H-1, finer pattern; H-2, almost uniform dark anteriorly; H-3, anterior-posterior gradient, lighter posteriorly; I-1, encroachment only a bit laterally behind arms; I-2, pattern extends to posterior belly; J-1, + a few [blotches] on lateral belly; J-2, everywhere except center of belly; J-3, light speckling over entire belly.

between sessions 2 and 3. Thus there is more consistency in scoring over a shorter time period (sessions 2 and 3) than for longer time periods between data taking (sessions 1–2, 1–3).

Given the low repeatability of scoring the 15 standards recorded for the Beltrán sample, the following two-step procedure was used to combine the standards to maximize repeatability on scoring of the data.

The first step was to visually inspect the 15 standards and combine states that were most similar. This resulted in recognition of seven states: (1) anterior mottling only, (2) belly mottled with an anterior–posterior gradient, (3) uniform mottling over the entire belly, (4) belly with anterior speckling only, (5) belly speckled with an anterior–posterior gradient, (6) belly uniformly speckled, and (7) no pattern (no melanophores on belly). The Beltrán sample data were rescored using these seven states. This resulted in 17% exact matches between session 1 and session 2, 25% between session 1 and session 3, and 56% between session 2 and session 3.

The second step was to examine the raw occurrence data from step 1 to determine which further combinations of character states would result in the greatest repeatability in the data set. Combining mottling and speckling states did not appreciably improve repeatability. However, combining these states with either of two other combinations did result in noticeable improvement. When mottling + speckling were combined with anterior only + anterior–posterior gradient states, the states were identical in 50% of the time between sessions 1 and 2 and between sessions 1 and 3, and 80% between session 2 and 3 scorings. When mottling + speckling were combined with anterior–posterior gradient + uniform states, the states were identical 42% of the time between session 1 and 2 data, 53 % between session 1 and 3 data, and 69% between session 2 and 3 data.

Thus, for the Beltrán data, the following final states are recognized: (1) belly speckled or mottled anteriorly only or with an anterior–posterior gradient, (2) belly speckled or mottled with the same intensity over the entire belly, and (3) belly patternless (no melanophores).

Finally, the total data set was evaluated visually using the above results as a guideline. Thirty-four belly pattern states occur in the total data set. Among the states that were not recorded in the Beltrán samples are two distinct features: spotted bellies and boldly mottled bellies. However, there are very few times the spotted belly state was recorded. The variation found, including these new features, result in the recognition of the following states used in analyses of belly patterns: (1) belly speckled, spotted, or mottled anteriorly only or with an anterior–posterior gradient, (2) belly boldly mottled anteriorly only or with an anterior–posterior gradient over the entire belly, (3) belly speckled or mottled with the same intensity over the entire belly, (4) belly boldly mottled with the same intensity over the entire belly, and (5) belly without a pattern (no melanophores). These states are assumed to have around 50% repeatability over the entire study and around 75% repeatability within data-taking sessions at single institutions or for multiple institutions visited consecutively during the same trip. Thus any belly pattern differences in data comparisons must exceed 50% (for adequate sample sizes) to be meaningful.

Dorsal Pattern

Eight dorsal pattern standards were recorded during the three Beltrán sample data-taking sessions. The same standards were used for all sessions. One transcription error was made where a nonexistent dorsal pattern standard was recorded.

Fifty-six percent of the standards were recorded as identical between sessions 1 and 2, 58% between sessions 1 and 3, and 75% between sessions 2 and 3. There is greater consistency in scoring over a shorter time period (sessions 2–3) than over a longer time period (sessions 1–2, 1–3).

The dorsal pattern standards were examined visually, and the most similar patterns were combined to see if the increase in repeatability offset the loss of potential information on variation. The following three states were recognized and used to recode the three session data: (1) dorsum uniform between interocular bar (when present) and sacrum; (2) single chevron between interocular bar and sacral chevron (when present) without additional spots lateral to dorsal chevrons; and (3) complex pattern with two dorsal chevrons and spotting all over dorsum, chevrons often elongate and fused with each other and interorbital bar. The recoded data yield 77% exact match of states between data for sessions 1 and 2, 67% between session 1 and 3 data, and 82% between session 2 and 3 data.

The increase in repeatability of the combined standards is considered worth the cost of losing information on variability of the dorsal patterns. However, further reduction of the states would be too great a loss of information on variability. For dorsal pattern variation, any adequate size sample comparisons have to differ by more than 33% in order to be meaningful.

The three states defined above encompass the variation recorded in the total data set.

Lip Stripe

Two features were evaluated for the lip stripe: the overall lip stripe pattern and whether a distinct light patch was present immediately below the eye.

Eighteen pattern states were recorded for the overall lip stripe pattern in the three Beltrán sample data sets. Nine percent of the first and second session data elements were scored identically, 29% were identical between session 1 and 3 data, and 42% were identical between session 2 and 3 data. One standard was added after the first session, which was scored at a 3% occurrence in the combined session 2 and 3 data.

The lip pattern standards were examined visually and combined to minimize mismatches in the original pattern scoring results. Four states were used to recode the data for the three sessions: (1) a noticeable broad light stripe extending from the tip of the snout to through the commissural gland, bordered below by continuous or broken darker markings on the upper lip and either bordered above by a dark line under the eye or not; (2) a narrow light stripe from at least the nostril to the lower posterior end of the eye; (3) a narrow light stripe from below the front of the eye through the commissural gland; and (4) central lip region uniform (about same color intensity as background dorsal color) with or without a dark stripe under the eye and/or dark bars/mottling on the upper lip. Identical pattern states were scored 53% of the time for the session 1 and 2 data, 69% for session 1 and session 3 data, and 57% for session 2 and session 3 data.

Further reduction of states would result in very little variation in the data set. For the overall lip pattern variation, any adequate sample size comparisons have to differ by more than 50% in order to be meaningful.

Three conditions of expression of a light patch under the eye (subocular light spot) were recorded in the three Beltrán data sets: (1) distinct, (2) indistinct, and (3) absent. Ninety-four percent of the first and second session data states were scored identically, 94% were identical between sessions 1 and 3 data, and 92% were identical between sessions 2 and 3 data. The level of observer error is considered acceptable. There are no further states for this character in the entire data set. For light subocular patch variation, adequate sample size comparisons have to differ by more than 10% to be meaningful.

Posterior Thigh Pattern

Thirteen posterior thigh pattern standards were scored for the three Beltrán data-recording sessions. One pattern was added after the first session but was not recorded in either the session 2 or 3 data. Two transcription errors were made where a nonexistent standard was recorded.

Identical pattern standards were recorded 28% of the time between sessions 1 and 2, 31% between sessions 2 and 3, and 31% between sessions 2 and 3. The scoring was quite consistent, but with high observer error in scoring, among sessions.

The standards were examined visually to combine the most similar patterns and to minimize scoring differences found in the raw scoring results. The following five states were recognized and used to recode the three session data: (1) posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) over the entire surface of the posterior thigh; (2) posterior thigh with a darker patch with small, irregular light spots on the distal half of the thigh; (3) posterior thigh with a dark vermiculated pattern on the distal half of the thigh; (4) posterior thigh pattern ranges from boldly mottled with large light irregular spots or vermiculations on a dark background to the light markings being more extensive than the dark areas, the light markings sometimes coalesced; and (5) posterior thigh uniformly dark with only a very few light irregular markings. Identical states of the rescored data between session 1 and session 2 occurred 69% of the time, 92% of the time between sessions 1 and 3, and 64% of the time between sessions 2 and 3.

Patterns 2 and 3 defined above were only scored twice each in the entire data set. Pattern 5 defined above (created from examination of specimens of the *L. latrans* complex) was scored once in the rescoring of the Beltrán sample specimens. In addition, two other patterns were scored once in the entire data set. All of these single- or double-occurrence states are combined with their closest patterns with the result that only two states are recognized for this character: (1) posterior thigh with a uniform or variable dispersion of melanophores (weakly to moderately mottled) usually over entire surface of posterior thigh, and (2) posterior thigh boldly mottled with large light irregular spots/vermiculations on a dark background to the light marks being more extensive than the dark areas, the light markings sometimes coalesced.

Adequate sample size comparisons have to differ by more than 33% in order to be meaningful.

Dorsal Shank Pattern

Six patterns were scored in the three data-recording sessions of the Beltrán sample. No patterns were added after the first session.

Identical pattern standard states were recorded 42% of the time in sessions 1 and 2 data, 25% in sessions 1 and

3 data, and 69% in sessions 2 and 3 data. Scoring between sessions was quite inconsistent.

Only minimal combining of pattern states was possible by visual examination of the standards, resulting in recognition of the following three character states: (1) uniform, (2) short dark transverse bars extending less than halfway across shank, and (3) at least one dark transverse bar extending halfway or more across the shank. Identical states in the rescored data occurred 50% of the time in sessions 1 and 2 data, 31% in sessions 1 and 3 data, and 72% in sessions 2 and 3 data. These values are only marginally better with respect to the values based on uncombined pattern character states. Further combining of states would result in little variation of the upper shank patterns. There is no additional variation for this character in the entire data set. Adequate sample size comparisons have to differ by more than 70% in order to be meaningful.

Dorsolateral Folds

Six states were recognized in the Beltrán specimen data. One state was added after the first data-recording session, which was recorded in 11% of the session 2 and 3% of the session 3 data.

Fifty percent of the specimens were recorded with identical states between sessions 1 and 2, 47% between sessions 1 and 3, and 53% between sessions 2 and 3.

The initial states were combined into two states to determine whether the increase in repeatability was worth the loss of information concerning variation: (1) dorsolateral fold distinct and (a) continuous from supratympanic fold to end of body, or (b) with a brief interruption of the fold, or (c) the fold is distinct only from the supratympanic fold to the sacral region; and (2) dorsolateral fold with several interruptions to indiscernible (including lack of fold probably due to poor preservation). The rescored comparisons resulted in 78% exact matches between data sessions 1 and 2, 65% between sessions 1 and 3, and 76% between sessions 2 and 3. The gain in repeatability is considered worth the loss of information concerning variability.

The redefined states encompass all the variation observed in the entire data set. Differences in dorsolateral fold states must exceed 33% in adequate sample sizes to be meaningful.

Lateral Folds

Six states were recognized in the three data-taking sessions. One state was added after the first session and was recorded in 3% of the individuals for both the session 2 and 3 data.

Identical states were scored 39% of the time between session 1 and session 2 data, 44% between sessions 1 and 3, and 53% between sessions 2 and 3.

The initial states were combined into two states: (1) lateral fold distinct or slightly interrupted from supratympanic fold to leg and (2) lateral fold distinct only in groin region, indistinct overall, or indiscernible (probably due to poor preservation). The rescored data between sessions 1 and 2 were identical in 61% of the comparisons, 71% between sessions 1 and 3, and 88% between sessions 2 and 3. The increase in repeatability is considered worth the loss of variation information.

There is no further variation in this character in the entire data set. Differences in lateral fold states must exceed 40% among adequate size samples to be meaningful.

The new states are used in subsequent analyses.

There are two extremes of repeatability of character states represented by belly and lip patterns at one extreme and the light subocular patch at the other. The belly and lip patterns involve several features that together represent a total gestalt. In retrospect, WRH focused on different components of the belly and lip pattern standards at different times. For example, during one session more attention might have been focused on the nature of the markings on the upper lip adjacent to the jaw; during another session the focus might have been on how distinctive the broad light stripe was between the upper lip and the eye; and yet another session may have focused more on the nature of the dark markings immediately below the eye. Such unintentional shifts in focus could explain why there was more consistency in observations made over short periods of time and much greater inconsistency when data were taken after a long hiatus between sessions. In contrast, the light subocular patch is a single feature for which the variation was recorded much more consistently.

There are at least two ways that complex patterns could be analyzed differently than was done in this study to minimize observer error. The first would be to take all the data in one continuous session, where on a day-to-day basis, there would likely be carryover of which features in complex patterns were being focused on. The second would be to pick a series of specimens that likely encompass the range of variation in the entire study sample and prepare a series of sketches that represent that range of variation for each general character to be evaluated, such as belly pattern. On the basis of those sketches, individual features could be identified and character states defined that would then be used to evaluate the entire data set. The second approach would seem more effective to minimize observer error.

MALE SECONDARY SEXUAL CHARACTERS

There are only two adult males in the Beltrán sample. The only variation among the three data sessions was a one-step difference out of a four-state range in evaluation of the degree of male arm hypertrophy.

The male secondary sexual characteristics can be defined and described in terms of individual features as follows.

Male Arm. (1) Upper arm not hypertrophied and (2) upper arm hypertrophied.

Male Lower Jaw. (1) Male lower jaw lacking keratinized tubercles and (2) male lower jaw with keratinized tubercles.

Male Chest. (1) Male chest lacking a patch of keratinized tubercles, (2) male chest with a central patch of keratinized tubercles, and (3) male chest with a central patch and two elongate lateral patches of keratinized tubercles just posterior to the central patch.

Male Thumb. (1) Male thumb with one weakly chisel-shaped keratinized spine, (2) male thumb with one well-developed chisel-shaped keratinized spine, and (3) male thumb with two round keratinized spines. The spines have keratinized sheaths at least during the reproductive season. Spines lacking sheaths still demonstrate the differences between the chisel-shaped and rounded features.

The above definitions would be expected to have the same repeatability as those for the light subocular patch character. Erring on the conservative side, adequate sample size comparisons for the male secondary sexual characters should differ by more than 15% to be meaningful.

Appendix 2. Molecular Sample Locality Data

LEPTODACTYLUS BOLIVIANUS COMPLEX LOCALITY DATA USED IN MOLECULAR ANALYSIS

Leptodactylus bolivianus. USNM 268966 (USFS 152368)—PERU: Madre de Dios, Tambopata Reserve, 12°50′S, 69°17′W, 66 m (collection of Jiri Moravec); BOLIVIA: Pando, settlement of Nacebe, 11°00′S, 67°25′W.

Leptodactylus guianensis. AMNH145130–131 (JC 7056–57)—GUYANA: Corontyne Region, Dubulay Ranch on the Berbice River, 5°40′5″N, 57°51′32″W, UC 128, 130; BRAZIL: Roraima, proximity of Vila Surumu, 04°11′40.9″N, 60°47′25.1″W.

Leptodactylus insularum. MUJ 2187—COLOMBIA: no other data. JDL 24816—COLOMBIA: Sucre, San Marcos, vereda La Florida, 8°37′N, 75°11′W, 45 m. JDL 24887—COLOMBIA: San Marcos, finca Crocodilia, 8°36′N, 75°09′W, 34 m. JDL 26573, JDL 26591—COLOMBIA: Cordoba; Lorica, estacion pisicola CVS, 9°13′N, 75°50′W, 20 m. CH 4955–56—PANAMA: Panamá, Río Indio, camino hacia Las Minas. ULABG 5111–13, 5304—VENEZUELA: Mérida; Río Limones.

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