UNIVERSIDAD DE GRANADA

TAXONOMÍA INTEGRATIVA DE LAS ESPECIES DE *ELEUTHERODACTYLUS* Y *OREOBATES* DE BOLIVIA (AMPHIBIA:ANURA)

TESIS DOCTORAL

José Manuel Padial Fregenal

Granada, 2007

TAXONOMÍA INTEGRATIVA DE LAS ESPECIES DE *ELEUTHERODACTYLUS* Y *OREOBATES* DE BOLIVIA (AMPHIBIA: ANURA)

Memoria que el Licenciado José Manuel Padial presenta para aspirar al Grado de Doctor por la Universidad de Granada

Esta memoria ha sido realizada bajo la dirección de:

Dr. Ignacio J. De la Riva de la Viña

Ldo. José Manuel Padial Fregenal
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De: Ignacio De la Riva

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Como director de la tesis doctoral de D. José Manuel Padial Fregenal, titulada "Taxonomía integrativa de las especies de *Eleutherodactylus* y *Oreobates* de Bolivia (Amphibia: Anura)", expongo que la misma ha sido llevada a cabo según el calendario previsto desde sus inicios, se han incluido todos los aspectos relevantes que se habían programado, y el nivel de consecución de resultados ha sido óptimo. Asimismo, se han cumplido todos los requisitos para optar al Doctorado Europeo. Por todo lo expuesto, autorizo la presentación de dicha tesis ante esa comisión para proceder a su defensa en la fecha que sea estipulada.

Firmado: Dr. Ignacio J. De la Riva
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Pocas veces tiene un hijo
la ocasión de agradecer el enorme esfuerzo
de unos buenos padres,
esta es una de ellas:

a María Victoria Fregenal
a Manuel Padial

por procrear
y cuidar con empeño
a su prole

Las ciencias históricas son diferentes, no inferiores.
Sus métodos son comparativos, no siempre experimentales;
explican, pero no suelen intentar predecir;
reconocen la irreductible tendencia a lo insólito inherente a la historia
y el poder limitado de las circunstancias presentes
para imponer u obtener soluciones óptimas.
La reina entre todas sus disciplinas es la taxonomía, la Cenicienta de las ciencias.

Stephen Jay Gould

Encontrar una nueva especie es, seguramente,
el descubrimiento más importante
que un biólogo puede hacer

Edward O. Wilson

Padial, el día que te presentes a una plaza,
te acordarás de tus salidas…

Javier Castroviejo
Sevilla, noche de un viernes del 2002

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RESUMEN

Los análisis filogenéticos de dos fragmentos de genes mitocondriales (16S y Cytb) junto con sinapomorfías morfológicas y bioacústicas, apoyan la monofilia del género *Oreobates*. Las especies del grupo de *Eleutherodactylus discoidalis* pertenecen a *Oreobates*, que pasa así a incluir las siguientes especies: *O. choristolemma*, *O. cruralis*, *O. discoidalis*, *O. granulosus*, *O. heterodactylus*, *O. ibischi*, *O. lehri*, *O. madidi*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*, *O. saxatilis*, *O. simmonsi* y *O. zongoensis*. se describen como nuevas especies *Oreobates sanderi*, *O. madidi* y *O. lehri*, se redescubren y se redescriben *O. heterodactylus* y *O. granulosus*, y *O. cruralis* es también redescrita. Los miembros de *Oreobates* se distribuyen desde la Amazonía colombiana (*O. quixensis*) hasta el norte de Argentina (*O. discoidalis*), con una especie vicariante en los afloramientos rocosos del escudo precámbrico del Cerrado brasileño-boliviano (*O. heterodactylus*). *Oreobates* es el grupo hermano de las series *E. martinicensis* y *E. conspicillatus* del subgénero *Eleutherodactylus* y no está cercanamente relacionado con los grupos *E. dolops*, *E. nigrovittatus* o la serie *E. binotatus*, como había sido propuesto por otros autores. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S, junto con sinapomorfías morfológicas y bioacústicas, también apoyan la validez y monofilia del grupo de *E. fraudator* y su independencia del género centroamericano *Craugastor*. Dicho grupo, endémico de la Cordillera Oriental de los Andes centrales, es elevado a rango de subgénero bajo el nombre de *Yunganastes*, e incluye *E. ashkapara*, *E. bisignatus*, *E. fraudator*, *E. mercedesae* y *E. pluvicanorus*. Los análisis filogenéticos indican además que el género *Eleutherodactylus* debería restringirse al clado formado por las series *E. martinicensis* y *E. conspicillatus*. Finalmente, los análisis basados en un fragmento del gen 16S, junto a análisis morfométricos y bioacústicos, apoyan la independencia de dos linajes crípticos que representan sendas nuevas especies, una del grupo *E. conspicillatus* y una del grupo *E. unistrigatus*. *Eleutherodactylus karcharias* es puesto en la sinonimia de *E. skydmainos*. Junto a 10 especies del género *Oreobates,* en Bolivia habitan 22 especies de *Eleutherodactylus* (cinco del grupo *E. conspicillatus,* doce del grupo *E. unistrigatus* del subgénero *Eleutherodactylus* y cinco del subgénero *Yunganastes*).

ABSTRACT

Phylogenetic analyses of mtDNA (partial 16S and Cytb) together with morphological and bioacoustical synapomorphies support the monophyly of *Oreobates*. This genus contains the following 14 species (including former members of the *E. discoidalis* species group): *O. choristolemma*, *O. cruralis*, *O. discoidalis*, *O. granulosus*, *O. heterodactylus*, *O. ibischi*, *O. lehri*, *O. madidi*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*, *O. saxatilis*, *O. simmonsi* and *O. zongoensis*. *Oreobates sanderi*, *O. madidi* and *O. lehri* are described as new species, *O. heterodactylus* and *O. granulosus* are rediscovered and redescribed, and *O. cruralis* is redescribed. Members of *Oreobates* occur from Amazonian Colombia to northern Argentina, with an isolated vicariant species in the Precambrian Brazilian Shield (*O. heterodactylus*). *Oreobates* is the sister group of the *E. martinicensis* and *E. conspicillatus* series of the subgenus *Eleutherodactylus* and it is not closely related to members of the *E. dolops* or *E. nigrovittatus* groups, nor it is closely related to members of the *E. binotatus,* as proposed by former authors. Also, morphological, molecular and bioacoustical characters support the monophyly of the *E. fraudator* group, containing *Eleutherodactylus ashkapara*, *E. bisignatus*, *E. fraudator*, *E. mercedesae* and *E. pluvicanorus*. We describe this clade of *Eleutherodactylus* as the subgenus *Yunganastes*, which is endemic to the Eastern Cordillera of the Central Andes. Finally, morphometrics, bioacoustics, and phylogenetic analyses of partial mtDNA 16S gene support the independence of two new, cryptic species taxa; one is assigned to the *E. conspicillatus* Group and one to the *E. unistrigatus* Group. *E. karcharias* is placed in the synonymy of *E. skydmainos*. Besides ten members of *Oreobates,* Bolivia contains 22 species of the genus *Eleutherodactylus*, five belonging to the *E. conspicillatus* group, twelve of the *E. unistrigatus* group of the subgenus *Eleutherodactylus*, and five of the subgenus *Yunganastes*.

INTRODUCCIÓN

Los estudios sobre la sistemática de anfibios neotropicales han experimentado un notable auge durante las últimas décadas (Glaw y Köhler, 1998). Sin embargo, muchos problemas taxonómicos permanecen sin resolver, numerosas especies aún no han sido descritas, existen aún grandes áreas con un escaso nivel de exploración (Kress *et al.*, 1998) y faltan secuencias de ADN para muchas especies (Vences y Köhler, 2006).

Las ranas de los géneros *Eleutherodactylus* Duméril y Bibron, 1841 y *Oreobates* Jiménez de la Espada, 1872 son endémicas de la región Neotropical. Allí, *Eleutherodactylus* ha sufrido una intensa radiación que ha llevado a este género a convertirse en el grupo de vertebrados terrestres con mayor número de especies (Lynch y Duellman, 1997; Glaw y Köhler, 1998). Hasta el año 2005 se habían descrito unas 500 especies de *Eleutherodactylus* (Frost, 2006). Por el contrario, los miembros de *Oreobates* son escasos (tan sólo catorce especies), y han estado confundidos frecuentemente bajo los nombres de *Eleutherodactylus* e *Ischnocnema* (Apéndice 9).

La mayoría de los miembros del género *Eleutherodactylus* habitan en los bosques húmedos de montaña de las laderas de los Andes, aunque también se los encuentra con una notable diversidad en los bosques húmedos de la llanura amazónica, y en mucho menor número y abundancia en los boques secos del Cerrado (Heyer y Muñoz, 1999) y los valles secos interandinos (Köhler, 2000a). Su rango de distribución se extiende desde las islas del Caribe hasta Bolivia y la Mata Atlántica (Frost, 2006).

Los *Eleutherodactylus* son famosos entre los herpetólogos debido a su gran diversidad y a su incompleta y compleja taxonomía. Las dificultades para localizar en el campo individuos de muchas de sus especies (lo cual ha motivado que, frecuentemente, hayan sido descritas con escasos ejemplares), unido a que la mayoría de ellas se identifican por pequeñas diferencias en caracteres morfológicos sutiles, ha dado lugar a que sean pocos los herpetólogos que hayan dedicado un esfuerzo real y considerable para resolver su taxonomía.

El conocimiento de la diversidad de especies de *Eleutherodactylus* de Bolivia ha cambiado drásticamente desde la primera lista de los anfibios del país (De la Riva, 1990), cuando se citaron sólo seis especies. Poco después, De la Riva (1993) citó ocho especies para Bolivia, y consideró otras dos *(E. andicola y E. bokermanni)* como dudosas. De estas dos especies dudosas, la segunda resultó ser un sinónimo de *E. bokermanni* (De la Riva, 1997), mientras que la primera, cuyo holotipo se ha perdido, permanece con un estatus incierto y puede que ni siquiera sea un *Eleutherodactylus* (Lynch y McDiarmid, 1987). Tras posteriores campañas de muestreo y revisión de colecciones, el número de especies del país aumentó hasta 17, tanto por las nuevas citas como por el descubrimiento de algunas especies nuevas (De la Riva *et al.*, 2000). Ese mismo año, Köhler (2000) reconocía la presencia de 19 especies en Bolivia, añadiendo una nueva cita de una especie del Cerrado brasileño adyacente a Bolivia (*E. dundeei*), mientras que resucitaba otra especie, *E. bisignatus*, a la que Lynch (1980) había puesto bajo la sinonimia de *E. fenestratus*; asimismo, anunciaba la pronta descripción de *E. ibischi* por Reichle *et al.* (2001), y eliminaba de la lista a *E. peruvianus*, citado por primera vez en Bolivia por De la Riva (1994). Tanto De la Riva *et al.*, (2000) como Köhler (2000) manifestaron que aún quedaban muchas especies por descubrir en Bolivia, que muchas especies que habitaban en la frontera con Bolivia podrían ser pronto registradas en el país, y que muchos problemas taxonómicos permanecían a la espera de ser resueltos.

Es por ello que, bajo la dirección de Ignacio De la Riva, comenzó el presente trabajo, cuyo principal objetivo ha sido el estudio de la diversidad, filogenia y taxonomía del género *Eleutherodactylus* en Bolivia.

LOS GRUPOS DE ESPECIES

Los grupos de especies son agrupaciones fenéticas subgenéricas, cuya intención es facilitar el trabajo taxonómico mediante la subdivisión de grandes géneros en subunidades más pequeñas y abordables (Mayr, 1942). Dentro del género *Eleutherodactylus*, las divisiones en grupos de especies se plantearon como pasos previos para definir unidades monofiléticas (Lynch y Duellman, 1997). Lynch (1976) realizó una reordenación fenética de las especies sudamericanas de *Eleutherodactylus* en subunidades numéricas que dividió en grupos de especies. Estos grupos se caracterizaban por la combinación de caracteres morfológicos internos y externos. Dado que la intención era facilitar la organización y el estudio de estas ranas, los caracteres fueron escogidos sin testar su validez como sinapomorfías. Sin embargo, en una reorganización más detallada y rigurosa por Lynch y Duellman (1997) se planteó la posible validez de algunos de estos caracteres como sinapomorfías y la posible monofilia de algunos grupos.

Otros estudios más amplios no han contribuido a resolver los problemas de clasificación y relaciones filogenéticas de *Eleutherodactylus* y otros taxones cercanos. Sin embargo, tanto Darst y Cannatella (2004) como Frost *et al.* (2006) ponían de manifiesto que el género *Eleutherodactylus* no era monofilético bajo la clasificación actual. Una de las acciones de Frost *et al.* (2006) fue la división de la familia Leptodactylidae, a la que habían pertenecido históricamente los *Eleutherodactylus* (Lynch, 1975), y su traslado en bloque a la familia Brachycephalidae junto con miembros de lo que ahora consideramos *Oreobates*. Sin embargo, el estatus taxonómico de la mayoría de los grupos de especies de *Eleutherodactylus*, su monofilia y sus hipótesis de parentesco quedaban pendientes de ser analizadas. Así, en tiempos más recientes, se produjo una búsqueda de sinapomorfías dentro de los grupos de "*Eleutherodactylus*" (Lynch, 1993; Lynch, 2001; Guayasamín, 2004) y se definieron y reclasificaron algunos grupos (Lynch, 1986, 1989; Hedges, 1989). Además, la filogenia molecular de Crawford y Smith (2005) reconoció como género aparte a las especies de Centroamérica y el Caribe, que formaban el antiguo subgénero *Craugastor*. Por otro lado, los análisis comparativos de la morfología de algunos grupos (Guayasamín, 2004) arrojaron luz sobre la clasificación y las relaciones filogenéticas de estas diversas ranas tropicales. Sin embargo, tanto las hipótesis de parentesco y monofilia, como la validez de los caracteres usados como sinapomorfías para muchos grupos permaneció a la espera de análisis más rigurosos.

En este estado quedaron los miembros del grupo *Eleutherodactylus discoidalis* (Lynch, 1989) y de sus supuestos parientes, los miembros de *Oreobates* [que incluía algunas de las especies anteriormente incluidas en *Ischnocnema* (Caramaschi y Canedo, 2006)]. Igualmente, permanecían sin resolver algunas cuestiones como la monofilia, validez, y relaciones del grupo de *Eleutherodactylus fraudator*, definido por Köhler (2000) para algunas especies de Bolivia.

Los *Eleutherodactylus* citados en Bolivia por De la Riva *et al.*, (2000) y Köhler (2000) pertenecen a cuatro grupos de especies: *E. conspicillatus*, *E. discoidalis*, *E. fraudator* y *E. unistrigatus*. Todas las especies de dos de ellos, las del grupo *E. discoidalis* y las del grupo *E. fraudator*, se encuentran en Bolivia. Por el contrario, los otros dos grupos son de distribución mucho más amplia y sólo unas pocas de sus especies alcanzan Bolivia. Es por ello que este estudio se centra en las especies de los grupos eminentemente bolivianos. Las investigaciones de esta tesis sobre los grupos de *E. conspicillatus* y *E. unistrigatus* se centraron en aquellas especies que presentaban una problemática especial. Además, ya que los miembros del grupo de *E. discoidalis* (Lynch, 1989) estaban muy cercanamente emparentados con miembros de *Ischnocnema*, se decidió inicialmente incluir a los miembros *Ischnocnema* en este estudio. Sin embargo, durante la elaboración del mismo, Caramaschi y Canedo (2006) sinonimizaron la especie tipo de *Ischnocnema* con *Eleutherodactylus* y trasladaron a algunos miembros de *Ischnocnema* al género *Oreobates*. De este modo, el género *Oreobates* pasaba a tener seis especies, cuatro de las cuales (*O. choristolemma*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*) eran endémicas de Bolivia, y otra de ellas alcanzaba los bosques amazónicos de llanura del norte de Bolivia. Es por ello que se decidió incluir todo el género en el presente estudio.

CONSIDERACIONES METODOLÓGICAS

Taxonomía integrativa

Tras la incorporación de la teoría evolutiva en la ciencia taxonómica durante la llamada “modern synthesis" (1937-1948), los taxónomos comenzaron a darse cuenta de que la diversidad de especies del planeta no podía ser descrita mediante el uso exclusivo de caracteres morfológicos y sin englobar la tarea dentro de un contexto evolutivo. Sin embargo, la mayoría de los taxónomos han seguido describiendo las especies basándose sólo en las diferencias morfológicas. Los cambios en este sentido no han dejado de suscitar ciertas críticas. Por ejemplo, algunos ecólogos y biólogos de la conservación consideran que aquella fracción de taxónomos que se apoya en la filogenética molecular para describir especies promueve la inestabilidad taxonómica (ver Padial y De la Riva, 2007). En los últimos años, el auge de la filogenética dentro de la taxonomía y, concretamente, del llamado “DNA-barcoding”, ha impulsado un intenso debate científico sobre los criterios y objetivos a seguir en la taxonomía moderna.

Respecto a esta tesis, se ha preferido aquí seguir la aproximación integrativa a la diversidad de especies propuesta por Dayrat (2005) y Will *et al.* (2005), que considera el uso de múltiples líneas de evidencia para establecer los límites de las especies. La convergencia de diferentes líneas de evidencia permite proponer hipótesis taxonómicas más estables, mientras que la divergencia permite detectar puntos débiles en las taxonomías.

El concepto de especie

En la inmensa mayoría de las descripciones de especies, se hayan basado sólo en caracteres morfológicos o no, se obvia algo tan fundamental como el concepto de especie utilizado, algo paradójico cuando el problema del concepto de especie constituye uno de los temas centrales y más controvertidos de la biología evolutiva. Sin embargo, el intenso debate sobre el concepto de especie podría estar llegando a su fin (Hey, 2006). Esto se debe a que las propiedades biológicas usadas tradicionalmente para separar especies y que han dado lugar a, por ejemplo, el concepto biológico de especie o el concepto filogenético de especie, no son necesarias o suficientes para definir a la especie como categoría fundamental de la organización biológica (De Queiroz, 2005a-c). Esta perspectiva procede de considerar a la especies como linajes de metapoblaciones que pueden ser identificados por las propiedades biológicas contingentes que aparecen durante la evolución de dichos linajes (por ejemplo, caracteres morfológicos, aislamiento reproductivo, monofilia recíproca, etc.). De este modo, las especies pueden ser definidas y testadas mediante el estudio de cualquiera de esas propiedades.

Las especies son consideradas aquí como hipótesis sobre especies-taxa (ver De Queiroz, 2005c), que asignan grupos de individuos a determinados linajes de metapoblaciones que corresponden a la categoría de especie. Dependiendo de qué tipo de evidencia apoye una hipótesis, se las considerará, implícitamente, especies morfológicas (diferencias morfológicas), especies genéticas (divergencias en fragmentos de genes), o especies biológicas (barreras reproductivas precigóticas).

Sin embargo, muchos biólogos aún no han captado esta aproximación, lo que ha dado lugar a los actuales debates sobre cuál es la mejor metodología para definir especies. Así han surgido los conflictos entre los biólogos de diferentes ramas, que intentan enfatizar aquellas propiedades de las especies que son más evidentes en su campo de estudio. Por ejemplo, aquellos más familiarizados con las secuencias de ADN intentan promover como el mejor método de investigación taxonómica el estudio de las diferencias entre fragmentos de genes. En el otro extremo, los taxónomos clásicos siguen defendiendo el uso casi exclusivo de los caracteres morfológicos.

La realidad es que el método más ampliamente usado para describir especies es el de la comparación y descripción de caracteres morfológicos cualitativos. Cada hipótesis de especie es representada por un binomio linneano que hace referencia a un espécimen o un conjunto de ellos (holotipo o sintipos). Las descripciones originales actúan como la fuente científica de cada hipótesis, ya que incluyen la descripción morfológica original (evidencias), la designación de tipos y la denominación de la especie. Estas hipótesis son testables y modificables mediante el estudio y comparación de los tipos, y el estudio de la variabilidad de los caracteres en ejemplares adicionales de la localidad tipo o zonas adyacentes. Las re-evaluaciones de las hipótesis aparecen en forma de revisiones taxonómicas (Wheeler, 2004), como las que incluye esta tesis (ver más abajo).

En este estudio se utilizaron las especies morfológicas propuestas por otros autores y por el autor de esta tesis como base sobre la que comparar otros criterios para definir la especie (aislamiento reproductivo y monofilia). Para testar las especies morfológicas propuestas por otros autores se revisaron los ejemplares tipo de todas las especies consideradas en este estudio y material adicional, que aparece en los apéndices de los diferentes Apéndices.

Las especies biológicas son aquellas definidas principalmente por sus barreras reproductoras. Esta propiedad biológica fue la base del Concepto Biológico propuesto por Mayr (1942), que se convirtió en el más ampliamente defendido concepto de especie para organismos sexuales durante la segunda mitad del siglo XX. En anuros se han usado los cantos de apareamiento como evidencias de aislamiento reproductivo, ya que actúan como barreras reproductivas precigóticas (Bickford *et al.* 2007; Padial *et al.* 2007). De hecho, algunas de las especies consideradas en este estudio, fueron descubiertas inicialmente sólo por sus diferencias en el canto (Köhler 2000b; Reichle *et al.*, 2001; Apéndices 5 y 11).

Las divergencias entre marcadores moleculares mitocondriales han sido ampliamente empleadas en los últimos años como fuentes de evidencia taxonómica (Vences *et al.* 2005a) y filogenética (e. g. Darst y Cannatella, 2004; Frost *et al.* 2006). Uno de los marcadores mitocondriales más ampliamente utilizado y con mayor homogeneidad en los resultados para las especies de anuros es el gen mitocondrial 16S (Vences *et al.*, 2005a, b). En este estudio se seleccionó un fragmento de dicho gen para determinar las distancias genéticas de las especies y su monofilia recíproca respecto a especies cercanas. La monofilia recíproca es la base de algunas de las definiciones filogenéticas de especie, y una línea de evidencia de la independencia de linajes a nivel de especie (De Queiroz, 1999). Adicionalmente, incluimos otro marcador, un fragmento del gen Cytb, para reforzar una de las hipótesis filogenéticas.

En definitiva, este estudio es fruto de numerosos muestreos en el campo para colectar ejemplares, tejidos y grabaciones de cantos; del estudio de la morfología de los ejemplares tipo de las especies y de ejemplares adicionales de diversas colecciones europeas y americanas; y del análisis cuantitativo y cualitativo comparado de las diferencias en la morfología, los cantos y los marcadores mitocondriales. Los resultados de este estudio encajan por tanto en lo que se ha denominado taxonomía integrativa.

Esta memoria se divide en dos partes, que incluyen un total de once Apéndices correspondientes a siete artículos científicos, tres manuscritos aceptados para su publicación y un manuscrito enviado a revisar. A continuación se describen brevemente los antecedentes y objetivos de los diferentes Apéndices.

PARTE PRIMERA

UNA APROXIMACIÓN A LA DIVERSIDAD DE *ELEUTHERODACTYLUS* DE BOLIVIA

La primera parte de la memoria es una aproximación general a la diversidad de *Eleutherodactylus* de Bolivia. Se revisó la lista de especies pertenecientes a este género para el país y se resolvieron algunos de los problemas más evidentes; además, se identificaron los principales problemas a abordar en una segunda fase de estudio más pormenorizado. En la primera fase se contribuyó al aumento en la lista de especies del país con cinco citas nuevas de especies desconocidas previamente en Bolivia; se redescubrió y redescribió *Eleutherodactylus heterodactylus*; se estudió la morfología externa de *E. skydmainos* y *E. karcharias* y se sinonimizó la segunda bajo la primera; se describieron una nueva especie de *Ischnocnema* (actualmente *Oreobates*) de Bolivia y una nueva especie del grupo de *Eleutherodactylus discoidalis* de Bolivia (actualmente *Oreobates*); se estudió la variación morfológica y la distribución de *Eleutherodactylus mercedesae*, una de las especies de *Eleutherodactylus* más desconocidas; y, por último, se describió una nueva especie del grupo de *E. discoidalis* del sur de Perú (actualmente en *Oreobates*).

Apéndice 1. Nuevas citas de Eleutherodactylus *para Bolivia (Padial et al., 2004).*

Este trabajo es el resultado del estudio de diversas colecciones de *Eleutherodactylus*. El objetivo principal era realizar una aproximación a la diversidad de *Eleutherodactylus* de

Bolivia tras los trabajos de De la Riva *et al.* (2000) y Köhler (2000), y proporcionar así un nuevo punto de partida más actualizado. Se publican cinco nuevos registros de *Eleutherodactylus* (Anura: Leptodactylidae) para Bolivia y se incorpora una especie previamente eliminada por Köhler (2000). La diversidad de *Eleutherodactylus* en Bolivia ascendió a 25 especies.

Capitulo 2. Redescubrimiento y redescripción de Eleutherodactylus heterodactylus, *descripción de su canto y notas sobre otros* Eleutherodactylus *de Bolivia (Padial y De la Riva, 2005a).*

Se redescubre y redescribe *Eleutherodactylus heterodactylus*, una especie descrita en 1937 del Cerrado brasileño cercano a Bolivia y de la que sólo se conocían sus ejemplares tipo, mal preservados. Los objetivos son redescribir la morfología de esta especie, citarla por primera vez en Bolivia, describir por primera vez su canto de apareamiento, y discutir su posible asignación a un grupo de especies (ver Apéndices 8 y 9). Además, se confirma el estatus taxonómico de *E. crepitans* y *E. dundeei* como especies bien diferenciadas de *E. fenestratus*, todas ellas pertenecientes al grupo de *E. conspicillatus*. Se comenta por primera vez que las poblaciones de los Andes bolivianos asignadas por Köhler (2000a) a *E. dundeei* podrían corresponder en realidad a una especie no descrita (ver Apéndice 11).

Apéndice 3. El estado taxonómico de Eleutherodactylus skydmainos *y* E. karcharias *(Padial y De la Riva 2005b).*

En este Apéndice se estudia la variabilidad morfológica de *Eleutherodactylus skydmainos* (ver también Apéndice 1) y la de *E. karcharias*, y se concluye que ambas son conespecíficas, por lo que el objetivo de este trabajo es sinonimizar la segunda bajo el nombre de la primera (ver también el Apéndice 11).

Apéndice 4. Una nueva especie de Ischnocnema *de los bosques nublados de los Andes de Bolivia (Padial et al., 2005a).*

El objetivo es describir una nueva especie del género *Ischnocnema* (ahora *Oreobates;* ver Apéndice 9) de los bosques nublados de los Andes del norte de Bolivia. La descripción se basa en caracteres morfológicos externos. Se discute su posible relación con *I. sanctaecrucis* (Apéndice 9) y también sobre su parecido con algunas especies del grupo de *Eleutherodactylus discoidalis*.

Apéndice 5. Una nueva especie de Eleutherodactylus *del grupo de* E. discoidalis *de los bosques húmedos de montaña de Bolivia (Padial et al., 2005b).*

El objetivo es describir una nueva especie del grupo de *Eleutherodactylus discoidalis* de los bosques húmedos andinos del Departamento de La Paz, en el norte de Bolivia. La nueva especie se diferencia de otras especies del grupo por su canto de apareamiento, por tener piel dorsal uniformemente rugosa, tímpano pequeño, discos digitales redondeados y ligeramente agrandados y color marrón oscuro bastante uniforme. Se compara el canto con el de *E. cruralis*, la especie que parece ser más cercana a la nueva (ver también los Apéndices 8 y 9). Se proporciona por primera vez una clave de identificación para las especies del grupo de *E. discoidalis.*

Apéndice 6. Variación morfológica y distribución de E. mercedesae *(Padial et al., 2006).*

El objetivo es contribuir al conocimiento de la distribución de *Eleutherodactylus mercedesae*, que se consideraba hasta ahora endémica para Bolivia, y se cita por primera vez en Perú. Su rango conocido de distribución comprende actualmente unos 1000 km de los bosques nublados de las laderas amazónicas de los Andes, desde el sur de Perú al centro de Bolivia. Se describe por primera vez la hembra de esta especie basándose en dos ejemplares, que son comparados con el holotipo y el paratipo. Añadimos algunas observaciones sobre la descripción original y la variación morfológica de la especie (ver también Apéndice 10).

Apéndice 7. Una nueva especie de Eleutherodactylus *del grupo de* E. discoidalis *de los bosques húmedos de montaña de Perú (Padial et al., 2007).*

El descubrimiento de esta especie de Perú es resultado de los estudios sobre la variabilidad de *E. cruralis*, una especie del grupo de *E. discoidalis* que habita en Bolivia y Perú, ya que la revisión de algunas colecciones de ejemplares de Perú identificados como *E. cruralis* dió lugar a su descubrimiento (ver también el Apéndice 9). Se discuten las hipótesis propuestas sobre las relaciones del grupo *E. discoidalis* con los miembros de *Ischnocnema* trasladados a *Oreobates* por Caramaschi y Canedo (2006).

PARTE SEGUNDA

Esta parte consta de cuatro manuscritos en los que se profundiza en las relaciones entre las diferentes especies, se comparan sus cantos, se redescriben y recolocan especies en sus respectivos grupos, se reorganiza la clasificación del grupo de *E. fraudator* mediante la descripción de un subgénero (*Yunganastes*), se integra a los antiguos miembros del grupo de *E. discoidalis* en *Oreobates* y, por último, se describen dos especies crípticas de los grupos de *E. conspicillatus* y *E. unistrigatus* de Bolivia y Perú.

Apéndice 8. El estudio taxonómico de ranas tropicales con técnicas bioacústicas: variación geográfica en el canto de apareamiento de las especies del grupo de Eleutherodactylus discoidalis *(Padial et al., en prensa).*

Los cantos de apareamiento de anuros son una fuente de información muy importante para identificar y solventar problemas taxonómicos, así como para localizar especies crípticas e incluso híbridos (Heyer *et al.*, 1996; Wycherley *et al.*, 2002; Angulo *et al.*, 2003; Gergus *et al.*, 2004). A las llamadas de apareamiento de los machos responden sólo las hembras de su especie; son, así, altamente selectivas y, por tanto, útiles para el taxónomo a la hora de distinguir especies. De hecho, en el caso de ausencia de caracteres morfológicos consistentes, los cantos ha sido de gran ayuda para detectar y describir nuevas especies crípticas, incluyendo algunas de *Eleutherodactylus* (e. g. Köhler y Lötters, 1999; Köhler, 2000b; Reichle *et al.*, 2001).

Dentro del grupo de *E. discoidalis*, sólo se conocen los cantos de *E. cruralis*, *E. ibischi* y *E. madidi*. Márquez *et al.* (1995) describieron el canto de una población entonces asignada a *E. cruralis*, que resultó más tarde corresponder a *E. ibischi* (Reichle *et al.*, 2001). Estos últimos autores compararon los cantos de *E. cruralis* de la Amazonia con los de *E. ibischi* y con supuestas poblaciones de *E. cruralis* de los valles secos interandinos y todas ellas presentaban diferencias. Köhler (2000a) describió un canto de una población asignada a *E. cruralis* en una zona de transición entre los valles secos interandinos y los bosques húmedos de montaña y también encontró diferencias con las poblaciones amazónicas de esta especie. Posteriormente, Padial *et al.* (2005) describieron una especie morfológicamente similar *E. cruralis* pero con un canto muy distintivo.

En este manuscrito se reanalizan y comparan distintos parámetros de los cantos de los diferentes miembros del grupo de *E. discoidalis*, incluido el de la especie *E. discoidalis*, que permanecía desconocido. Esta comparación tiene la finalidad de evaluar el estado taxonómico de las especies del grupo y detectar diferencias que pudieran indicar la presencia de especies crípticas. Además, esto permite, a su vez, determinar cuáles son los parámetros cualitativos y cuantitativos del canto con mayor poder discriminatorio entre especies. Por ultimo, se discuten las hipótesis de parentesco del grupo de *E. discoidalis* e *Ischnocnema* , y del estatus de *E. heterodactylus*, basándose en las similitudes del canto entre ellos.

Apéndice 9. Sistemática del género Oreobates *y del grupo de* Eleutherodactylus discoidalis *(Padial et al., aceptado-a).*

El grupo de especies de *E. discoidalis* fue propuesto por Lynch (1976) para incluir a cinco especies: *E. cruralis* (Boulenger, 1902), *E. discoidalis* (Peracca, 1895), *E. elassodiscus* Lynch, 1973, *E. granulosus* (Boulenger, 1903) y *E. nigrovittatus* (Anderson, 1945). La definición de este grupo se basó en una combinación de supuestas sinapomorfías y fue considerado monofilético. Sin embargo, Lynch (op. cit.) también destacó que las especies de Bolivia y Perú (*E. cruralis*, *E. discoidalis* y *E. granulosus*) diferían de las restantes (*E. elassodiscus* y *E. nigrovittatus*). Posteriormente, Lynch (1989) sinonimizó *E. granulosus* con *E. cruralis* y escindió el grupo dejando como únicos miembros a *E. discoidalis* y *E. cruralis.* El mismo autor consideraba a *Ischnocnema* filogenéticamente más primitivo que el grupo de *E. discoidalis* y al grupo *E. binotatus* lo consideraba cercano a *Ischnocnema* y ancestral al grupo de *E. discoidalis*. Por último, Lynch (1989) rechazó la hipótesis de Savage (1987) sobre la relación del género mexicano *Tomodactylus* e *Ischnocnema*.

Mas recientemente, la filogenia molecular de Frost *et al.* (2006) apoyaba la posición de *Ischnocnema* dentro de *Eleutherodactylus*, y como grupo hermano del grupo de *E. binotatus*. Sin embargo, debido al escaso “taxon sampling” dentro de los eleuterodactilinos, la única conclusión apoyada por los datos era que *Eleutherodactylus* no era monofilético, la misma conclusión a la que habían llegado Darst y Cannatella

(2004). Por lo tanto, ambas filogenias solo concluían que *Ischnocnema* y *Eleutherodactylus* eran grupos cercanos una conclusión a la que ya había llegado Jiménez de la Espada (1872, 1875).

El género *Oreobates* fue descrito por Jiménez de la Espada (1872) para la especie *O. quixensis* de la amazonía ecuatoriana. Sin embargo, Lynch y Schwartz (1971) consideraron *Oreobates* sinónimo de *Ischnocnema*, un género descrito por Reinhardt y Lütken (1862) para acomodar a *Leiuperus verrucosus* Reinhardt y Lütken, 1862, procedente de Minas Gerais (SE de Brasil). Lynch y Schwartz (1971) también pusieron en sinonimia de *O. quixensis* dos *Hylodes* (=*Eleutherodactylus*) de Jiménez de la Espada (1875). Además, consideraron perdidos tanto los tipos de uno de los *Hylodes* como el de *O. quixensis*. El único tipo de *Ischnocnema*, un juvenil en muy mal estado, quedaba como la única evidencia disponible. Por eso, debido a esta gran carencia de material de estudio para realizar análisis taxonómicos adecuados, la sistemática de estas especies ha permanecido sin resolver. Además, el redescubrimiento de *Ischnocnema verrucosa* y su inclusión en *Eleutherodactylus* por Caramaschi y Canedo (2006), así como la resurrección de *Oreobates* para acomodar a los antiguos miembros andinos y amazónicos de *Ischnocnema*, anulaba muchas de las hipótesis filogenéticas de Lynch (1989) y dejaba en completa incertidumbre las relaciones de *Oreobates* y *Eleutherodactylus*. Surgieron algunas preguntas, como por ejemplo: ¿es *Oreobates* un grupo monofilético? ¿cuál es su relación con *Eleutherodactylus* y, sobre todo, con el grupo de *E. discoidalis*, cuyas especies son morfológicamente similares?.

Por todo ello, los objetivos de este trabajo fueron: (1) determinar si el grupo de *E. discoidalis* y el género *Oreobates* eran monofiléticos; (2) esclarecer las relaciones filogenéticas de ambos; (3) determinar la diversidad de especies de ambos grupos.

Apéndice 10. Revisión del grupo de Eleutherodactylus fraudator *(Padial et al., aceptado-b).*

Köhler (2000a, b) propuso y definió el grupo de *E. fraudator*, que incluía a varias especies morfológicamente similares del centro de Bolivia: *E. ashkapara* Köhler, 2000, *E. fraudator* Lynch y McDiarmid, 1987 y *E. pluvicanorus* De la Riva y Lynch, 1997. Otra especie (*E. mercedesae* Lynch y McDiarmid, 1987) fue marcada como posible candidata para ser incorporada al grupo en el futuro. La primera especie del grupo, *E. fraudator*, fue incluída tentativamente en el grupo de *E. conspicillatus* por sus autores, mientras que *E. mercedesae* no fue asignada a ningún grupo en la descripción original (Lynch y McDiarmid, 1987). Posteriormente, Lynch y Duellman (1997), realizaron una revisión de los grupos de especies de Sudamérica y no asignaron ni a *E. fraudator* ni a *E. mercedesae* a ningún grupo de especies. De la Riva y Lynch (1997) describieron *E. pluvicanorus* y lo consideraron muy cercano a *E. fraudator*, debido a su apariencia externa similar y a la presencia en ambos de un fontanela frontoparietal. Además, estos autores asignaron ambas especies al subgénero *Craugastor* debido a la presencia en ambas especies de la condición "E" del nervio trigémino de la rama mandibular del músculo abductor de la mandíbula, que era considerada como sinapomorfía por Lynch (1986). Köhler (2000a, b), al definir el grupo, puso en duda la relación de este conjunto de especies con el subgénero *Craugastor*. Aunque el origen y las relaciones filogenéticas de *Craugastor* fueron tratadas con detalle por Crawford y Smith (2005), las relaciones con el grupo de *E. fraudator* permanecieron sin resolver. Recientemente, Frost *et al.* (2006) rechazaron la hipótesis de De la Riva y Lynch (1997) sobre la relación entre *Craugastor* y el grupo de *E. fraudator*, basándose en análisis moleculares y diferencias en la condición del nervio trigémino observadas en *E. pluvicanorus*. Sin embargo, su análisis molecular fue erróneo, ya que la única especie del grupo que incluyeron (*E. pluvicanorus*) resultó estar mal identificada, al tratarse de *E. rhabdolaemus* (un miembro del grupo de *E. unistrigatus*).

En definitiva, varias cuestiones de interés estaban aún por resolver: ¿es el grupo de *E. fraudator* una mera agrupación fenética o un grupo natural monofilético?; ¿cuál es su relación con *Craugastor*?; ¿cuál es la diversidad de especies del grupo?; ¿es la condición del nervio trigémino diferente en los miembros de *Craugastor* y el grupo de *E. fraudator*? Si así fuese, ¿se trata de una sinapomorfía del grupo?.

Por todo esto, los objetivos de este trabajo se resumen en: (1) testar la monofilia del grupo de *E. fraudator* y determinar su diversidad de especies mediante el estudio de su morfología, diferencias en el canto de apareamiento y en un fragmento del gen mitocondrial 16S; y (2) esclarecer sus relaciones con *Craugastor* y otros *Eleutherodactylus*.

Apéndice 11. Una aproximación taxonómico-integrativa revela especies crípticas amazónicas de Eleutherodactylus *(Padial y De la Riva, en revisión).*

Las especies crípticas y las especies gemelas, ocultas ante los ojos del taxónomo clásico, podrían ser mucho más abundantes de lo que se creía (Bickford *et al.*, 2007). Por eso, el número de *Eleutherodactylus*, con unas 500 especies descritas en base, casi exclusivamente, a diferencias en caracteres morfológicos evidentes, podría elevarse rápidamente con la incorporación de otras líneas de evidencia. De hecho, los *Eleutherodactylus* son buenos candidatos para ocultar especies crípticas, debido a la sutileza de muchos de sus caracteres y a los patrones de especiación que han dado lugar a su diversidad (Lynch y Duellman, 1997). Esto se ejemplifica en este Apéndice, donde se incorporan la bioacústica y la taxonomía molecular para reanalizar antiguos problemas taxonómicos de *Eleutherodactylus* de Bolivia, y se describen dos nuevas especies crípticas, diferenciables por caracteres del canto, moleculares y morfométricos.

El estatus taxonómico de ciertas poblaciones *Eleutherodactylus* de los bosques húmedos amazónicos de las laderas andinas del centro de Bolivia, asignadas a *E. dundeei* por Köhler (2000a), fue considerado dudoso por Padial y De la Riva (2005a, Apéndice 2). Esas mismas poblaciones habían sido previamente asignadas a *E. fenestratus* (De la Riva, 1993) y posteriormente a *E. peruvianus* (De la Riva, 1994). Sin embargo, Köhler (2000a) redescribió *E. peruvianus* y lo eliminó de la lista boliviana de especies por no haber encontrado ningún ejemplar que coincidiese con el holotipo. Posteriormente, Padial y De la Riva (2005a, Apéndice 2) rechazaron la postura de Köhler (2000a), ya que una serie de ejemplares de diferentes localidades del sur de Perú, identificados como *E. peruvianus* presentaban variabilidad en los caracteres considerados por Köhler (2000a). En realidad, la variabilidad observada en los ejemplares de las tierras bajas de la Amazonía del sur de Perú incluía también a *E. danae*, una especie perteneciente a un grupo diferente, el de *E. unistrigatus* y que habita los bosques húmedos de montaña del sur de Perú y norte de Bolivia. Por lo tanto podría existir una especie cercana a *E. danae* oculta bajo el nombre de *E. peruvianus*.

Para intentar solventar este complejo panorama taxonómico, se seleccionaron como objetivos los dos taxa de dudoso estado taxonómico identificados por Padial y De la Riva (2005a; Apéndice 2) como *E.* cf. *peruvianus*. Uno correspondería a las poblaciones amazónicas y de los bosques húmedos del pie de monte del sur de Perú y norte de Bolivia, morfológicamente más similar a *E. danae*. El otro correspondería a las poblaciones del centro de Bolivia asignadas por Köhler (2000) a *E. dundeei*, un miembro del grupo *E. conspicillatus*.

Eleutherodactylus peruvianus es un miembro del grupo de *E. conspicillatus*. Los miembros de este grupo se distribuyen desde Centroamérica hasta Bolivia, tanto al este como al oeste de los Andes (Frost, 2006). Sólamente una especie, *E. w-nigrum* parece habitar a ambos lados de los Andes, mientras que el resto se distribuyen a uno u otro lado de la cordillera. La mayoría de las especies habitan los bosques húmedos de las laderas de los Andes y la llanura amazónica. Ya que nuestras especies de dudoso estado taxonómico se distribuyen al este de los Andes y en la Amazonía, nuestras comparaciones se redujeron a especies con distribución dentro de estas zonas. En otras, palabras, no incluimos especies de las laderas del oeste de los Andes, del escudo guayanés, ni de las cadenas montañosas del norte de Ecuador, Colombia y Venezuela. Las especies del grupo de *E. conspicillatus* incluidas en este estudio fueron: *E. avicuporum*, *E. bipunctatus*, *E. buccinator*, *E. caliginosus*, *E. citriogaster*, *E. condor*, *E. cosnipatae*, *E. conspicillatus*, *E. crepitans*, *E. cuneirostris*, *E. dundeei*, *E. fenestratus*, *E. lanthanites*, *E. lymani*, *E. malkini*, *E. metabates*, *E. peruvianus*, *E. samaipatae*, *E. skydmainos* [que incluye *E. karcharias* (ver Apéndice 3; Padial y De la Riva, 2005b)], *E. vilarsi* y *E. zeutoctylus*. Por otro lado, ya que uno de los taxa de dudoso estado taxonómico escondido bajo el nombre *E.* cf. *peruvianus* es muy similar a *E. danae*, incluímos a esta especie en las comparaciones correspondientes, y a otros miembros del grupo de *E. unistrigatus* que comparten caracteres con *E. danae* y habitan en la Amazonía y las laderas adyacentes de los Andes: *E. altamazonicus*, *E. carvalhoi*, *E. croceoinguinis*, *E. diadematus*, *E. eurydactylus*, *E. martiae*, *E. ockendeni*, *E. platydactylus*, *E. rhabdolaemus*, *E. salaputium*, *E. toftae*, y *E. ventrimarmoratus*. Las comparaciones de cantos y genéticas se restringieron a especies muy próximas en caracteres cualitativos.

DISCUSIÓN

Los Apéndices precedentes muestran que muchas de las hipótesis taxonómicas sobre especies y grupos de especies de *Eleutherodactylus, Ischnocnema* y *Oreobates* de Bolivia anteriores a este estudio no se mantienen. En consonancia con las evidencias expuestas en este estudio, se ha modificado el estado taxonómico de algunas especies, otras han sido redescubiertas y revalidadas, se han trasladado especies de unos grupos en otros, se han redefinido los grupos en base a sus sinapomorfías, y la distribución de algunos grupos y especies ha sido modificada acorde a los cambios taxonómicos. Con todo ello, Bolivia cuenta actualmente con 22 especies de *Eleutherodactylus* y 10 de *Oreobates* (Tabla 1). Los *Eleutherodactylus* se incluyen por un lado en dos grupos de especies, *E. conspicillatus* y *E. unistrigatus*, pertenecientes al subgénero *Eleutherodactylus*, y por otro en el subgénero *Yunganastes*. El grupo de *E. discoidalis* desaparece y sus miembros pasan a formar parte del género *Oreobates*, junto con las especies emplazadas en dicho género por Caramaschi y Canedo (2006).

OREOBATES

Los análisis filogenéticos de dos genes mitocondriales realizados en este estudio apoyan la monofilia de *Oreobates*. Este género incluye además a todas las especies del grupo de *Eleutherodactylus discoidalis*. Tres posibles sinapomorfías morfológicas y las similitudes en el canto apoyan esta hipótesis filogenética. El grupo hermano de *Oreobates* lo constituye un clado que incluye a los miembros del grupo de *E. conspicillatus* y a la serie *E. martinicensis* del subgénero *Eleutherodactylus*. La serie *E. binotatus* y los grupos de especies *E. dolops* y *E. nigrovittatus* del subgénero *Eleutherodactylus* no están cercanamente relacionados ni con la serie *E. martinicensis* ni con *Oreobates*. Por lo tanto, y en concordancia con los resultados preliminares de Darst y Cannatella (2004) y Frost *et al.* (2006), el género *Eleutherodactylus* no es monofilético. De hecho, este género debería restringirse a las series *E. martinicensi* y *E. conspicillatus*. Nuestros datos corroboran la hipótesis de monofilia de *Craugastor* (Crawford y Smith, 2005).

Según este estudio, la mayor diversidad y grado de endemismo de *Oreobates* se alcanza en la Cordillera Oriental de los Andes Centrales. Esta diversidad parece estar relacionada con eventos vicariantes producidos durante la orogenia andina (Lynch y Duellman, 1997; Doan, 2003), y/o por la fragmentación de los hábitats producida por cambios climáticos históricos (Weir, 2006; Hughes y Eastwood, 2006). Sólo dos especies se encuentran en las tierras bajas de la Amazonía (*O. quixensis*, *O. cruralis*) y una en el escudo precámbrico brasileño (*O. heterodactylus*). Nuestros datos sugieren que el origen de *Oreobates* estaría en el pie de monte andino de la cabecera de la cuenca amazónica. Por otro lado, al ser estas ranas de desarrollo directo, dependen de condiciones de humedad elevada para su desarrollo, lo que podría explicar la distribución observada de su mayor diversidad en los bosques montanos húmedos de montaña. Del mismo modo, las especies como *E. heterodactylus*, *E. ibischi* y *E. discoidalis*, que habitan bosques semideciduos, sólo están activas durante la época de lluvias y restringidas a los microhábitats más húmedos de esos ambientes. Además, las diferencias encontradas entre los cantos de algunos miembros de *Oreobates* parecen tener relación con los tipos de hábitat que ocupa cada especie, por lo que estas diferencias podrían deberse a una combinación de aislamiento en un determinado tipo de hábitat conjuntamente con las presiones selectivas impuestas por ese hábitat. El patrón de variación del canto también se ajusta a un eje latitudinal a lo largo de los Andes, lo que parece coincidir con otras hipótesis sobre especiación que relacionan la orogenia de los Andes y la diversidad y antigüedad de las especies (Doan, 2003). La especie más aislada de *Oreobates*, *O. heterodactylus,* se encuentra en arroyos húmedos de los afloramientos rocosos del escudo precámbrico del oeste de Brasil y este de Bolivia (Heyer y Muñoz, 1999; Padial y De la Riva, 2005a; Apéndice 2). La distribución de esta especie evidencia una antigua conexión biogeográfica de esta zona montañosa del Cerrado con las laderas andinas durante periodos más húmedos (ver también Pennington *et al.* 2000).

En definitiva, los miembros de *Oreobates* se distribuyen desde el norte de la Amazonía (*O. quixensis*) hasta Argentina (*O. discoidalis*), alcanzando una mayor diversidad en las laderas de los Andes, y siendo Bolivia (con una posición geográfica intermedia) el país con una mayor diversidad de especies de este género. Cabe destacar que los análisis bioacústicos realizados han señalado la posible existencia de especies crípticas ocultas bajo lo que hoy en día conocemos como *O. cruralis*. Sin embargo, se ha optado por no describirlas a la espera de un mayor número de muestras y evidencias adicionales.

El subgénero Yunganastes

Los miembros del antiguo grupo *Eleutherodactylus fraudator* (*E. ashkapara*, *E. fraudator* y *E. pluvicanorus*) más *E. mercedesae* y *E. bisignatus* forman ahora el subgénero *Yunganastes*, dentro de *Eleutherodactylus*. Estas especies forman un grupo monofilético endémico de los bosques húmedos de montaña de los Andes centrales (desde el centro de Bolivia hasta el sur de Perú). Todas las especies habitan en Bolivia, y tan sólo una (*E. mercedesae*) en Perú. La monofilia de *Yunganastes* está apoyada por análisis filogenéticos de un gen mitocondrial, y por sinapomorfías bioacústicas y morfológicas.

El género *Yunganastes* no pertenece ni está relacionado con *Craugastor*, como fue sugerido por De la Riva y Lynch (1997), en base a la posición del nervio trigémino. De hecho, en este estudio se describe un nuevo estado del nervio trigémino compartido por los miembros de *Yunganastes* y no con *Craugastor*. Este estudio tampoco apoya la monofilia de *Eleutherodactylus* como género ni la de *Craugastor* si se mantiene en él a la especie *E. bufoniformis*.

Se plantean dos hipótesis opuestas sobre el origen de *Yunganastes*. En la primera *E. mercedesae* sería una especie derivada que habría colonizado el bosque montano húmedo tras la aparición y diversificación de *Yunganastes* en los boques nublados de mayor altitud. La segunda hipótesis, aquella más apoyada por los datos presentados, considera a *E. mercedesae* la especie más primitiva del grupo. El ancestro de *Yunganastes* habría tenido entonces una distribución amplia altitudinal y latitudinal en las laderas de los Andes de Perú y Bolivia, y habría sufrido una radiación en el centro de Bolivia como consecuencia de la fragmentación y aislamiento de los boques nublados en esta zona de transición con otras más secas.

OTROS *ELEUTHERODACTYLUS* CON PROBLEMAS TAXONÓMICOS

Dos nuevas especies de *Eleutherodactylus*, una del grupo de *E. conspicillatus* (*Eleutherodactylus* sp. 1) y una del grupo de *E. unistrigatus* (*Eleutherodactylus* sp. 2) son descritas de las laderas amazónicas de los Andes y las llanuras contiguas del centro y norte de Bolivia y sur de Perú. *Eleutherodactylus* sp 1. había sido confundida tanto con *E. fenestratus* y *E. dundeei* como con *E. peruvianus*, mientras que *Eleutherodactylus* sp. 2 había sido confundida con *E. danae* y con *E. peruvianus*. Los problemas taxonómicos asociados a estas especies han podido ser resueltos gracias a la combinación de varias líneas de evidencia: morfometría, bioacústica y taxonomía molecular. Las tres líneas de evidencia apoyaron el estatus taxonómico y la independencia de las nuevas especies.

ELEUTHERODACTYLUS (22 spp.)
Subgénero *Eleutherodactylus* (17 spp.)
Grupo *E. conspicillatus* (5 spp.)
E. dundeei Heyer y Muñoz, 1999
E. fenestratus (Steindachner, 1864)
E. samaipatae Köhler y Jungfer, 1994
E. skydmainos Flores y Rodríguez, 1997
E. sp. nov. 1 Padial y De la Riva
Grupo *E. unistrigatus* (12 spp.)
E. altamazonicus Barbour y Dunn, 1921
E. carvalhoi Lutz y Kloss 1952
E. danae Duellman, 1978
E. llojsintuta Köhler y Lötters, 1999
E. ockendeni (Boulenger, 1912)
E. olivaceus Köhler, Morales, Lötters, Reichle, y Aparicio, 1998
E. platydactylus (Boulenger, 1903)
E. rhabdolaemus Duellman, 1978
E. toftae Duellman, 1978
E. ventrimarmoratus (Boulenger, 1912)
E. zimmermanae Heyer y Hardi, 1991
E. sp. nov. 2 Padial y De la Riva
Subgénero *Yunganastes* (5 spp.)
E. ashkapara Köhler, 2000
E. bisignatus (Werner, 1899)
E. fraudator Lynch y McDiarmid, 1987
E. mercedesae Lynch y McDiarmid, 1987
E. pluvicanorus De la Riva y Lynch, 1997
OREOBATES (10 spp.)
O. choristolemma (Harvey y Sheehy, 2005)
O. cruralis (Boulenger, 1902)
O. discoidalis (Peracca, 1895)
O. heterodactylus (Miranda-Ribeiro, 1937)
O. ibischi (Reichle, Lötters y De la Riva, 2001)
O. madidi (Padial, González y De la Riva, 2005)
O. quixensis Jiménez de la Espada, 1872
O. sanctaecrucis (Harvey y Keck, 1995)
O. sanderi (Padial, Reichle y De la Riva, 2005)
O. zongoensis (Reichle y Köhler, 1997)

Tabla 1. Especies de *Eleutherodactylus* y *Oreobates* conocidas en Bolivia.

El origen de ambas especies parece estar relacionado con las diferentes condiciones ambientales que reinan en los hábitat ocupados por las especies más próximamente relacionadas. Por ejemplo, *Eleutherodactylus* sp. 1 es la especie hermana de *E. fenestratus*. Mientras que *E. fenestratus* ocupa una distribución amplia en la cuenca amazónica y las laderas húmedas andinas a baja altitud, *Eleutherodactylus* sp. 1 se restringe a los bosques de los valles secos interandinos y penetra levemente en el límite sur de los bosques húmedos del pie de monte andino. Por otro lado, *Eleutherodactylus* sp. 2 es la especie hermana de *E. danae*. Mientras que *E. danae* habita en los bosques húmedos de montaña del sur de Perú y norte de Bolivia, *Eleutherodactylus* sp. 2 habita los bosques de menor altitud y las llanuras amazónicas, que se caracterizan por su estacionalidad más marcada, su menor humedad y su mayor temperatura. En definitiva, dada la distribución parapátrica de esas cuatro especies en hábitat contiguos y diferentes, la hipótesis de especiación mediante conservacionismo de nicho, basada en las diferencias ambientales de los hábitat que ocupan las especies (Wiens *et al.*, 2006), podría explicar el origen de las divergencias de estas especies. Esto se habría visto favorecido por la reducción del tamaño y aislamiento de los ambientes ocupados actualmente por estas especies durante períodos más fríos y secos (Pennington *et al.*, 2000; Killeen *et al.*, 2007).

Durante 250 años de taxonomía linneana, el descubrimiento y descripción de especies se ha basado principalmente en el uso de caracteres morfológicos cualitativos. Es por ello que este estudio es uno de los pocos ejemplos en los que se realiza una aproximación taxonómico-integrativa en un grupo complejo de oganismos tropicales. En él se ha mostrado como la combinación de diferentes líneas de evidencia, morfología, aislamiento reproductivo y diferencias genéticas, son una poderosa herramienta para resolver antiguos problemas taxonómicos y para descubrir especies crípticas dentro de grupos complejos y diversos. Es muy probable que estudios similares a gran escala dentro de lo que hoy conocemos como *Eleutherodactylus*, con casi 500 especies descritas según el procedimiento taxonómico clásico, den como resultado un considerable aumento del número de especies y un mejor conocimiento de sus relaciones filogenéticas.

CONCLUSIONES

1. En Bolivia habitan 22 especies de *Eleutherodactylus*: por un lado, cinco del grupo de *E. conspicillatus* y doce del grupo de *E. unistrigatus,* ambos dentro del subgénero *Eleutherodactylus*, y, por otro lado cinco del subgénero *Yunganastes*. El género *Oreobates* está representado por diez especies.

2. Los análisis filogenéticos de dos fragmentos de genes mitocondriales (16S y Cytb) junto con sinapomorfías morfológicas y bioacústicas, apoyan la monofilia de *Oreobates* y de las especies que lo componen.

3. Los miembros del grupo de especies de *Eleutherodactylus discoidalis* no pertenecen en realidad a *Eleutherodactylus*, sino a *Oreobates*, que incluye las siguientes 14 especies: *O. choristolemma*, *O. cruralis*, *O. discoidalis*, *O. granulosus*, *O. heterodactylus*, *O. ibischi*, *O. lehri*, *O. madidi*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*, *O. saxatilis*, *O. simmonsi* y *O. zongoensis*.

4. Los miembros de *Oreobates* se distribuyen desde la Amazonía colombiana (*O. quixensis*) hasta el norte de Argentina (*O. discoidalis*), con un vicariante (*O. heterodactylus*) en los afloramientos rocosos del escudo precámbrico del Cerrado brasileño-boliviano. La mayor diversidad de especies se encuentra en los bosques montanos húmedos de la Cordillera Oriental de los Andes bolivianos.

5. *Oreobates* es el grupo hermano de las series *E. martinicensis* y *E. conspicillatus* del subgénero *Eleutherodactylus*, y no está cercanamente relacionado con los grupos de especies *E. dolops* o *E. nigrovittatus*, ni con la serie *E. binotatus*, con los que se le había asociado anteriormente.

6. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S, así ciertas como sinapomorfías morfológicas y bioacústicas, apoyan la validez y monofilia del grupo de *Eleutherodactylus fraudator*, que es aquí considerado con rango de subgénero bajo el nombre de *Yunganastes*, e incluye las siguientes especies: *E. ashkapara*, *E. bisignatus*, *E. fraudator*, *E. mercedesae* y *E. pluvicanorus*.

7. *Yunganastes* es endémico de la Cordillera Oriental de los Andes centrales. Todas las especies de *Yunganastes* habitan los bosques nublados del centro y norte de Bolivia, con una única especie (*E. mercedesae*) presente en Perú.

8. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S y determinadas diferencias en la condición del nervio trigémino rechazan la hipótesis de pertenencia y/o relación de *Yunganastes* con el género centroamericano *Craugastor*. La monofilia del género *Craugastor* sólo se mantiene si se excluyen los miembros del grupo de *C. bufoniformis*.

9. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S no apoyan la hipótesis de monofilia de *Eleutherodactylus*. Este género debería restringirse al clado formado por las series *E. martinicensis* y *E. conspicillatus*.

10. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S, junto a análisis morfométricos y bioacústicos, apoyan la independencia de dos linajes crípticos que serán descritos como nuevas especies, una del grupo de *E. conspicillatus* y una del grupo de *E. unistrigatus*.

11. El alto grado de endemismo en la Cordillera Oriental de los Andes centrales parece haber sido producido por una combinación de eventos vicariantes promovidos por la orogenia andina y cambios climáticos que han fragmentado los hábitats y creado condiciones ambientales diferentes. La deriva genética y el conservacionismo de nicho son las hipótesis de especiación favoritas para ser testadas en un futuro.

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APÉNDICE I

Graellsia, 60(2): 167-174 (2004)

FIRST RECORDS OF FIVE SPECIES OF THE GENUS *ELEUTHERODACTYLUS* DUMÉRIL AND BIBRON, 1841 (ANURA, LEPTODACTYLIDAE) FOR BOLIVIA

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ABSTRACT

Five species of the genus *Eleutherodactylus* (Anura, Leptodactylidae) are reported from Bolivia for the first time: *E. altamazonicus* from Departments Pando and La Paz; *E. carvalhoi* from Cochabamba; and *E. ockendeni*, *E. skydmainos* and *E. zimmermanae* from Pando. New departmental records are provided for other species: *E. cruralis* for Department Pando, *E. danae* for Beni and La Paz, *E. platydactylus* for Beni, *E. toftae* for Pando and La Paz and *E. ventrimarmoratus* for La Paz. Currently, 25 species of *Eleutherodactylus* are known in Bolivia. The taxonomic status of some species remains uncertain, new species are being described and others are still to be discovered. Therefore, the actual number of Bolivian *Eleutherodactylus* species is still unknown.

Key words: *Eleutherodactylus*, Bolivia, new records, Anura, Leptodactylidae

RESUMEN

Nuevas citas de *Eleutherodactylus* Duméril y Bibron, 1841 (Anura, Leptodactylidae) para Bolivia

En este trabajo se publican los primeros registros de cinco especies del género *Eleutherodactylus* (Anura, Leptodactylidae) para Bolivia: *E. altamazonicus* en los Departamentos de Pando y La Paz; *E. carvalhoi* en Cochabamba; y *E. ockendeni*, *E. skydmainos* y *E. zimmermanae* en Pando. Además, se aportan las primeras citas de *E. cruralis* para el Departamento de Pando, de *E. danae* para Beni y La Paz, de *E. platydactylus* para Beni, de *E. toftae* para Pando y La Paz y de *E. ventrimarmoratus* para La Paz. La diversidad conocida de *Eleutherodactylus* en Bolivia es de 25 especies, aunque los problemas taxonómicos sin resolver, las especies en descripción y el alto número de especies aún no citadas que se considera probable encontrar, hacen que aún desconozcamos la verdadera diversidad de este género en Bolivia.

Palabras clave: *Eleutherodactylus*, Bolivia, nuevas citas, Anura, Leptodactylidae.

Introduction

Frogs of the genus *Eleutherodactylus* occur in the Neotropical Region, where they are the most diverse genus of terrestrial vertebrates (Glaw & Köhler, 1998). Most *Eleutherodactylus* inhabit the humid mountain forests of the Andes, but many species occupy the lowland Amazonian forest, the Cerrado open forest, the Mata Atlantica, Middle America and most Caribbean islands (Frost, 1985; 2004). Currently, nearly 700 species have been recognized (Duellman, 1993; Lynch & Duellman,

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1997; Duellman & Pramuk, 1999; AmphibiaWeb, 2004), but several new species are described every year (Glaw *et al.*, 1998).

In Bolivia, 17 species have been formally recognized by De la Riva *et al.* (2000) and 19 by Köhler (2000). These authors expected that additional species would be reported in subsequent years. During the examination of unidentified specimens of *Eleutherodactylus* deposited in three collections of Bolivia, we identified some species that had not yet been recorded for the country, or were only known from single previous records. The aim of this work is to report and comment on these specimens and provide an overview of the species diversity of the genus *Eleutherodactylus* in Bolivia.

Methods

New records were established upon the basis of the last published lists (De la Riva *et al.*, 2000; Köhler, 2000; Reichle, 2003). Specimens were collected by different naturalists (specified in each case) and deposited in three Bolivian collections: Museo de Historia Natural Noel Kempff Mercado (MNKA, Santa Cruz de la Sierra), Colección Boliviana de Fauna (CBF, La Paz) and Colección de Fauna del Centro de Biodiversidad y Genética (CBG, Cochabamba). Other institutions consulted were: Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Perú (MHNSM, formerly MUSM) and Museo de Zoología, Universidade de São Pulo, (MZUSP). JMP identified specimens by comparing them with museum specimens, author's notes on type specimens, and original descriptions and/or redescriptions. The snout-vent length (SVL) of adult specimens was measured to the nearest 0.01 mm by a single author (JMP) using a digital caliper. For each species we provide complete distributional information (when available) that consists of department (Fig. 1), pro-

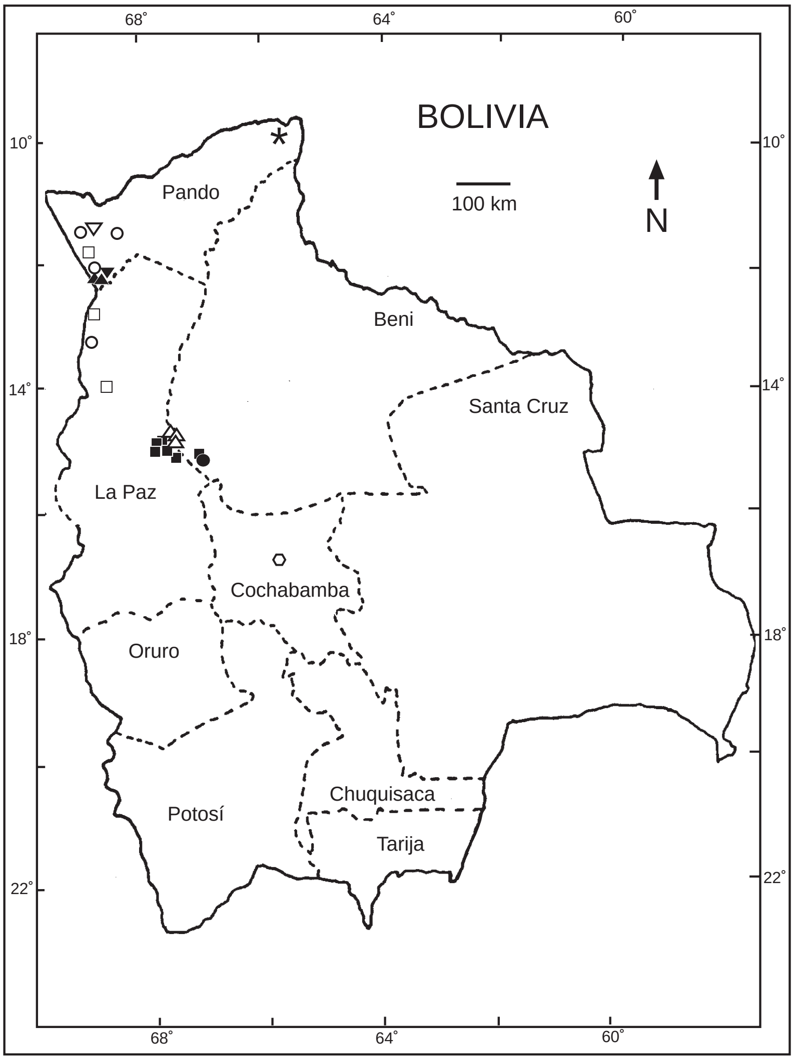

Fig. 1.— Map of Bolivia showing the Departments and new locality records for: *Eleutherodactylus altamazonicus* (white circles), *E. carvalhoi* (hexagon), *E. cruralis* (black triangles), *E. danae* (black squares), *E. ockendeni* (black inverted triangle), *E. platydactylus* (dot), *E. skydmainos* (asterisk), *E. toftae* (white squares), *E. ventrimarmoratus* (white triangles), and *E. zimmermanae* (white inverted triangle).

Fig. 1.— Mapa de Bolivia mostrando los departamentos y las nuevas localidades para: *Eleutherodactylus altamazonicus* (círculos blancos), *E. carvalhoi* (hexágono), *E. cruralis* (triángulos negros), *E. danae* (cuadrados negros), *E. ockendeni* (triángulo invertido negro), *E. platydactylus* (círculo negro), *E. skydmainos* (asterisco), *E. toftae* (cuadrados blancos), *E. ventrimarmoratus* (triángulos blancos), and *E. zimmermanae* (triángulo invertido blanco).

vince and locality (coordinates in parentheses). For the habitat characteristics we followed De la Riva *et al.* (2000).

New records

Eleutherodactylus altamazonicus Barbour & Dunn, 1921

PANDO: Province Manuripi: Reserva Nacional de Vida Silvestre Amazónica Manuripi, Luz de América (12º07'16.6"S/ 68º36'37.5"W), MNKA 3637 (immature, SVL: 12.07 mm), collected on 8 June 2000 by L. Gonzáles (Fig. 2); Curichón, Reserva Nacional de Vida Silvestre Amazónica Manuripi Heath (11º46'S/68º18'W), MNKA 3878 (male, SVL: 14.13 mm), collected on 28 August 2002 by M. Guerrero; Province Nicolás Suárez: Reserva Nacional de Vida Silvestre Tahuamanu: Luz de América, Maravilla (11°24'27"S/69°01'07"W), MNKA 6879 (young female, SVL: 15.80), collected on 2 July 2002 by M. Guerrero; Province Federico Román: MNKA 6880 (adult male, SVL: 15.69) collected during 1999 by J. Cadle.
LA PAZ: Province Iturralde: Candelaria (13º35'12.5"S/ 68º40'48"W), CBF 5311(sex undetermined, SVL: 13.31 mm), collected on 23 April 2001 by C. Cortez.

These five specimens constitute the first Bolivian and the southernmost records for this species. *Eleutherodactylus altamazonicus* is known to inhabit the upper Amazon Basin from Colombia to southern Peru (Lynch, 1980). In southern Peru, it has been found at Tambopata (Doan & Arizábal, 2002) and at Cuzco Amazónico (Duellman & Salas, 1991), both near the Bolivian border, and its presence in Bolivia was previously suspected (De la Riva *et al.*, 2000). The specimens were found in the Amazonian lowland forest and Andean foothills.

Fig. 2.— *Eleutherodactylus altamazonicus* from Luz de América, Reserva Manuripi, Pando, Bolivia (LG).

Fig. 2.— *Eleutherodactylus altamazonicus* de Luz de América, Reserva Manuripi, Pando, Bolivia (LG).

Eleutherodactylus carvalhoi Lutz, 1952

COCHABAMBA: Province Chapare: El Palmar, Carrasco National Park (17º06'S, 65º34'W), CBG 332 (adult female, SVL: 20.80), CBG 334 (adult male, SVL: 15.77), CBG 335 (adult male, SVL: 15.35), collected on 15 September 1998 by R. Aguayo.

These three specimens represent the first country record and the southernmost record for this species. It is known to occur in Huánuco, Loreto, Pasco and Madre de Dios (Cocha Cashu), Peru (Rodríguez *et al.* 1993; Rodríguez and Cadle, 1990). It has also been reported from Tambopata (Doan & Arizábal, 2002), near the Bolivian border, and its presence in Bolivia was not suspected (De la Riva *et al.*, 2000). Specimens were found in the Amazonian forest at the Andean foothills.

Eleutherodactylus cruralis (Boulenger, 1902)

PANDO: Province Manuripi: Laguna Bay, Reserva Nacional de Vida Silvestre Amazónica Manuripi (11º57'20"S /68º39'32.5"W), MNKA 6120 (adult female, SVL: 30.63) and MNKA 6121 (adult female, SVL: 30.67), collected on 01 February 2002 by L. Gonzáles and J. Condori; Florida, Reserva Nacional de Vida Silvestre Amazónica Manuripi (approx. 12º10'S/68º34'W), MNKA 5086 (adult male, SVL: 21.51mm), 5122 (adult female, SVL: 26.19 mm), collected on 17-18 July 2001 by L. Gonzáles.

These are the first records for this department. This species was previously recorded for Departments Beni (Padial & Castroviejo, 2000), La Paz, Cochabamba and Santa Cruz (De la Riva *et al.*, 2000). The specimens were found in the Amazonian lowland forest.

Eleutherodactylus danae Duellman, 1978

BENI: Province Ballivián: Serranía del Pilón, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°16'06"S/67°04'17"W), MNKA 4178 (juvenile), MNKA 4181(juvenile), MNKA 4203-4205 (juvenile), MNKA 4206 (gravid female, SVL: 32.00 mm), MNKA 4207 (juvenile), collected on 19-20 July 1999 by L. Gonzáles.
LA PAZ: Province Sud Yungas: Serranía de Chepete, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°05'24"S/67°31'42"W), MNKA 4743 (adult male, SVL: 30.69), collected on 24 September 1998 by L. Gonzáles; Serranía Beu, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°06'36"S/67°32'33"W), MNKA 3710, MNKA 3717, MNKA 3692, MNKA 3703, MNKA 3705, MNKA 3714 (adult males, average SVL =27.53 mm, range=24.27-29.59 mm, n=6), collected on 16-18 September 1998 by L. Gonzáles; San Ignacio, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°23'20"S/ 67°10'25"W), MNKA 4081-2 (juveniles), MNKA 4119-22 (juveniles), MNKA 4126-27 (juveniles), MNKA 4128 (adult male, SVL= 27.93), MNKA 4129-32 (juveniles), MNKA 4139-43 (juveniles), collected on 11-16 July 1999 by L. Gonzáles;

Quebrada Boquerón, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°22'19"S/67°11'41"W), MNKA 4112-4113 (juveniles), collected on 13 July 1999 by L. Gonzáles; Boquerón, Tierra Comunitaria de Origen y Reserva de Biosfera Pilón Lajas (15°22'37"S/ 67°12'04"W), CBG 844, CBG 846-853 (adult males, average SVL = 26.58, range = 25.37-27.51, n = 7), CBG 845 (adult female, SVL:31.79), CBG 853 (gravid female, SVL:37.06), collected on September 2002 by R. Aguayo.

Eleutherodactylus danae occurs from the Cosñipata Valley in Peru to Bolivia (Frost, 2004). In Bolivia, it was cited for the first time in Department Cochabamba (Köhler & Jungfer, 1995). Its presence was suspected for La Paz and Santa Cruz by De la Riva *et al.* (2000). These are the first records for Beni and La Paz. Specimens were found in the humid montane forest and the Amazonian lowland forest from about 200 to 1400 m.

Eleutherodactylus ockendeni (Boulenger, 1912)

PANDO: Province Manuripi: Florida, Reserva Nacional de Vida Silvestre Amazónica Manuripi (Aprox. 12º10'S/68º34'W), MNKA 5111 (adult male, SVL: 17.94 mm), collected on 17 July 2001 by L. Gonzáles, M. Guerrero and G. Calderón,

This is the first country record and southernmost record for this species. *Eleutherodactylus ockendeni* is a lowland species occurring from Colombia to Peru, in the Upper Amazon Basin (Lynch, 1980). It has been found at Tambopata (Doan & Arizábal, 2002) and at Cuzco Amazónico (Duellman & Salas, 1991), both near the Bolivian border. Its presence in Bolivia was previously suspected (De la Riva *et al.*, 2000). In Bolivia, a single specimen was found in the Amazonian lowland forest.

Eleutherodactylus platydactylus (Boulenger, 1903)

BENI: Province Ballivián: Serranía del Pilón, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°16'06"S/ 67°04'17"W), MNKA 4156-7, MNKA 4177, MNKA 4187-4198 (all immature; largest specimen, MNKA 4190, SVL: 18.49 mm), collected on 19 July 1999 by L. Gonzáles.

This is the most common species in the Andean Humid Forest of Bolivia. It has been recorded in the departments of Cochabamba, La Paz, and Santa Cruz (De la Riva *et al.*, 2000). The only previous record from Beni was the holotype of *E. bockermanni* Donoso-Barros (a junior synonym of *E. platydactylus),* purportedly from Rurrenabaque, although this locality is doubtful (see De la Riva, 1987). These 15 specimens from humid montane forest constitute the first confirmed departmental record.

Fig. 3.— *Eleutherodactylus skydmainos* from Río Negro, Pando, Bolivia (LG).

Fig. 3.— *Eleutherodactylus skydmainos* de Río Negro, Pando, Bolivia (LG).

Eleutherodactylus skydmainos Flores & Rodríguez, 1997

PANDO: Province Federico Román: Río Negro (9º50'S/ 65º39'W), MNKA 6911(an adult female, SVL: 31.09 mm), MNKA 6912 (subadult female, SVL: 23.75 mm), collected on 5-8 November 2002 by L. Gonzáles and M. Guerrero (Fig. 3).

This is the first country record of this species and it extends the species' known distributional range 700 km airline northeastward. This species was recently described from specimens found in localities in the upper Amazon Basin of Peru. The nearest records to Bolivia are: Cocha Cashu, Manu National Park and the west bank of Tambopata River, Zona Reservada Tambopata-Candamo (Flores & Rodríguez, 1997). The presence of this species in Bolivia was previously suspected by De la Riva *et al.* (2000). The specimens were collected in the Amazonian lowland forest.

Eleutherodactylus toftae Duellman, 1978

PANDO: Province Manuripi: Campamento Malecón, Reserva Nacional de Vida Silvestre Amazónica Manuripi (11º56'S/ 68º49'W), MNKA 6041, MNKA 6068 (adult females, SVL: 25.32 mm, 24.97 mm respectively), collected on 23-26 January 2002 by L. Gonzáles & J. Condori; Nueva América, Reserva Nacional de Vida Silvestre Amazónica Manuripi, MNKA 6082 (adult female, SVL: 24.1 9mm), collected on 29 January 2002 by L. Gonzáles & J. Condori.
LA PAZ: Province Iturralde: Pampas del Heath, Madidi National Park (13º01'S/68º50'W), CBF 4485 (adult female, SVL:24.22), collected on 20 April 1997 by F. Guerra, E. Pérez and J. Pérez; Serranía de Tequeje (approx. 13º50'S/68º05'W), CBF 5098-5099 (gravid females, SVL: 24.49 mm and 24.97 mm respectively), CBF 5100-5102 (three juveniles), CBF 5103 (adult male, SVL: 16.23).

Table 1.— Species of *Eleutherodactylus* recorded in Bolivia with their distribution by Departments. LP: La Paz; CO: Cochabamba; SC: Santa Cruz; BE: Beni; PA: Pando; CH: Chuquisaca; TA: Tarija.

Tabla 1.— Especies de *Eleutherodactylus* registradas en Bolivia, con su dstribución por departamentos. LP: La Paz; CO: Cochabamba; SC: Santa Cruz; BE: Beni; PA: Pando; CH: Chuquisaca; TA: Tarija.

Species of *Eleutherodactylus*	**Departments**						
	LP	**CO**	**SC**	**BE**	**PA**	**CH**	**TA**
E. ashkapara	-	X	-	-	-	-	-
E. altamazonicus	X	-	-	-	X	-	-
E. bisignatus	X	-	-	-	-	-	-
E. carvalhoi	-	X	-	-	-	-	-
E. cruralis	X	X	X	X	X	-	-
E. danae	X	X	-	X	-	-	-
E. discoidalis	-	X	X	-	-	X	X
E. cf. *dundeei*	-	-	X	-	-	-	-
E. fenestratus	X	X	X	X	X	-	-
E. fraudator	-	X	X	-	-	-	-
E. ibischi	-	-	X	-	-	-	-
E. llojsintuta	-	X	X	-	-	-	-
E. mercedesae	X	X	-	-	-	-	-
E. ockendeni	-	-	-	-	X	-	-
E. olivaceus	-	X	X	-	-	-	-
E. cf. *peruvianus*	X	X	X	-	-	-	-
E. platydactylus	X	X	X	X	-	-	-
E. pluvicanorus	-	X	X	-	-	-	-
E. rhabdolaemus	X	X	X	-	-	-	-
E. samaipatae	-	-	X	-	-	-	X
E. skydmainos	-	-	-	-	X	-	-
E. toftae	X	X	-	-	X	-	-
E. ventrimarmoratus	X	-	-	-	X	-	-
E. zimmermanae	-	-	-	-	X	-	-
E. zongoensis	X	-	-	-	-	-	-

These are the first departmental records for *E. toftae* Duellman, 1978 in Pando and La Paz. This species inhabits the peri-Andean forests from southern Peru to central Bolivia (Frost, 2004). In Bolivia, it had only been recorded in Cochabamba (De la Riva et. al., 2000). The specimens reported herein were adult, and they were found in the Amazonian lowland forest.

Eleutherodactylus ventrimarmoratus (Boulenger, 1912)

LA PAZ: Province Larecaja: Serranía de Muchanes, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°08'31"S/67°33'01"W), MNKA 3689 (adult male, SVL: 19.31 mm), collected on 15 September 1998 by L. Gonzáles; Serranía de Chepete, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°02'24"S/67°31'42"W), MNKA 3742 (adult female, SVL: 37.20 mm), collected on 24 September 1998 by L. Gonzáles; Boquerón, Tierra Comunitaria de Origen y Reserva de la Biosfera Pilón Lajas (15°22'37"S, 67°12' 4"W), CBG 775 (adult female, SVL: 40.00 mm), CBG 776 (adult male, SVL: 22.45), CBG 777 (juvenile, SVL: 18.44), collected on 21 September 2002 by R. Aguayo.

This is the first departmental record and the southernmost record for this species. It is known to inhabit the cloud forests in northern Ecuador and the rainforests from northern Ecuador to southern Peru, adjacent to Bolivia, and western Brazil (Frost, 2004). In Bolivia it has been recently cited in Pando, and is suspected to occur in Depts. La Paz and Beni (De la Riva *et al.*, 2000). The specimens were found in humid montane forest.

Eleutherodactylus zimmermanae Heyer & Hardy, 1991

PANDO: Province Manuripi: San Sebastián, Tahuamanu Biological Station (11º24'27"S/69º01'07"W), CBF 2537 (adult female, SVL: 25.68 mm), CBF 2539 (adult male, SVL: 17.40 mm), collected on 16-18 October 1999 by S. Reichle and J. Cadle.

This is the first country record and the southernmost record for the species, which is known to occur in the lower Amazon Basin of Colombia and Brazil (Heyer & Hardy, 1991). The nearest records are from Acre, in Estrada Rio Branco-Abuná and from Santa Cruz da Serra and Alto Paraiso, Rondônia (Heyer & Hardy, 1991). The specimens were found in the Amazonian lowland forest.

Discussion

Our knowledge of the diversity of Bolivian *Eleutherodactylus* has changed dramatically since De la Riva's (1990) preliminary list of seven species was published (six recognized species and one of doubtful taxonomic status). Subsequently, in a synopsis of the genus in Bolivia, De la Riva (1993) recognized eight species and considered two doubtful: *E. andicola* (Boettger, 1891) and *E. bockermanni* Donoso-Barros, 1970. The status of the first of these species continues to be uncertain and, most likely, it is not an *Eleutherodactylus* (see Lynch & McDiarmid, 1987). The second was synonymized with *E. platydactylus* by De la Riva (1997). Subsequently, with further fieldwork conducted in poorly known areas, more species were added to the list, and 17 species were formally recognized by De la Riva *et al.* (2000). The same year, Köhler (2000) recognized 19 species for the country. He added a new country record (*E. dundeei* Heyer and Muñoz, 1999), resurrected a synonym [*E. bisignatus* (Werner, 1899)], pointed out a new species under description (later described as *E. ibischi* by Reichle *et al.*, 2001), and deleted one from the list [*E. peruvianus* (Melin, 1941)]. These criteria were followed by Reichle (2003). In summary, with the species herein reported, the current number of *Eleutherodactylus* in Bolivia stands at 25 species (see Table 1). Nevertheless, the taxonomic status of some species remains uncertain, several new species are being described and many others are still expected to be found (De la Riva *et al.*, 2000). Therefore, the actual number of species is still far from being known.

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Appendix.— Other specimens examined.

Apéndice.— Otros especímenes examinados.

Eleutherodactylus acuminatus. PERU: Department Amazonas: Pagua, Caroill, Cordillera del Cóndor (MHNSM 582).

Eleutherodactylus altamazonicus. PERU: Department Madre de Dios: Cocha Cashu, Manu National Park (MHNSM 9143-9147); Tambopata (MHNSM 7352).

Eleutherodactylus carvalhoi. PERU: Department Loreto: Estirón, Río Ampiyacu (MZUSP 23090-23091); Madre de Dios: Cocha Cashu, Manu National Park (MHNSM 2077, MHNSM 3864, MHNSM 9202, MHNSM 10073-5).

Eleutherodactylus danae. PERU: Department Cuzco: 2.5 Km WSW Santa Isabel (MHNSM 13957, paratype).

Eleutherodactylus lacrimosus. ECUADOR: Department Pastaza: Puyo (MZUSP 54621-54624). PERU: Department Pasco: Oxapampa, Castilla Forest (MHNSM 11184-5); Department Ucayali: Parque Humboldt (MZUSP 11274).

Eleutherodactylus ockendeni. PERU: Department. Madre de Dios: Cocha Cashu, Manu National Park (MHNSM 3863, MHNSM 9064, MHNSM 9269-9272, MHNSM 15507); La Colpa-Tambopata (MHNSM 14009). BRAZIL: Department Amazonas: Reserva Ducke (MZUSP 58669).

Eleutherodactylus olivaceus. BOLIVIA: Department Cochabamba: road from Paracti to El Palmar (CBF 3329, holotype).

Eleutherodactylus skydmainos. PERU: Department Madre de Dios: Cocha Cashu, Manu National Park. (MHNSM 1930-1932, MHNSM 3850-3859, MHNSM 9090-9091, MHNSM 9095-9101, paratypes).

Eleutherodactylus zimmermanae. BRAZIL: Department Amazonas: Reserva INPA-WWF (MZUSP 64539-64541, type specimens).

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Rediscovery, Redescription, and Advertisement Call of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937) (Anura: Leptodactylidae), and Notes on Other *Eleutherodactylus*

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ABSTRACT.—***Eleutherodactylus heterodactylus* was rediscovered in Cerrado montane forest of eastern Bolivia, 250–300 km airline from its type locality in Brazil, in similar habitat. The advertisement call is described for the first time. This species shares morphological features with species of the *Eleutherodactylus binotatus* and *Eleutherodactylus discoidalis* groups but is not assigned to either group pending further study. We confirm that *Eleutherodactylus crepitans* and *Eleutherodactylus dundeei* are valid species easily distinguishable from *Eleutherodactylus fenestratus*. Bolivian populations of the Andes previously assigned to *E. dundeei* correspond to an undescribed species allied to *Eleutherodactylus peruvianus*.**

Although a great part of the nearly 700 recognized species of *Eleutherodactylus* inhabit humid mountain forest of the Andes, the lowland Amazonian forest, the Mata Atlantica and the Caribbean islands, some of them also occur in the South American open formation of the Cerrado (Frost, 1985; D. R. Frost, Amphibian species of the world 3.0, http://research.amnh.org/herpetology/amphibia/index.php, 2004; AmphibiaWeb, http://elib.cs.berkeley.edu/aw/, 2004). Among these Cerrado species is *Eleutherodactylus heterodactylus* (Miranda-Ribeiro), a form hitherto known only from the original description, based on two specimens collected in a sandstone cave near the town of Cáceres (Matto Grosso, Brazil) (A. Miranda-Ribeiro, 1937; Heyer and Muñoz, 1999). A. Miranda-Ribeiro (1937) created the genus *Teletrema* to accommodate the species, which he called *Teletrema heterodactylum*. P. Miranda-Ribeiro (1955) designated as lectotype MN106A. Myers (1962) put *Teletrema* under the synonymy of *Eleutherodactylus* and provided some morphological notes on the two syntypes. Heyer and Muñoz (1999) studied the types and wrote: "Both types are faded such that most features of any color patterns are no longer discernible. The lectotype is in poor condition, the paralectotype is in worse condition. The paralectotype is very brittle and fragile and disintegrates more each time it is handled. The lectotype is the (noticeably) larger of the two . . . ." Based on the original description and illustrations of Miranda-Ribeiro (1937), and on the examination of the specimens, Heyer and Muñoz (1999) compared *E. heterodactylus* with other *Eleutherodactylus* inhabiting Mato Grosso: *Eleutherodactylus fenestratus* (Steindachner, 1864), *Eleutherodactylus dundeei* Heyer and Muñoz 1999, and *Eleutherodactylus crepitans* Bokermann, 1965. They recognized the species status of *E. heterodactylus* and described the morphological differences with the other species. Lynch and Duellman (1997) included this species in the *Eleutherodactylus binotatus* species group, from the Atlantic Forest.

During the revision of museum specimens from Museo de Historia Natural Noel Kempff Mercado (Santa Cruz de la Sierra, Bolivia), we surprisingly found some distinctive, unidentified specimens of *Eleutherodactylus* collected in the

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semideciduous forest of the Cerrado mountain area of the Serranía de Santiago, in Eastern Bolivia. These specimens did not belong to any of the known species of *Eleutherodactylus* in Bolivia listed by De la Riva et al. (2000). This finding encouraged us to organize an expedition to the Serranía de Santiago, and we found *Eleutherodactylus* calling at night in the forest of the hills and near a sandstone cave where our colleages D. Embert and S. Reichle had mentioned the occurrence of these frogs previously. Comparisons of the specimens obtained with species from Bolivia and from the Cerrado area of Brazil led us to conclude they were *E. heterodactylus*. This is the first record after its original description (Heyer and Muñoz 1999; D. R. Frost, Amphibian species of the world 3.0, http://research.amnh.org/herpetology/amphibia/index.php, 2004) and the first time it is reported for Bolivia, 250–300 km airline distance from the type locality.

Because of the bad condition of the type specimens (Heyer and Muñoz, 1999) and the brief original description, we redescribe *E. heterodactylus* based on our new material. We also describe its advertisement call, provide some data on its biology and ecology, and discuss on its taxonomic affinities. In addition, we include some taxonomic notes on other species from the Cerrado, namely *E. crepitans* (Bokermann, 1965) and *E. dundeei* Heyer and Muñoz, 1999.

MATERIALS AND METHODS

Specimens were fixed in 10% formalin and preserved in 70% ethanol. For morphological and color characteristics, we follow Lynch and Duellman (1997). Measurements were taken with a digital caliper to the nearest 0.01 mm, but following Hayek et al. (2001), we rounded all measurements to one decimal point. Measurements of the lectotype of *E. heterodactylus* are taken from Heyer and Muñoz (1999). Abbreviations are as follows: snout-vent length, SVL; head length (from rictus to tip of snout), HL; head width (measured at level of rictus), HW; interorbital distance, IOD; eye length, EL; upper eyelid width, EW; eye to nostril distance, EN; eye to eye distance (distance between the anterior margins of eyes), EE; tympanic membrane height, TYH; tympanic membrane length, TYL; width of the terminal disk of third finger, FIII; width of the terminal disk of fourth finger, FIV; thigh length, THIGH; tibia length, TL; foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL; width of the terminal disk of fourth toe, TIV; and forearm length (from elbow to the proximal margin of thenar tubercle), ARM. Color characteristics were taken in life. Recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone. Recordings were processed on an Apple Macintosh computer. The sounds were digitized and edited at a sampling frequency of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board and Peak 3.2 (OSX) software. Raven 1.1 (Cornell University, Ithaca, New York) software was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through fast Fourier transform (FFT; width, 512 points). Digitized calls were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (Madrid, Spain; track number 2634). Voucher specimens and material studied correspond to the herpetological collections of: Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA, formely MNK and NKA); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Peru (MHNSM); Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Museo de Zoologia da Universidade de São Paulo, Brazil (MZUSP); Museu Nacional, Rio de Janeiro, Brazil (MN); Naturhistoriska Museet, Göteborg, Sweden (NHMG); and Natural History Museum, the University of Kansas, Lawrence (KU).

SYSTEMATICS

Redescription of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937)

Teletrema heterodactylum Miranda-Ribeiro, 1937; syntypes: MN 106 (2 specimens); type locality: "gruta dita Facendinha," Matto-Grosso, Cáceres, Brazil.

Teletrema heterodactylum: Miranda-Ribeiro, 1955; MN 106A, designated lectotype.

Eleutherodactylus heterodactylus: Myers, 1962.

Eleutherodactylus (*Eleutherodactylus*) *heterodactylus*: Lynch and Duellman, 1997.

Eleutherodactylus heterodactylus: Heyer and Muñoz, 1999.

Diagnosis.—A medium-sized species of *Eleutherodactylus* (Fig. 1) characterized by (1) skin on dorsum smooth, venter smooth, posterior surfaces of limbs smooth, groin granular, discoidal fold conspicuous, almost reaching the groin, no dorsolateral folds, postrictal glands well developed; (2) tympanic membrane and annulus distinct, its length about two-thirds of eye length, supratympanic fold well developed; (3) head longer than wide, snout slightly pointed in dorsal view, round in lateral profile, canthus rostralis sharp, slightly concave; (4) cranial crests absent, upper eyelid tubercles absent; (5) vomerine odontophores medial to choanae; (6) males with vocal slits and a single faint nuptial pad on thumb; (7) first finger longer than second,

FIG. 1. *Eleutherodactylus heterodactylus* MNK A7177, female (SVL, 30.79 mm) from Serranía de Santiago, Santa Cruz, Bolivia.

subarticular tubercles enlarged and subconical, supernumerary tubercles small, rounded, terminal discs of Fingers I and II rounded, not enlarged, those of Fingers III and IV markedly enlarged, ovate (Fig. 2); (8) lateral fringes and keels on fingers absent; (9) two to four ulnar tubercles small, white; (10) no tubercles on heel, small, slightly elongate proximal tubercle on tarsus; (11) inner metatarsal tubercle small, high, ovoid, outer more rounded, subequal; (12) toes lacking lateral fringes or keels, webbing absent, fifth toe shorter than third, discs of toes slightly enlarged, rounded (Fig 2); (13) dorsal coloration light reddish-brown with dark brown to black spots and marks, outlined by cream; in preservative, dorsal regions brownish-gray, snout dark gray with darker marks, throat cream with dense and fine brown mottling, venter immaculate.

Eleutherodactylus heterodactylus can be distinguished from the other species of the open formations in Mato Grosso (*E. crepitans* and *E. dundeei*) by the following combination of characters: discs of Fingers III and IV greatly enlarged, ovate, much larger than any other finger or toe disc, approximately one-half to two-thirds the size of tympanum; vomerine odontophores situated between choanae; skin of venter smooth; first finger longer than second; and absence of well-developed toe fringes and tarsal fold. The two other species mentioned have triangular, moderately enlarged discs on Fingers III and IV, and a well-developed tarsal fold (small like a tubercle and almost indistinct in *E. heterodactylus*). Additionally, *E. crepitans* and *E. fenestratus* are larger than *E. heterodactylus*; *E. fenestratus* and *E. heterodactylus* demonstrate marked sexual size dimorphism, whereas *E. crepitans* does not. *Eleutherodactylus heterodactylus* resembles in general appearance the Andean *Eleutherodactylus discoidalis* and both share an uncommon character: the vomerine odontophores are situated between the choanae; however, *E. heterodactylus* has ovate and much more enlarged discs on Fingers III and IV (moderately enlarged and truncate in *E. discoidalis*).

Description.—Head longer than wide, slightly pointed in dorsal view and rounded in lateral profile; nostrils slightly protuberant, oriented posterolaterally; canthus rostralis distinct, slightly concave; loreal region flat; upper eyelid without tubercles; no cranial crests. Supratympanic fold distinct; tympanic membrane and tympanic annulus distinct; tympanic membrane nearly round, its length about two-thirds of eye length; one to two postrictal glands. Choanae rounded, very small, anterolateral, not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; vomerine odontophores medial to choanae, very small and almost in contact. Skin of dorsal surfaces and posterior parts of hind limbs smooth, with scarce granules and/or small flat warts only on the supratympanic fold and anterior margin of the flanks; ventral skin smooth; no dorsolateral folds; ventral discoidal fold distinct, almost reaching the groin.

Two to four small ulnar tubercles; two palmar tubercles, outer rounded, almost of the same size as inner; supernumerary tubercles small, rounded, smaller than subarticular tubercles; subarticular tubercles large, subconical; discs of Fingers I and II small, rounded, those of Fingers III and IV greatly enlarged, ovate; fingers lacking lateral fringes and keels; single white nuptial pad on thumb; relative length of fingers from shortest to longest, II < IV < I < III.

Heels lacking tubercles or folds; tarsus with a proximal small, inconspicuous tubercle-like fold; inner metatarsal tubercle ovoid, longer than wide, almost the same size as conical outer; subarticular tubercles large, conical, directed forward; supernumerary tubercles small, rounded; toes lacking lateral fringes and keels; discs of toes rounded, slightly enlarged; relative length of toes, from shortest to longest, I < II < V < III < IV.

In life, dorsum light reddish-brown, with many dark brown marks (triangular interorbital, arrowlike scapular, X-like middorsal) outlined with cream; dorsal surfaces of extremities with dark brown to black bars; flanks with dark brown spots and an irregular oblique band in the anterior part; loreal region and lips cream with dark bars; canthus rostralis dark brown to black; supratympanic fold dark brown to black; tympanic membrane brown, annulus cream; iris metallic yellow. Ventral surfaces white with fine brown mottling on throat; inner surfaces of hind limbs fleshy brown. The color in preservative is similar but more brownish-gray.

Variation.—There is little variation in morphological traits and color among specimens (Table 1). The single female is larger than the males. All

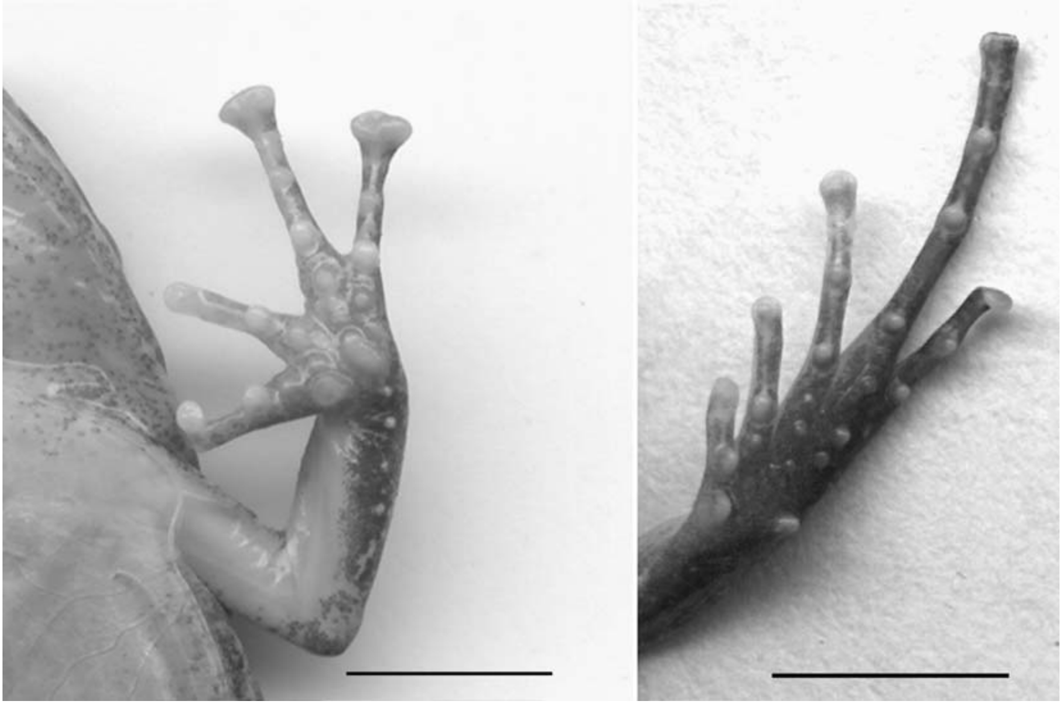

FIG. 2. Hand and foot of *Eleutherodactylus heterodactylus* MNK A7177; scale bars = 5 mm.

males have a single nuptial pad on the thumb and vocal slits. Toe fringes can be incipient (as in MNKA 6482). The contrast of the dorsal pattern varies from intense (MNKA 7175) to moderate (MNKA 7176), with more cream tonalities. A pair of occipital black spots or a "W" can be present.

Distribution and Ecology.—This species inhabits the semideciduous Cerrado forest of the mountainous regions of western Brazil and eastern Bolivia. It is only known from tree localities (Fig. 3): Fazendinha, near Pirizal, Cáceres, Mato Grosso, Brazil (type locality); Bella Boca, Province Angel Sandoval, Department Santa Cruz, Bolivia; and Cerro del Arco, Province Chiquitos, Department Santa Cruz, Bolivia. Bella Boca and Cerro del Arco are, respectively, about 250 km and 300 km airline from the type locality. Males were heard calling at night in the forest, from low

TABLE 1. Measurements (in millimeters) of the lectotype and new material of *Eleutherodactylus heterodactylus*.

	MN 5089	MNKA 6482	MNKA 6357	MNKA 6356	MNKA 7175	MNKA 7176	MNKA 7177	MNCN 495	MNCN 496
SVL	24.6	28.3	26.0	25.3	26.3	26.9	30.8	26.6	26.2
HL	8.6	10.6	11.4	11.0	10.4	10.2	11.1	10.1	9.9
HW	8.5	9.6	9.3	7.9	9.9	9.1	10.7	10.2	9.6
IOD	—	3.1	2.5	2.5	2.3	2.4	2.7	2.2	2.1
EL	—	3.3	3.4	3.5	3.9	3.5	4.2	3.5	3.3
EW	—	2.3	2.4	2.5	2.6	2.3	3.0	2.6	2.4
EN	3.5	3.1	3.2	3.5	3.1	3.0	3.7	3.3	3.4
EE	5.1	—	4.9	5.3	4.7	4.6	5.0	4.4	4.8
TYH	—	2.4	2.2	2.2	2.2	2.3	2.6	2.2	2.4
TYL	2.4	2.4	2.2	2.2	2.0	2.3	2.3	2.2	2.1
FIII	1.4	1.1	1.3	1.2	1.4	1.2	1.7	1.4	1.3
FIV	—	—	1.3	1.3	1.3	1.1	1.6	1.3	1.4
THIGH	14.1	—	9.2	10.3	13.1	11.2	13.3	12.8	12.8
TL	—	14.4	13.5	13.1	13.1	13.3	13.6	13.1	13.1
FL	12.0	12.1	12.2	11.9	11.3	12.2	12.5	12.2	11.7
TIV	0.7	—	1.0	0.8	1.1	1.0	1.0	1.0	0.9
ARM	—	—	6.1	6.0	5.8	5.9	5.9	5.5	5.6

FIG. 3. Type localities of *Eleutherodactylus crepitans* (triangle) and *Eleutherodactylus heterodactylus* (square) in Brazil, and new localities of *E. heterodactylus* in Bolivian Department of Santa Cruz (dots).

vegetation or from rocks or trunks, during a dry night of the rainy season. Apparently, in this area, during the dry season, specimens can be found in sandstone caves (D. Embert and S. Reichle, pers. comm.).

The female MNKA 7177 contained seven light-orange eggs in the left oviduct and five in the right; the mean size of the eggs was 2.9 mm (range: 2.5–3.6).

Advertisement Call.—The call of *E. heterodactylus* was recorded at Serranía de Santiago, Cerro del Arco, on 4 December 2003, 2330 h; the air temperature was 23°C. Forty-three calls from four specimens were analyzed. The call is a single pulsed note averaging 176 msec (range: 126–245), 6.8 pulses (5–9), and 38.8 pulses/sec (36.5–48.6). The call has 3–5 harmonics, but the fourth and fifth harmonics are not evident in all analyses; the call repetition rate is 26.9 calls/min (24.9–29.33); the dominant frequency is 3946.9 Hz (2876–4134) and usually corresponds to the second harmonic, but exceptionally is the first harmonic, 2043.2 Hz (1981—2153; Fig. 4).

DISCUSSION

The Bolivian populations of *E. heterodactylus* are separated from the type locality by the wetlands of the Pantanal. Nevertheless, all these populations might be connected via the mountainous arc formed to the north by the low Serranías Chiquitanas, all belonging to the Precambrian shield.

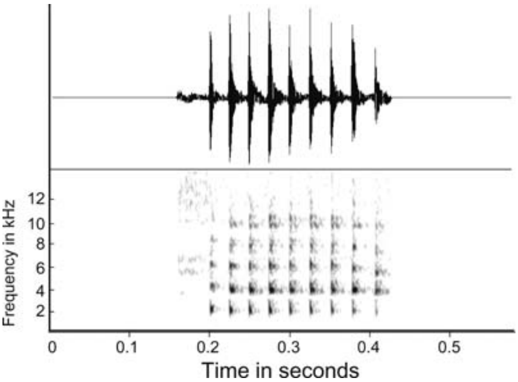

FIG. 4. Oscillogram and spectrogram of the call of *Eleutherodactylus heterodactylus* recorded at Serranía de Santiago, Santa Cruz, Bolivia; air temperature, 23°C.

Despite the simplicity of the original description of *E. heterodactylus* (Miranda Ribeiro, 1937), the drawings that accompanied it are good enough as to clearly identify the species. The characteristics of the specimens collected by us in Bolivia match well both the description and the illustrations and also are consistent with the data provided by Myers (1962) and Heyer and Muñoz (1999). The great development of the discs of Fingers III and IV, large hands, dorsal pattern, and general morphology, make it easy to distinguish *E. heterodactylus* from *E. fenestratus* or *E. dundeei*, both belonging to the *Eleutherodactylus conspicillatus* species group. Although the original description of *E. crepitans* describes the specimens from Bolivia reasonably well, examination of the type specimens of *E. crepitans* indicated that the Bolivian material did not belong to this species and that the coincidence was caused by the relative simplicity of the description. There is a possibility that the Bolivian specimens belong to an undescribed species similar to *E. heterodactylus*. The bad preservation of the types (Heyer and Muñoz, 1999), and the absence of new specimens collected at the type locality render this possibility difficult to explore. Nevertheless, considering that the Cerrado is an area of low diversity of *Eleutherodactylus* and that *E. heterodactylus* is a quite distinctive species, we consider Bolivian and Brazilian populations as conspecific; this assumption should be corroborated by collecting of new specimens at the type locality and comparisons of them with Bolivian material.

The assignment of *E. heterodactylus* to any of the species groups of *Eleutherodactylus* currently recognized is not possible with the information at hand. Lynch and Myers (1983) placed this species within the *Eleutherodactylus fitzingeri* group, and Lynch and Duellman (1997) included it in the

Eleutherodactylus binotatus species group, from the Atlantic Forest, something that Heyer and Muñoz (1999) considered lacking zoogeographic sense. We found that this species shares morphological features with species of the *E. binotatus* (Duellman and Lynch, 1997) and *E. discoidalis* (sensu Lynch, 1989) groups. However, these groups are defined mainly on the basis of plesiomorphic characters, and we prefer not to include *E. heterodactylus* in any group until the intrageneric phylogenetic relationships are better understood.

Besides *E. heterodactylus*, *E. crepitans* and *E. dundeei* also inhabit the Cerrado mountain areas of Mato Grosso. *Eleutherodactylus crepitans* is known only from the type locality ("São Vicente, Cuiabá, Mato Grosso, Brasil"). Heyer and Muñoz (1999) removed this species from the synonymy of *E. fenestratus*, where it had been placed by Lynch (1980). Lynch and Duellman (1997) did not assign it to any species group. The holotype and paratypes were collected in a dry area covered by grass-scrub vegetation in the middle of large blocks of granite (Bokermann, 1965). *Eleutherodactylus dundeei* belongs to the *Eleutherodactylus conspicillatus* species group. It was described from gallery forest of the Cerrado region of Chapada dos Guimaraes (Heyer and Muñoz, 1999).

We examined the type specimens of *E. dundeei* and *E. crepitans* and agree with Heyer and Muñoz (1999) that these are valid species well distinguishable from *E. fenestratus*. Köhler (2000) examined paratype specimens of *E. dundeei* and compared a single call from the type locality with calls of certain Bolivian specimens of *Eleutherodactylus*. Based on this, he reported *E. dundeei* for the humid Andean Amazonian slopes of Departmento de Santa Cruz, Bolivia, approximately 850 km airline from the type locality and in a completely different kind of habitat. Köhler (2000) failed to observe that those Bolivian specimens lack basal webbing on toes, possessed by all type specimens of *E. dundeei*. Although preliminary comparisons indicated that calls were similar between both populations (Köhler, 2000), a more comprehensive analysis is required (Reichle provided recordings of these frogs from Paractito, Departamento Cochabamba [2002]). Convergences in general morphology and advertisement calls are common in anurans including many *Eleutherodactylus* species, and this could explain the superficial similarity of *E. dundeei* and Andean populations assigned to this species. Furthermore, occurrence of *E. dundeei* in humid rain forest at the foot of the Andes is biogeographically discordant. No other anuran species has a disjunct distribution in the open formations of the Cerrado and in the humid rain forests at the foot of the Andes; the anuran fauna of the intervening areas (wet savannas and semihumid transitional rain forests in Beni and Santa Cruz Departments) is fairly well known, and no species of *Eleutherodactylus* occurs there (De la Riva, 1993a; De la Riva et al., 2000). Bolivian populations of *Eleutherodactylus* treated as *E. dundeei* by Köhler (2000) had been traditionally considered to be *E. fenestratus* (see De la Riva, 1993b); later, they were identified as *E. peruvianus* by De la Riva (1994), based on the examination of large Peruvian samples identified as *E. peruvianus*, from seven localities in central and southern Peru (see Appendix 1). Fifteen of 78 Peruvian specimens (19.2%) from different localities had morphology and color patterns equal to those of Bolivian specimens. It seems that populations from northern Peru and Ecuador consistently show a well-marked dorsal pattern, presence of orange spots on the posterior surface of thighs and dorsolateral folds (Lynch and Duellman, 1980; Rodríguez and Duellman, 1994; Duellman and Pramuk, 1999), whereas populations from southern Peru and Bolivia often have a less marked pattern and lack orange spots and dorsolateral folds (De la Riva, 1994; De la Riva et al., 2000). Thus, these characters are subject to considerable variation in what is currently known as *E. peruvianus*.

Köhler (2000) and Padial et al. (2000) examined the holotype of *E. peruvianus* (NHMG 490, type locality: Roque, Departamento San Martín, Peru; indicated as NHMG type number 0030:1 by Padial et al. [2000]). Köhler (2000) concluded that the absence of dorsolateral folds in Bolivian specimens and differences in finger lengths and coloration clearly indicate that they belong to a different species, which he considered to be *E. dundeei*; consequently, he deleted *E. peruvianus* from the list of anurans of Bolivia, although he did not discard its presence in that country. However, he examined only the holotype but not a large sample of specimens to assess the range of variation of these characters, a range into which Bolivian specimens fall perfectly. We consider plausible that more than one species is involved in what is currently known as *E. peruvianus*. If this is the case, then the Bolivian populations might represent an undescribed species related to *E. peruvianus*; this, however, would not exclude the possibility that "true" *E. peruvianus* also occur in the country, as suggested by Köhler (2000). Until a taxonomic study is done to confirm or discard the presence of more than one species, the mentioned populations from Bolivia and central and southern Peru should be referred to as *E. peruvianus* or *E.* cf. *peruvianus*; at the same time, *E. dundeei* should be deleted from the list of amphibians of Bolivia, although its presence is still plausible in the Cerrado formations of northeastern Santa Cruz (De la Riva et al., 2000).

A species similar to *E. peruvianus* s. l. is *Eleutherodactylus danae* Duellman, from which *E. peruvianus* was purported to differ from by having the skin of the venter smooth and the first finger longer than the second (Duellman, 1978). Comparisons of the holotype of *E. danae* (KU 162307) with the previously mentioned large samples identified as *E. peruvianus* render the diagnosis of *E. danae* quite inconsistent (IDLR, pers. obs.). This fact is probably due to the existence in these samples of the putative new species mentioned above, which would be less different from *E. danae* than the true *E. peruvianus*. *Eleutherodactylus danae* is indeed a valid species with a distinctive advertisment call (Köhler and Jungfer, 1995), and it inhabits the Andean cloud forests of Peru and Bolivia; *E. danae* and *E. peruvianus* might be sympatric at intermediate elevations.

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APPENDIX 1

Specimens Examined

Eleutherodactylus crepitans: BRAZIL: Mato Grosso, São Vicente, MZUSP 85628 (holotype), MZUSP 73671 (allotype, same data as holotype).

Eleutherodactylus danae: PERU: Dept. Cusco, Río Cosñipata, 4 km (by road) southwest of Santa Isabel, KU 162307 (holotype).

Eleutherodactylus dundeei: BRAZIL: Mato Grosso, Rio Coxipozinho, Veu da Noiva, Chapada dos Guimarães, MZUSP 79834–7 (type series).

Eleutherodactylus heterodactylus: BOLIVIA: Dept. Santa Cruz, Prov. Angel Sandoval, Bella Boca (17°50′S, 59°00′W), MNKA 6482. Dept. Santa Cruz, Prov. Chiquitos, Serranía de Santiago, Cerro del Arco (18°20′50.7″S, 59°53′37.06″W), MNKA 6356–7, 7175–7, MNCN 42014–5.

Eleutherodactylus cf. *peruvianus*: BOLIVIA: Dept. Santa Cruz, Prov. Andrés Ibáñez, Espejillos (17°50′S, 63°25′W), MNKA 7172–4, MNCN 42017–8. Dept. Santa Cruz, Prov. Ichilo, La Chonta (17°39′36″S, 63°42′06.6″W), MNCN 42016. PERU: Dept. Cusco, approximately 40 km east Quincemil on Puerto Maldonado road above Marcapata, KU 196475. Dept. Huánuco, Casa Campa, southern slope of Serranía de Sira, KU 154848–52. Dept. Huánuco, Laguna, 1280 m, southern slope of Serranía de Sira, KU 154863–65. Dept. Huánuco, Río Llullapichis, 4–5 km upstream from Río Pachitea, KU 154835–47, 154858–62, 171867–91. Dept. Madre de Dios, Cocha Cashu, P. N. Manu, KU 154856–57. Dept. Madre de Dios, Cuzco Amazónico, KU 194909, 205107, 205132–5, 205137–8, 205142, 207715–7, 215481–8. Dept. Madre de Dios, Manu, 365 m, KU 154853–5.

Eleutherodactylus peruvianus: PERU: Dept. San Martín, Roque, NHMG 490 (holotype).

APÉNDICE III

The taxonomic status of *Eleutherodactylus skydmainos* Flores & Rodríguez, 1997 and *E. karcharias* Flores & Rodríguez, 1997 (Anura: Leptodactylidae)

José M. Padial, Ignacio De la Riva

Eleutherodactylus skydmainos and *Eleutherodactylus karcharias* were both described by Flores and Rodríguez (1997) and no information on these species has been published since then. *Eleutherodactylus skydmainos* was described from Amazonian Lowland Forest and Andean slopes in Southern and Central Peru, while *E. karcharias* was described from Andean slopes in northern Peru. Both species were included in the *E. conspicillatus* group *sensu* Lynch (1976). Although Flores and Rodríguez (1997) compared separately the two new species with other members of the *E. conspicillatus* group, they failed to compare the two new species to each other. We studied the following well preserved specimens of the type series of *E. skydmainos*: MCZ 88304 (holotype), MCZ 88305-9 (paratopotypes), USNM 298914-5 (paratypes), USNM 324334-6 (paratypes), MHNSM (Museo de Historia Natural, Universidad Mayor de San Marcos, Lima, Peru) 9081, 9091, 9090 (paratopotypes). We also studied the topotype series of *E. karcharias*: MCZ 89074 (subadult female), 89076-80 (juvenile females); the holotype, MCZ 89075, a subadult female, is destroyed and no longer recognizable. We follow Lynch and Duellman (1997) for the description of characters. Based on our observations, the high coincidence in the diagnostic characters of *E. skydmainos* and *E. karcharias* does not support species separation. Following the International Code of Zoological Nomenclature (ICZN, 1999) the name of the species first described in the paper (*E. skydmainos*) has priority.

In the original description, Flores and Rodríguez (1997) did not explicitly compare *E. skydmainos* and *E. karcharias*. Therefore, we summarise the main differences found in the text of both descriptions and compare them with our data (table 1). As can be seen in table 1, there are no relevant characters that can unequivocally distinguish both putative species. Most of the differences are due to errors of appreciation and/or also to the partial desiccation of the types of *E. karcharias*.

In the original diagnosis, *E. skydmainos* was compared with three other members of the *E. conspicillatus* group: *Eleutherodactylus fenestratus* (Steindachner, 1864), *E. conspicillatus* (Günther, 1858), and *E. peruvianus* (Melin, 1941), and the authors stated that *E. skydmainos* was distinguished by a combination of the following characters: “small size, prominent interocular fold, finely shagreened dorsal skin with dorsolateral ridges, very large tympanum, lateral keels on the fingers, short tarsal fold, basal toe webbing, dark spots on the anterior corner of the eye, middorsum, forearm, knee, inner shank, and heel, brown posterior thigh surfaces with minute pale flecking, and immaculate belly”. As noted by Flores and Rodríquez (1997), none of

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Table 1. Main characters used in the original descriptions of *Eleutherodactylus skydmainos* Flores and Rodríguez, 1997 and *Eleutherodactylus karcharias* Flores and Rodríguez, 1997 compared with our observations of the type series of these species. HL = head length; HW = head width.

Characters	*E. skydmainos*	*E. karcharias*	Observations
1. HL to HW	Equal	Longer	Longer in both
2. Interocular fold	Present	Absent	At least incipient in both
3. Dorsal skin	Finely shagreened	Coarsely shagreened	Shagreened, variable intensity in both
4. Finlike tubercle	Absent	Present	Present in both
5. Dorsal ridges	Two	Several	Two in both
6. Arm granules	Absent	Present	Present in both
7. Discoidal fold	Present	Absent	Present in both
8. Belly texture	Smooth	Areolate	Slightly granular in both
9. Supernumerary tubercles on hands	One/finger	Numerous	One in each finger, rarely two
10. Tarsal fold	Present	Absent	Present in both
11. Finger fringes	Present	Broad	Incipient in both
12. Finger 1 vs. 2	Longer	Equal	Longer in both
13. Throat	Mottled	Heavily mottled	Variable
14. Cranial crests	Absent	Low	Absent in both

these three species have a prominent interocular fold. Nevertheless, the interocular fold is not so prominent in all types and it can even be poorly elevated or inconspicuous. Moreover, at least in some specimens of *E. fenestratus*, an incipient interocular fold can be observed, but this species is larger in size, lacks dorsal ridges, and the posterior surfaces of the thighs are brown. The distinction of *E. skydmainos* from the two other species mentioned is more problematic. *Eleutherodactylus conspicillatus* and *E. peruvianus* have dorsolateral ridges and pale flecks on the posterior surfaces of the thighs (although even with the diagnostic differences between the two species stated by Lynch (1980), and the redescription of *E. peruvianus* by Lynch and Duellman (1980), the separation of both is still very difficult). Flores and Rodríguez (1997) based the separation between *E. skydmainos* and *E. conspicillatus*-*E. peruvianus* on the presence of a tarsal fold in the new species, differences in thigh flecking, presence *vs.* absence of interocular fold, and differences in ventral pattern (pigmented in *E. peruvianus* and immaculate in *E. skydmainos*). The venter is usually pigmented in populations assigned to *E. peruvianus* and sometimes also in *E. conspicillatus*, but in some populations either of them can show almost immaculate venter; the thigh pattern and the presence of tarsal fold are also highly variable characters. The interocular fold, and two other characteristics present in the type specimens (but overlooked by Flores and Rodríguez), can help in assessing the species status and distinction of *E. skydmainos*: the granular texture of posterior and lateral margins of the belly and a “fin-like” middorsal tubercle or bold black spot (fig. 1). The posterior and lateral margins of the belly and dorsum are shagreened in *E. skydmainos*. The dorsal skin texture, ridges, tubercles, and interocular fold can be more or less developed depending on the fixation, desiccation, and age.

We examined the types of another species inhabiting Southern Peru, *E. buccinator* Rodríguez, 1994. The holotype (MHNSM 3842) and paratypes (MHNSM 3845, USNM 299779) have a prominent interocular fold (this character was noted as coalescent interorbital granules in the original description), but differ from the aforementioned species in some traits; among them, the most conspicuous are the “X” or inverted “Y” shaped middorsal fold, very prominent dorsal ridges, coarsely shagreened to warty dorsal skin, and very different colour pattern. Recently, Köhler (2000) resurrected *Eleuthero-*

Figure 1. Dorsal view of *Eleutherodactylus skydmainos* (paratype, USNM 324335); below, detail of the fin-like middorsal black tubercle, as seen in lateral view from the right side.

dactylus bisignatus (Werner, 1899) from the synonymy of *E. fenestratus* and placed it in the *E. conspicillatus* group. This species inhabits montane rainforests in adjacent Bolivia, and can be differentiated from *E. skydmainos* at least by the absence of dorsolateral folds and its larger size.

Eleutherodactylus karcharias was described from a series composed of two immature females and five small juvenile females. For the diagnosis, Flores and Rodríguez (1997) wrote, "No other *Eleutherodactylus* has a fin-shaped middorsal tubercle. In addition, no other species in the *E. conspicillatus* group has a combination of a rugose venter, basal toe webbing, and dorsolateral ridges". This distinction no longer supports its species status, since the examination of the type series of *E. skydmainos* shows not only the general characteristics mentioned for *E. karcharias* (including granular belly), but also the fin-like middorsal tubercle, at least in the specimens USNM 298914-15, and 324334-6 (table 1; fig. 1). This tubercle can be substituted by a single bold black spot in other type specimens. Moreover, the interocular fold is at least incipient in the juveniles MCZ 89074 and MCZ 89078 of *E. karcharias*. This character is not well developed in the juveniles of the type series of *E. skydmainos* although it was a highly relevant character in the original description.

Failures in detecting characters as the aforementioned ones can be common when studying frogs of a complex group as the *E. conspicillatus* group, whose species can usually be distinguished only by traits highly influenced by preservation and time since collection. Some researchers familiarised with *Eleutherodactylus* of the *E. unistrigatus* group (sensu Lynch, 1976), whose species have a coarsely granular or areolate belly, tend to consider the belly as smooth when they do not observe a highly granular or areolate ventral surface. But in the species of the *E. conspicillatus* group, belly texture can range from totally smooth (as in most populations of *E. fenestratus*) to partially granular, especially in posterior and lateral margins (as in *E. dundeei* Heyer and Muñoz, 1999). Bad preservation, hyper-hydration, or dehydration can induce superficial changes in the original skin texture (whatever dorsal or ventral); certain features can be lost, mitigated or intensified, and only careful observation (under binocular) can give us accurate information on the original condition.

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APÉNDICE IV

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New Species of *Ischnocnema* (Anura: Leptodactylidae) from the Andes of Bolivia

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Abstract.—**A new species of *Ischnocnema* is described from cloud forests in the Andes of northwestern Bolivia (La Paz Department). The new species can be distinguished from all other *Ischnocnema* except *Ischnocnema sanctaecrucis* by the presence of a pale sacral stripe from the level of the caudal tip of the coccyx to the level of the sacrum, well-developed occipital and sacral folds, rounded narrow terminal discs 1.3 times as wide as the corresponding phalange, and dorsal skin moderately warty. It can be distinguished from *I. sanctaecrucis*, also of the Bolivian cloud forests, by having head longer than wide, well-marked occipital and sacral folds, longer subacuminate to rounded snout, ventral regions mostly cream with little brown mottling, and dorsal region without reddish to scarlet mottling or spots. The new species shares some characteristics with other species of *Ischnocnema* and members of the *Eleutherodactylus discoidalis* group, and it appears closely related to *I. sanctaecrucis*.**

The Neotropical leptodactylid genus *Ischnocnema* consists of five species. Three species (*Ischnocnema simonsi* Lynch, *Ischnocnema saxatalis* Duellman, and *Ischnocnema sanctaecrucis* Harvey and Keck) inhabit Andean cloud forests (Duellman, 1990; Lynch, 1974; Harvey and Keck, 1995). *Ischnocnema quixensis* (Jiménez de la Espada) has a broad distribution in the lowland rainforests of the upper Amazon basin, from southern Colombia to northern Bolivia, and reaches altitudes of 1000 m in the Andean slopes (Lynch and Lescure, 1980; Harvey and Keck, 1995; De la Riva et al., 2000). The fifth species, *Ischnocnema verrucosa* (Reinhardt and Lütken), is endemic to the Atlantic Forest of eastern Brazil (Lynch, 1972). The genus *Ischnocnema* is in the subfamily Eleutherodactylinae, which is speciose in mountain forest regions, and the diversity of *Ischnocnema* seems higher than previously expected in the tropical Andes. Therefore, it is not surprising that additional species are being discovered in poorly surveyed remote areas.

Until now, only two species of *Ischnocnema* were known for Bolivia. The first to be found was *I. sanctaecrucis*, a species endemic to the cloud forest of the Andes in central Bolivia (Harvey and Keck, 1995; Reichle, 1999; Köhler, 2000). Subsequently, specimens of *Ischnocnema quixensis* were collected in the lowland Amazonian forest from the northern part of the country (De la Riva et al., 2000). During fieldwork in 1999 in a mountainous area of the Madidi National Park in northwestern Bolivia, specimens of an unidentified *Ischnocnema* were found. Later, additional specimens were obtained by other field parties at two other localities of montane forest in La Paz Department. The study of their external morphology revealed that they belong to a new species described herein.

Materials and Methods

Specimens were fixed in 10% formalin and preserved in 70% ethanol. For description of measurements, morphology, and color characteristics, we follow Lynch and Duellman (1997) and Harvey and Keck (1995). Specimens of *Ischnocnema quixensis*, the holotype of *I. sanctaecrucis*, and one paratype of *I. saxatalis* were examined; also, specimens of *Eleutherodactylus cruralis*, *Eleutherodactylus discoidalis*, *Eleutherodactylus ibischi*, and *Eleutherodactylus zongoensis* were studied (see Appendix 1). Specimens were measured with a digital caliper to the nearest 0.01 mm; following Hayek et al (2001), we rounded all measurements to one decimal place.

Museum abbreviations refer to Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (NKA, formerly MNK); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Perú (MHNSM); Museo Nacional de Ciencias Naturales, Madrid,

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FIG. 1. Female of *Ischnocnema sanderi* sp. nov. (holotype, CBF 5385).

Spain (MNCN); Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK).

SYSTEMATICS

Ischnocnema sanderi sp. nov.

Figures 1–3

Holotype.—CBF-5385, an adult female (Fig. 1), from Arroyo Bilunto (14°38′24″S, 68°31′45″W), Chunirumi Valley, Bilunto Mountains, Area Natural de Manejo Integrado Madidi, near Santa Cruz de Valle Ameno, Province Franz Tamayo, Departament La Paz, Bolivia, 1800 m, one of a series collected on 24 November 1999 by Ignacio De la Riva, Jaime Bosch, Steffen Reichle, Hans Pröller, and Amira Apaza.

Paratypes.—Four specimens, with the same data as the holotype: MNCN 42016 (adult male; Fig. 2); MNCN 42017 (subadult male); and CBF-5383-4 (juveniles). Other paratypes are: ZFMK 80600 (adult female), ZFMK 80601 and NKA 6563 (juveniles) from Colonia Eduardo Avaroa (15°41′S/67°27′W), approximately 30 km north of Caranavi on the road from Caranavi to Yucumo, Province Nor Yungas, Departament La Paz, Bolivia, approximately 1300 m, collected on 18 April 2003 by Andreas John; CBF 4119 and CBF 4220-2 (adult males), CBF 4218-9 (adult females), and CBF 4223 (juvenile) from Bajo Hornuni (16°12′54″S/67°53′09″W), Province Nor Yungas, La Paz Department, Bolivia, 1935 m, collected in 1999 by Claudia Cortez; NKA 6695 (juvenile) and NKA 6696 (adult male) from the road from Apolo to Sarayo (14°42′34.4″S/68°18′33.4″W), Province Franz Tamayo, Departament La Paz, Bolivia, 1990 m, collected on 21–22 February 2004 by Dirk Embert.

FIG. 2. Male of *Ischnocnema sanderi* sp. nov. (paratype, MNCN 42016).

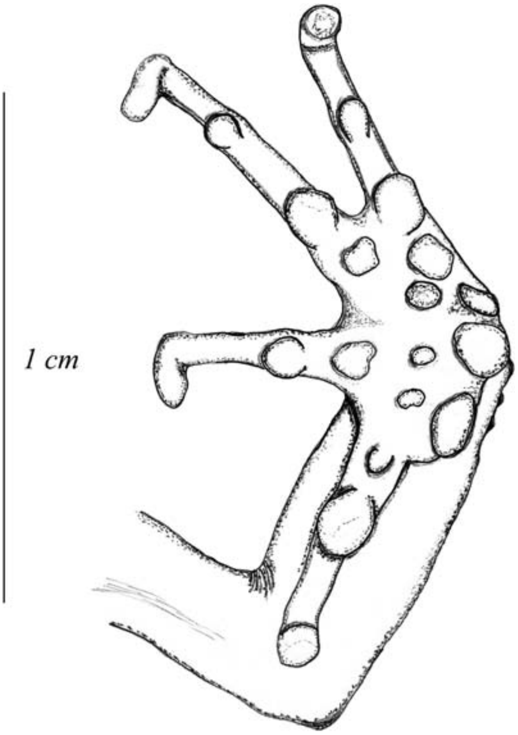

FIG. 3. Right palm of the holotype of *Ischnocnema sanderi* sp. nov.

Diagnosis.—The new species is a member of the genus *Ischnocnema* based on external characters (see Discussion). *Ischnocnema sanderi* can be distinguished from all other *Ischnocnema* except *I. sanctaecrucis* by having: a pale sacral stripe from the level of the caudal tip of the coccyx to the level of the sacrum; rounded, indistinct terminal disc 1.3 times as wide as the corresponding phalange; and dorsal skin moderately warty with scattered small tubercles. From *I. simmonsi*, it also differs in ventral coloration, being cream in *I. sanderi* and dark gray with white spots in *I. simmonsi*, and in dorsal skin texture, irregulary warty in *I. sanderi* and smooth with small spicules in *I. simmonsi*. The dorsum of *I. quixensis* has many large, subconical tubercles, whereas in *I. sanderi* the tubercles are small; furthermore, *I. quixensis* has a dark brown groin and heavily mottled venter, whereas in *I. sanderi* the venter is cream to white with some pale gray mottling in some specimens. From *I. saxatilis*, *I. sanderi* differs mainly by having smaller tubercles and warts, and by lacking a pale orange groin.

Ischnocnema sanctaecrucis is the only other *Ischnocnema* in Bolivian cloud forests, and although it occurs in Cochabamba and Santa Cruz departments, it has not been found in La Paz Department. The new species can be distinguished from *I. sanctaecrucis* by the following characteristics (*I. sanctaecrucis* in parentheses): brownish-gray coloration and no red spots or marks in life (red to scarlet spots in axiles, dorsum and shanks); well-defined W-shaped occipital fold (absent or ill defined); well-defined sacral fold (absent); head about as long as wide (wider than long); long, subacuminate to rounded snout (short and rounded to truncate); presence of a single white nuptial pad on thumb of adult male (absent); small and low granules or warts in dorsal skin (conical, pungent granules); presence of incipient fringes in the inner margin of Finger III (fringes well developed on Fingers II and III); venter mostly cream with slight gray mottling (dark brown with cream spots); dorsum brownish-gray (dark brown); in general, both species are quite similar in proportions (see Table 2).

Ischnocnema sanderi shares some characters with members of the *E. discoidalis* species group. Four species of that group inhabit the Andean region of Bolivia and southern Peru: *E. cruralis* (Boulenger), *E. discoidalis* (Peracca), *E. ibischi* Reichle, Lötters and De la Riva, and *E. zongoensis* Reichle and Köhler. *Ischnocenema sanderi* can be distinguished from these species by the W-shaped occipital fold, presence of at least incipient dorsolateral folds in males, rounded and not developed finger tips (developed and truncated at least in some finger), and different color pattern in life and in alcohol. It can be distinguished from *E. cruralis* by the warty dorsal skin (smooth to finely areolate with few to many small scattered warts in *E. cruralis*) and by having rounded toe tips (truncated). From *E. discoidalis*, *I. sanderi* differs by having warty dorsal skin (shagreened in *E. discoidalis*), vomerine odontophores posterior to choanae (medial), and lacking toe fringes (toe fringes present). From *E. ibischi*, *I. sanderi* differs mainly by having a smaller tympanum, subacuminate snout in lateral profile (truncate in *E. ibischi*), and first finger longer than second (equal). From *E. zongoensis*, *I. sanderi* is distinguished by having irregularly warty skin (heavily granular skin, with granules equal in size and evenly spaced), and head longer than wide (wider than long).

Description of Holotype.—Head longer than wide; snout moderately long, rounded in dorsal view, subacuminate to rounded in profile; canthus rostralis distinct, straight; loreal region slightly concave, sloping gradually to the lips; lips with warty skin; nostrils prominent, oriented dorsolaterally; eye large, its length greater than both interorbital and eye–nostril distances; tympanum visible, vertically ovoid; tympanic annulus visible, prominent, only partially overlapped dorsally by weak black supratympanic fold; anterior three fourths of tongue attached; males with vocal slits posterolateral to tongue; choanae not concealed by the palatal shelf of maxillae, rounded, in anterolateral position and separated by a distance of five times diameter of choana; vomerine odontophores posterolateral and close to choanae, prominent, rounded, their length twice that of choana. Skin on dorsum granular to warty; warts of different sizes, small and rounded; granules small and subconical; W-shaped occipital fold; well-developed postrictal glands posteroventral to tympanum; fine middorsal fold from tip of the snout to sacrum. Belly smooth; discoidal fold present. Forelimbs long, slender; forearm with incipient white tubercles; thenar tubercle oval, posterolateral to and about three-fourth size of palmar tubercle; palmar tubercle rounded and divided in two parts, smaller elongate, and larger oval (Fig. 3); subarticular tubercles prominent, conical, larger than supernumerary tubercles; supernumerary tubercles prominent, rounded to subconical. Fingers slender; terminal disc poorly developed, only slightly expanded, approximately 1.3 times width of finger; webbing absent; finger III with incipient fringes on inner margin; relative length of fingers, from shortest to longest, 2<4<1<3. Hind limbs moderately robust, tibia short; heel without tubercles; tarsus with scattered, small, white granules; inner metatarsal tubercle prominent, oval, twice the size of conical outer metatarsal tubercle; toes slender, fringes and webbing absent; terminal discs rounded, poorly developed, approximately 1.5 times width of toe; relative length of toes, from shortest to longest, 1<2<5<3<4; subarticular tubercles prominent, conical; supernumerary tubercles small, rounded. For measurements and proportions, see Tables 1 and 2.

In preservative, color of dorsum mostly pale gray; a dark gray W-shaped mark outlined by black in occipital region; interorbital region dark gray; dark gray or brown irregular X-shaped mark from middorsal to sacral region; transverse dark gray spot in sacral region; narrow cream stripe extending from terminus of coccyx to middle of level of sacrum; dorsolateral region gray with two or three oblique irregular rows of small dark spots; dorsal warts pale cream; dorsal

TABLE 1. Measurements (in millimeters) of the type material of *Ischnocnema sanderi* (except CBF 4223, a small juvenile). Abbreviations are snout–vent length, SVL; head width, HW; head length, HL; interorbital distance, IOD; eye length, EL; upper eyelid width, EW; tympanic membrane length, TYL; tympanic membrane height, TYH; eye to nostril distance, EN; tibia length, TL; foot length, FL; M = male; F = male; A = adult; S = subadult; J = juvenile.

	CBF 4119	CBF 4218	CBF 4219	CBF 4220	CBF 4221	CBF 4222	CBF 5383	CBF 5384	CBF 5385 (holotype)	MNCN 42016	MNCN 42017	ZFMK 80600	ZFMK 80601	NKA 6563	NKA 6695	NKA 6696
SVL	29.8	36.8	37.3	28.6	30.7	28.0	20.3	17.7	33.9	28.4	26.6	38.0	24.6	23.8	23.2	27.3
HW	11.0	14.3	13.8	10.2	11.1	10.2	8.1	7.2	12.9	10.2	9.5	15.9	9.4	9.7	8.6	10.7
HL	10.8	13.7	13.3	10.4	10.6	9.9	8.7	7.6	13.5	10.6	10.7	15.3	9.7	9.5	10.7	11.0
IOD	2.7	3.3	2.9	2.6	2.7	2.7	2.2	1.8	3.0	2.8	2.5	3.2	2.4	2.6	3.1	3.5
EL	3.7	4.1	4.4	3.6	3.6	3.4	2.8	3.1	4.8	4.1	3.7	4.8	3.2	3.0	3.5	3.6
EW	2.6	3.0	2.9	2.7	2.3	2.6	1.9	1.7	2.9	2.6	2.2	3.5	2.6	2.5	—	—
TYL	2.2	2.7	2.4	2.4	2.4	2.3	1.5	1.5	2.3	2.2	1.0	2.8	1.8	1.9	1.8	2.2
TYH	2.8	3.0	2.7	2.3	2.4	2.2	1.7	1.6	2.7	2.2	1.8	3.3	2.2	2.1	2.0	2.4
EN	3.3	3.8	4.3	3.0	3.2	3.0	2.7	2.3	4.0	3.0	2.8	4.6	2.8	3.0	3.7	3.6
TL	16.4	20.6	20.7	15.9	16.9	15.9	12.1	10.0	18.3	15.1	14.5	21.8	12.8	13.3	13.1	14.5
FL	15.2	19.5	19.1	15.0	15.2	14.5	10.4	9.3	17.4	15.0	14.3	19.4	10.7	10.9	—	—
SEX	AM	AF	AF	AM	AM	AM	J	J	AF	AM	SM	AF	J	J	J	AM

region of the head pale gray with a dark gray spot on the snout; lips pale gray to white with irregular dark gray vertical bands; anterior margin of canthus rostralis dark gray, becoming paler near eye; tympanic membrane dark brown; tympanic annulus pale gray; dorsal regions of extremities reddish-brown with some irregular gray bars and spots; proximal region of forelimbs reddish-cream; hidden regions of extremities reddish cream with fine gray mottling. Belly and ventral surfaces of hind limbs translucent, immaculate cream, with some brown mottling laterally; throat cream with fine brown mottling and anastomosed cream irregular spots; white spots on ventral margin of the lips.

In life, dorsum pale brown with irregular dark gray or brownish-gray spots and marks surrounded by beige; no red spots. Dorsolateral region mostly cream with two or three oblique rows of dark brown spots. Belly almost transparent, throat yellowish-beige reticulated with gray; inner surfaces of limbs flesh colored; plantar and palmar surfaces gray; limbs barred. Iris golden green, the ventral two-thirds darker and reticulated with black.

Variation.—Ischnocnema sanderi shows strong sexual dimorphism in size but not in proportions (see Tables 1 and 2). Males have a single white nuptial pad on each thumb and vocal slits. Males resemble more *Eleutherodactylus* (Fig. 2); they are more slender than females and present incipient dorsolateral folds, composed by a line of tubercles that extends from the eyelid to the groin; tympanum is more rounded in males. Dorsal skin is less granular in males than in females. Color patterns in alcohol are similar, but pinkish tones are dominant as ground color in males. Dark markings are more common and darker in males; they usually have a dark brown W-shaped mark on the occipital region and a X- or Y-shaped mark middorsally to sacral region. Ventral coloration is similar in both sexes, and it varies

TABLE 2. Mean and range (in parentheses) of some morphometric proportions of adult specimens of *Ischnocnema sanderi* sp. nov. and *Ischnocnema sanctaecrucis*; for abbreviations, see Table 1.

	Ischnocnema sanderi			*Ischnocnema sanctaecrucis*
Proportions	Females (*N* = 4)	Males (*N* = 5)	Combined (*N* = 9)	Combined (*N* = 5)
TL/SVL	0.6 (0.5–0.6)	0.6 (0.5–0.6)	0.6 (0.5–0.6)	0.5 (0.5–0.5)
FL/SVL	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.5 (0.5–0.5)
HL/SVL	0.4 (0.4–0.4)	0.4 (0.3–0.4)	0.4 (0.3–0.4)	0.4 (0.4–0.4)
HW/SVL	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.4 (0.4–0.4)
HW/HL	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.1 (1.1–1.1)
IOD/EL	0.7 (0.7–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.9 (0.7–1.1)
IOD/SVL	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)
EN/EL	0.8 (0.7–0.9)	1.0 (0.8–1.0)	0.9 (0.7–1.0)	1.0 (0.8–1.1)
EN/SVL	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)
TYL/TYH	1.0 (0.8–1.0)	0.9 (0.8–1.0)	0.9 (0.8–1.0)	0.9 (0.6–1.1)

from immaculate cream to cream with some gray reticulation anteriorly and laterally. Some adult males have well-defined pale gray spots on the tip of the snout, on the posterior margin of the snout, near the eyelids, and on both heels. Dorsolateral stripes can be associated to the dorsolateral folds; these stripes are pale gray in the anterior margin and become pinkish at the level of the arms. Dorsal gray spots are more irregular and anastomosed in juveniles, giving them a more cryptic appearance. One specimen (MNCN 42016) has incipient dorsolateral pale stripes, the same color that a small interocular and snout spot; it has a hard rectangular protuberance in the belly that seems to have been caused by parasites.

Distribution and Life History.—*Ischnocnema sanderi* inhabits humid montane forests and cloud forests of the Cordillera Oriental of the Andes, where it is known from four areas in the Yungas of the La Paz Department, at approximately 1300–2000 m elevation (Fig. 4). The airline distance between the two most separate localities (Arroyo Bilunto and Bajo Hornuni) is approximately 190 km. The species might occur in Peru, whose frontier is only 60 km from the type locality. At Bajo Hornuni, the frogs were mostly on the forest litter near streams (Cortéz, 2001). At the type locality, the frogs were on the ground near a stream, within a remnant of cloud forest (about 3 km in length). Other anurans found at the type locality were *Bufo veraguensis* Schmidt, *Cochranella bejaranoi* (Cannatella), *Hyla armata* Boulenger, *Hyla balzani* Boulenger, *Leptodactylus rhodonotus* (Günther), *Eleutherodactylus* cf. *peruvianus* (Melin), *Eleutherodactylus platydactylus* (Boulenger), and *Eleutherodactylus rhabdolaemus* Duellman.

Etymology.—The name is a patronym for Holger Sanders (Germany), in recognition of his support of taxonomic research and conservation in Bolivia.

DISCUSSION

Species of the genus *Ischnocnema* are difficult to separate solely by their external features. For example, the best discriminating characters for *I. simmonsi* are the ventral coloration coupled with the skin texture (Lynch, 1974). Almost all species of *Ischnocnema* are mostly brown or gray without any remarkable color pattern. Only *I. sanctaecrucis* has red to scarlet marks in the groin, axillae and on the dorsum, and *I. saxatalis* has a pale orange groin. *Ischnocnema sanderi* and *I. sanctaecrucis* are more similar to each other than to any other species of the genus because they share some morphological characters and body proportions (Table 2), as well as habitat preferences and general ecology.

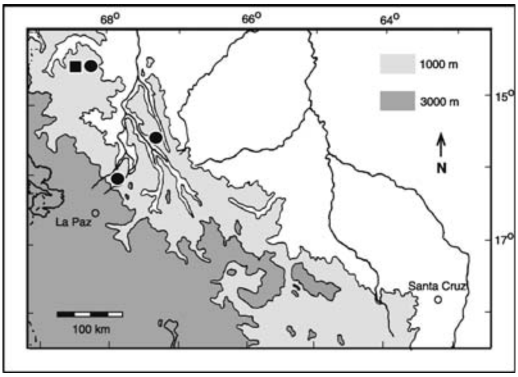

FIG. 4. Map of central Bolivia showing localities for *Ischnocnema sanderi* sp. nov. The square represents the type locality of the species.

The genus *Ischnocnema* was recognized and diagnosed by Lynch (1971, 1972), who pointed out that *Ischnocnema* could be phylogenetically more primitive and perhaps ancestral to frogs placed in the genus *Eleutherodactylus*. The generic boundaries between *Ischnocnema* and *Eleutherodactylus* are feeble, and similarities are especially remarkable when members of the *E. discoidalis* group are considered. The *E. discoidalis* group was redefined and diagnosed by Lynch (1989). It is restricted to southern Peru, Bolivia, and northern Argentina. All of the species (*E. cruralis*, *E. discoidalis*, *E. ibischi*, and *E. zongoensis*) occur in Bolivia and, especially *E. cruralis*, share many morphological characteristics with both *I. sanderi* and *I. sanctaecrucis*. Harvey and Keck (1995) assigned *I. sanctaecrucis* to the genus *Ischnocnema* because they found it had most nonosteological characters used by former authors to include species in this genus, namely, lack of T-shaped digital tips and transverse grooves, first finger markedly longer than second, three palmar tubercles, subarticular and supernumerary tubercles of hands conical or subconical, and barred lips and limbs (Lynch, 1974; Duellman, 1990; Harvey and Keck, 1995). All of these characters are present in *I. sanderi* as well; therefore, we place it in *Ischnocnema*. However, the putative monophyly of both *Ischnocnema* and the *E. discoidalis* group has not been demonstrated, and the phylogenetic relationships between them remain unknown. If *Ischnocnema* is more primitive than *Eleutherodactylus*, as proposed by Lynch (1971, 1972, 1989), it would be plausible that the *E. discoidalis* species group had evolved from the ancestral stock of *E. sanctaecrucis* and *I. sanderi*. More data are necessary to address these problems.

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APPENDIX 1

Specimens Examined

Ischnocnema sanctaecrucis. BOLIVIA: Santa Cruz Department: El Chapé, 2060 m, NKA 1198 (holotype) and MNCN 42010-13.

Ischnocnema quixensis: BOLIVIA: Pando Department: San Sebastián, CBF 2528–2529; Río Negro, NKA 6525–6527. *Ischnocnema saxatilis*: PERU: San Martín Department: Ponga de Shilcayo, about 4 km North Northwest of Tarapoto, 470 m (6°31′S/76°53′), MHNSM-8431 (paratype).

Eleutherodactylus cruralis: BOLIVIA: La Paz Department: Camino maderero el Chaval, Arroyo Mikai, Reserva Pilón Lajas, NKA 3759–3761; Cochabamba Department: Valle de Sajta, NKA 3633; Villa Tunari, NKA 1492; Los Guácharos, NKA 6617–6619; Pando Department: Lago Bay, Reserva Manuripi, NKA 6120–6121; Beni Department: Laguna Azul, Reserva Pilón Lajas, NKA 3975–3976, 3979, 3985–3987, 4003.

Eleutherodactylus discoidalis: BOLIVIA: Tarija Department: 12.3 km Northwest of Entre Ríos on the road to Tarija, NKA 3877-3897.

Eleutherodactylus ibischi: BOLIVIA: Santa Cruz Department: km 68.5 on Santa Cruz de la Sierra-Samaipata road, 750 m, CBF 3341 (holotype); El Fuerte, Samaipata, 1800 m, ZFMK 60402 (paratype); km 60 on Santa Cruz de la Sierra-Samaipata road, NKA 6612.

Eleutherodactylus zongoensis: BOLIVIA: La Paz Department: Valle de Zongo, 1250 m, CBF 2503 (holotype).

Ischnocnema sanderi Padial, Reichle & De la Riva, 2005 (Amphibia, Anura, Leptodactylidae): A nomenclatural note

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In a recent contribution, Padial *et al.* (2005) described a new species of frog, *Ischnocnema sanderi,* from humid forests of the Bolivian Andes. The section of etymology of the species description states that the specific name "…is a patronym for Holger Sanders…". After this statement, and following the rules of the ICZN (2000), it seems that the proper specific term for this species should be *sandersi,* instead *sanderi.* We could not examine the proofs of the corresponding manuscript and, consequently, the text was eventually published without any latest search for typographical errors or necessary changes. Thus, a mistake at this point could have had nomenclatural consequences. The species appears consistently named as *I. sanderi* throughout the text, and, by means of this note, we wish to state that such specific term is correct, and that a typographical mistake affected the name of the person to whom the species was dedicated, Holger Sander.

ACKNOWLEDGMENTS

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Padial, J. M., Reichle, S. & De la Riva, I., 2005. A new species of *Ischnocnema* from Bolivian Andean forests. *Journal of Herpetology,* 39(2): 186-191.

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Publicado, 14-VII-2005

APÉNDICE V

B. mirandaribeiroi: USNM 28934: Brazil: Para to Manaos; *Bufo guttatus* group: *B. blombergi*: KU 69843: Colombia: Santander; KU 59763: Ecuador, Collari; *B. guttatus*: KU 167631: Venezuela, Bolívar; *Bufo margaritifer* group: *B. typhonius*: KU 93138: Brazil: Amapa; KU 104756 Ecuador: Napo; KU 127511: Brazil: Para; KU 205269: Peru: Madre de Díos; *Bufo marinus* group: *B. marinus*: KU 42566: Nicaragua: Managua; KU 69846: Mexico: Alta Verapaz; KU 84935–37: Nicaragua: Rivas; KU 152914: Ecuador: Napo; *B. schneider*: KU 160307: Argentina: Santiago del Estero; *B. poeppigii*: KU 183234–35: Bolivia: La Paz; *Bufo spinulosus* group: *B. amabilis*: KU 120365, 120371, 124587: Ecuador: Loja; *B. arequipensis*: KU 214793: Peru: Arequipa; *B. atacamensis*: KU 217351: Chile: Coquimbo; *B. chilensis*: KU 217363: Chile: Santiago; *B. cophotis*: KU 218517–18: Peru: Cajamarca [C&S]; 218525–26: Peru: Cajamarca; *B. limensis*: KU 209226: Peru: Lima; *B. amabilis*: KU 120365, 120365, 124587: Ecuador: Loja; *B. spinulosus*: KU 160270–72: Bolivia: Potosí; KU 163032; Peru: Puno; KU 163036: Peru: Puno; KU 163066, 163074: Peru: Ayacucho; *B. vellardi*: KU 136053: Peru: Cajamarca; *Bufo veraguensis* group: *B. chavin*: MTD 43786–87: Peru: Huánuco, Palma Pampa; *B. veraguensis*: KU 164084: Peru: Cuzco; *B. multiverrucosus*: MTD 44751: Peru: Pasco, Paucartambo de Pasco Valley.

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A NEW SPECIES OF THE *ELEUTHERODACTYLUS DISCOIDALIS* GROUP (ANURA: LEPTODACTYLIDAE) FROM ANDEAN HUMID MONTANE FORESTS OF BOLIVIA

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A new species of the *Eleutherodactylus discoidalis* group from the Andean humid montane forests of Departamento La Paz, northern Bolivia, is described. The new species differs from other species of the group by having regularly warty dorsal skin, a small tympanum, slightly enlarged, rounded digit discs, and uniformly dark brown coloration. Data on the advertisement call of the new species and an operational key for the species of the *E. discoidalis* group are provided.

Key words: Anura; Bolivia; *Eleutherodactylus*; Leptodactylidae; New species

The *Eleutherodactylus discoidalis* Group was defined by Lynch (1976) on the basis of several external characters: first finger longer than second, head width <45% of snout–vent length, no cranial crests, ungual flaps of digits not indented, digits not expanded, and toes lacking webbing. He recognized five species: *E. cruralis* (Boulenger, 1902), *E. discoidalis* (Peracca, 1895), *E. elassodiscus* Lynch, 1973, *E. granulosus* (Boulenger, 1903) and *E. nigrovittatus* (Andersson, 1945). Subsequently, Lynch, (1989) considered *E. granulosus* a junior synonym of *E. cruralis*, and split the former *E. discoidalis* Group, rendering it only with the two southern species: *E. discoidalis* (Argentina and Bolivia) and *E. cruralis* (southern Peru and Bolivia) (De la Riva, 1993). There were two synapomorphies proposed for this group: 1) conical subarticular tubercles, and 2) supernumerary plantar tubercles. Recent studies in poorly surveyed areas of Bolivia revealed two new species of *Eleutherodactylus* belonging to this group: *E. zongoensis* Reichle and Köhler, 1997 and *E. ibischi* Reichle, Lötters and De la Riva, 2001. Members of the *E. discoidalis* Group are terrestrial and inhabit different ecoregions: the Tucumanian and Yungas Forest (*E. discoidalis*), the Andean Montane Rainforest (*E. cruralis*, *E. zongoensis*), the Amazonian Lowland Rainforest (*E. cruralis*), and the Inner Andean Dry Forest (*E. ibischi* and *E. cruralis*) (De la Riva et al., 2000; Köhler, 2000). Recent fieldwork in the humid montane forests of Departamento La Paz, Bolivia, yielded new material of this group. We made morphological comparisons of some

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FIG. 1.—Male of *Eleutherodactylus madidi* sp. nov., holotype (SVL 28.87 mm).

FIG. 2.—Male of *Eleutherodactylus madidi* sp. nov., MNCN 42015 (SVL 28.30 mm).

specimens collected at two localities in Madidi National Park and Pilón-Lajas Biosphere Reserve, with specimens of all the species of the *E. discoidalis* group. We concluded that these specimens represent an unknown species, which is described herein.

MATERIALS AND METHODS

Specimens were fixed in 10% formalin and preserved in 70% ethanol. For morphological and color characteristics used in the diagnosis and description, we followed Lynch (1989) and Lynch and Duellman (1997). Specimens examined are listed in the Appendix. Recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone. Recordings were obtained on 18 December 2003 at the type locality, during the evening and at sunset; air temperature was 21 C. Eigth calls were analyzed. The sounds were digitized at a sampling frequency of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board and Peak 3.2 (OSX) software, and edited with Audacity 1.2.2 (OSX). Praat 4.2.22 (OSX) software was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through fast Fourier transform (FFT) (width, 1024 points). Digitized calls were deposited in the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, Madrid, Track Number 263. Measurements were taken with a digital caliper to the nearest 0.01 mm, but following Hayek et al. (2001), for avoiding pseudo precision, we rounded all measurements to only one decimal. Abbreviations are as follows: snout–vent length, SVL; head width (measured at level of rictus), HW; head length (from rictus to tip of snout), HL; interorbital distance, IOD; eye diameter (measured horizontally), ED; upper eyelid width, EW; tympanic membrane length, TYL; tympanic membrane height, TYH; eye to nostril distance, EN; tibia length, TL; foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL. Color characteristics were noted in life. Museum abbreviations refer to: Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNK-A [Amphibian Collection]); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Perú (MHNSM); United States National Museum of Natural History, Smithsonian Institution, Washington, USA (USNM); Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Cochabamba, Bolivia (CBG); Zoologisches Forchungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK); and Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN).

SYSTEMATICS

Eleutherodactylus ***madidi*** sp. nov.
(Figs. 1, 2)

Holotype.—MNK-A 7856, an adult male from Arroyo Huacataya, Serranía Eslabón, Área Natural de Manejo Integrado Madidi, Provincia Franz Tamayo, Departamento La Paz, Bolivia (14° 20′ 12″ S, 68° 05′ 57″ W), ca.

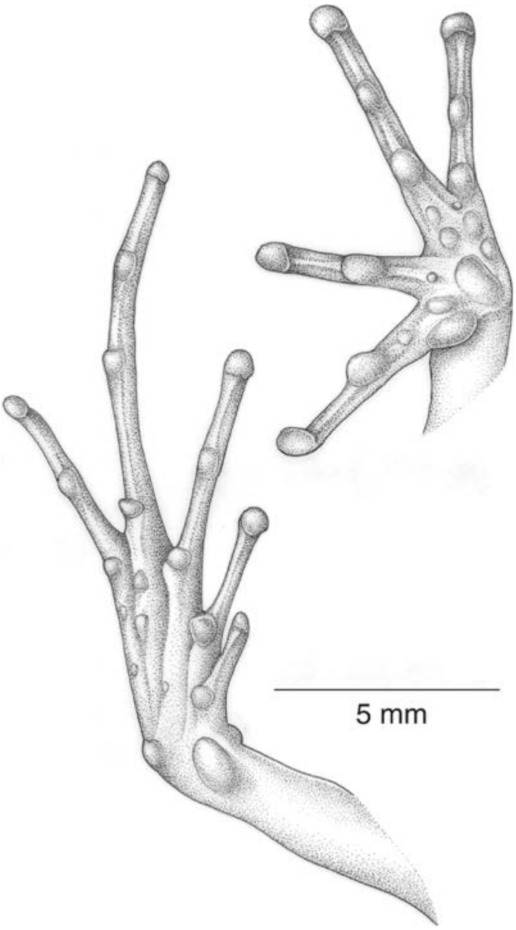

FIG. 3.—Hand and foot of the holotype of *Eleutherodactylus madidi* sp. nov.

1500 m, obtained on 17 December 2003 by J. M. Padial.

Paratypes.—MNK-A 7197 and MNCN 42014–15, (adult males) paratopotypes, same data as the holotype; MNK-A 4137 (adult male) and MNK-A 4138 (adult female), from La Cascada, Biosphere Reserve Pilón Lajas, Provincia Sud Yungas, Departamento La Paz, Bolivia (15° 24′ 00″ S, 67° 08′ 59″ W), 900 m, collected on 10 July 1999 by L. Gonzáles.

Diagnosis.—A member of the *E. discoidalis* Group, as defined by Lynch (1989), characterized by: (1) skin on dorsum heavily warty, warts regular in size, low, small, rounded, some of them enlarged on the posterior part of the head and scapular region; warts on flanks larger and more elongate than those of dorsum; venter smooth; posterior surfaces of limbs uniformly warty; discoidal fold present, barely visible; no dorsolateral folds; postrictal glands well developed; (2) tympanic membrane and annulus distinct, its length about 2/5 to 1/2 of eye length; supratympanic fold short; (3) head large, longer than wide; snout round to subacuminate in dorsal and lateral views; canthus rostralis rounded; (4) cranial crests absent; upper eyelid tubercles absent; (5) vomerine odontophores situated posteriormedial to choanae; (6) males with vocal slits and a single faint nuptial pad on thumb; (7) first finger longer than second; subarticular tubercles enlarged and subconical to conical in profile; supernumerary tubercles small; terminal discs of fingers rounded, not enlarged (Fig. 3); (8) lateral fringes and keels on fingers absent; (9) ulnar tubercles absent; (10) no tubercles on heel and tarsus; (11) inner metatarsal tubercle ovoid, outer more rounded, subequal; (12) toes lacking lateral fringes or keels; webbing absent; fifth toe shorter than third; discs of toes moderately enlarged, rounded (Fig. 3); (13) dorsal coloration dark brown to black, with diffuse dark marks; in preservative, dorsal regions brownish gray or purplish gray; snout dark gray with darker marks; throat cream with dense and fine brownish gray mottling; venter cream with scarce to dense brownish gray fine mottling.

Eleutherodactylus madidi can be distinguished from *E. cruralis* by having: dorsal skin uniformly warty (smooth or shagreen with few to many small, scattered warts in *E. cruralis*), rounded finger discs (truncate on Fingers III and IV), dorsum in life uniformly dark brown (brown color with many well defined dark and light marks and flecks), and larger size of males (SVL of adult males of *E. madidi* 27.9–29.4 mm [$n = 5$]; SVL of adult males of *E. cruralis* 18.3–25.0 mm [$n = 35$]). *Eleutherodactylus madidi* can be distinguished from *E. discoidalis* as follows: vomerine odontophores posterior to choanae (between choanae in *E. discoidalis*), finger and toe discs rounded (truncate), dorsal skin uniformly warty (shagreen), finger and toe fringes absent (present), and dorsal coloration dark brown with scarce pattern (variable, with intense pattern). *Eleutherodactylus madidi* differs from *E. ibischi* by having tympanic membrane equal or smaller than 1/2 of eye diameter (larger), first finger longer than second (equal), rounded finger discs (discs on

Fingers III and IV two times wider than the digits proximal to the disc), and dark brown dorsal coloration (light brown to beige). *Eleutherodactylus madidi* can be distinguished from *E. zongoensis* by its warty skin on dorsum (dorsum and extremities tuberculate in *E. zongoensis*), head longer than wide (wider than long), smaller tympanic membrane (TYL/ ED = 0.41–0.56 in *E. madidi* [*n* = 6], TYL/ ED = 0.60 in *E. zongoensis* [holotype]), toe discs round and moderately enlarged (truncate, small), labial bars present (absent), and ventral region cream with fine mottling (uniformly dark pinkish brown).

The species of the genus *Ischnocnema* share many morphological characters with the species of the *Eleutherodactylus discoidalis* Group (e.g., warty skin and lack of T-shaped digital tips). In Bolivia three species have been reported: *I. quixensis* (Jiménez de la Espada, 1872) in the lowland Amazonian rainforests of Departamento Pando, *I. sanctaecrucis* Harvey & Keck, 1995 in Andean cloud forests of Departamento Cochabamba and Departamento Santa Cruz, and *I. sanderi* Padial, Reichle and De la Riva, 2005 in cloud forests of Departamento La Paz (De la Riva et al., 2000; Padial et al., 2005). *Eleutherodactylus madidi* is easy to distinguish from *Ischnocnema quixensis* and *I. saxatilis* Duellman, 1990 (from the Andes of northern Peru); these species of *Ischnocnema* have irregularly warty and tuberculate dorsal skin, a different coloration, and are much more robust and larger. *Eleutherodactylus madidi* is smaller than *I. sanctaecrucis* (SVL of adult males of *E. madidi* 27.9–29.4 mm [*n* = 5]; SVL of adult males of *I. sanctaecrucis* 31.9–35.1 mm [*n* = 3]), has slender body and extremities, possess warty skin (tuberculate in *I. sanctaecrucis*), lacks scarlet marks on dorsum (present), and lacks toe and finger fringes (present). *Eleutherodactylus madidi* differs from *I. sanderi*, by lacking a W-shaped dorsal scapular fold (present in *I. sanderi*), lacking finger fringes (present), and having dorsum dark brown with darker diffuse marks (light brown with well defined dark marks).

Description of the holotype.—Head slightly narrower than body, longer than wide; snout rounded in dorsal view and lateral profile; nostrils slightly protuberant, oriented laterally; canthus rostralis indistinct, rounded in profile; loreal region slightly concave, sloping gradually to the lips; upper eyelid without tubercles; no cranial crests. Supratympanic fold distinct; tympanic membrane and tympanic annulus distinct; tympanic membrane nearly round, its length about 1/2 of eye length; tympanic membrane separated from eye by a distance of almost half the eye length; three postrictal glands. Choanae rounded, small, anterolateral, not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; vomerine odontophores triangular, posteromedial to choanae, well developed, their anterior margin at the level of the posterior margin of the choanae, their width about 1.3 times the diameter of the choanae; vocal slits present, posterolateral to tongue. Skin of dorsal surfaces and posterior parts of hind limbs finely warty, most warts of the same size, with larger ones on posterior surfaces of dorsum and on the flanks; ventral surfaces almost smooth, with a finely granular texture on the throat; a thin middorsal fold from the anterior margin of the snout to the sacral region; two faint, sinuous, parallel occipital folds; no dorsolateral folds; discoidal fold distinct.

No ulnar tubercles (only white spots); palmar tubercle moderately large, rounded, larger than elongate thenar tubercle; supernumerary tubercles small, subconical, smaller than subarticular tubercles; subarticular tubercles large, subconical to round; discs of fingers rounded, slightly wider than the corresponding digits; fingers lacking lateral fringes and keels; nuptial pads absent; relative length of fingers $2 < 4 < 1 < 3$ (Fig. 3).

Heel and tarsus lacking tubercles or folds; inner metatarsal tubercle ovoid, longer than wide, almost the same size as the conical outer; supernumerary tubercles small, rounded; subarticular tubercles large, conical, directed toward tip of digit; toes lacking lateral fringes and keels; discs rounded, slightly enlarged, about 1.3 times the width of the corresponding digit; relative length of toes $1 < 2 < 5 < 3 < 4$ (Fig. 3).

Measurements (in mm).—SVL 28.9; head width 9.4; head length 10.5; interorbital distance 2.8; eye diameter 3.8; upper eyelid width 2.3; tympanic membrane length 2.1; tympanic membrane height 2.2; eye to nostril distance 3.5; tibia length 15.2; foot length 14.5. For proportions, see Table 1.

Coloration.—In life, dorsum mostly dark brown, with some dark, diffuse marks; dorsal

TABLE 1.—Measurements and proportions of the type specimens of *Eleutherodactylus madidi* sp. nov. (M, male; F, female; for other abbreviations, see Material and Methods).

	MNK-A 7856 (M)	MNCN 42014 (M)	MNCN 42015 (M)	MNK-A 7197 (M)	MNK-A 4137 (M)	MNK-A 4138 (F)
SVL	28.9	27.9	28.3	28.5	29.4	33.0
HW	9.4	9.9	9.7	9.9	10.8	10.8
HL	10.5	12.1	10.9	10.1	10.7	11.3
IOD	2.8	2.4	2.5	2.1	2.6	3.0
ED	3.8	3.6	4.3	3.7	3.7	4.0
EW	2.3	2.6	2.8	2.2	2.8	2.4
TYL	2.1	2.0	1.8	1.5	1.6	1.7
TYH	2.2	2.3	2.5	2.2	1.6	1.7
EN	3.5	3.2	3.3	3.8	3.6	3.6
TL	15.2	15.6	14.5	15.2	16.3	16.9
FL	14.5	13.7	13.9	14.4	15.0	15.8
HL/HW	1.12	1.21	1.13	1.02	0.99	1.03
TYL/ED	0.55	0.57	0.43	0.41	0.43	0.42
EN/ED	0.93	0.90	0.78	1.01	0.98	0.90
IOD/ED	0.74	0.68	0.59	0.56	0.72	0.75
TL/SVL	0.53	0.56	0.51	0.55	0.55	0.58

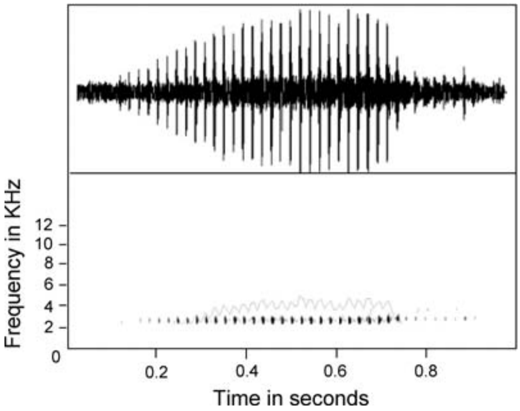

FIG. 4.—Oscillogram and spectrogram of a 1 s section of the call of *Eleutherodactylus madidi* sp. nov.; air temperature, 21 C.

surfaces of extremities dark brown with fine brown mottling and diffuse bars; loreal region and lips lighter, lips with diffuse dark brown bars (Fig. 1); supratympanic fold dark brown to black; ventral regions cream with fine brown mottling, more intense on extremities; inner surfaces of hind limbs brown; a whitish cream line departs from the proximal part of the posterior surface of each thigh and goes above the level of the vent, forming a parentheses-shaped pattern around it; plantar surfaces brown, with gray and white tubercles. The coloration in preservative is similar.

Variation.—There is little variation among specimens from Eslabón, but some differences do exist between them and the specimens from La Cascada. In the specimens from Eslabón, none has a nuptial pad, while the male from La Cascada has a faint nuptial pad on each thumb; one male from Eslabón (MNCN 42015), has a fine, bright yellow middorsal line from the tip of the snout to the coccyx (Fig. 2). The specimens from La Cascada had orange tones in the ventral regions, postrictal glands, axillary regions, and groins. The single male from La Cascada has larger thenar and palmar tubercles and more developed supernumerary tubercles; the tympanic membrane is less evident in the two specimens from this locality. The single female (MNK-A 4138) is larger (SVL 33.0 mm) than the males (SVL 27.9–29.4 mm; n = 5), has lighter dorsal coloration, the head is longer than wide, and the vomerine odontophores are rounded to slightly oval, low and almost in contact.

Advertisement call.—The call consists of a single pulsed note of variable duration (mean duration, 988.7 ms; range, 926–1075; n = 8) and regular pulse repetition, with 29.6 pulses per call (28–32) (Fig. 4); the pulse rate is 29.9 pulses/s (29.3–30.3) and the dominant frequency is at 2436.4 Hz (2411.7–2584); there are no harmonics. The call repetition rate is 4.3 calls/minute; often, two calls are emitted together in a sequence and, in this case, the first call is shorter than the second. The call of *E. madidi* is longer and has a much higher number of pulses than the call of *E. cruralis* (228–413 ms, 12–17 pulses) and *E. ibischi* (117–193 ms, 6–8 pulses); the pulse rate is higher in both *E. cruralis* (41–53 pulses/s) and *E. ibischi* (36–43 pulses/s), and the dominant frequency is higher in *E. cruralis* (3300–3420 Hz) but similar in *E. ibischi* (2000–2847 Hz) (see Márquez et al., 1995; Reichle et al., 2001). These characteristics allow a clear distinction of the three species by their call.

Distribution and ecology.—*Eleutherodactylus madidi* inhabits the humid montane forests of the Andean slopes of northern Bolivia at elevations of 900–1500 m (Fig. 5). The species is only known from La Cascada and Serranía Eslabón, two localities of Departmento La Paz separated by an airline distance of 150 km. It is a terrestrial species that calls mainly at dusk, from natural cavities in the ground, and it is difficult to find. The single female (MNK-A 4138), collected on 10 July (dry season) is gravid.

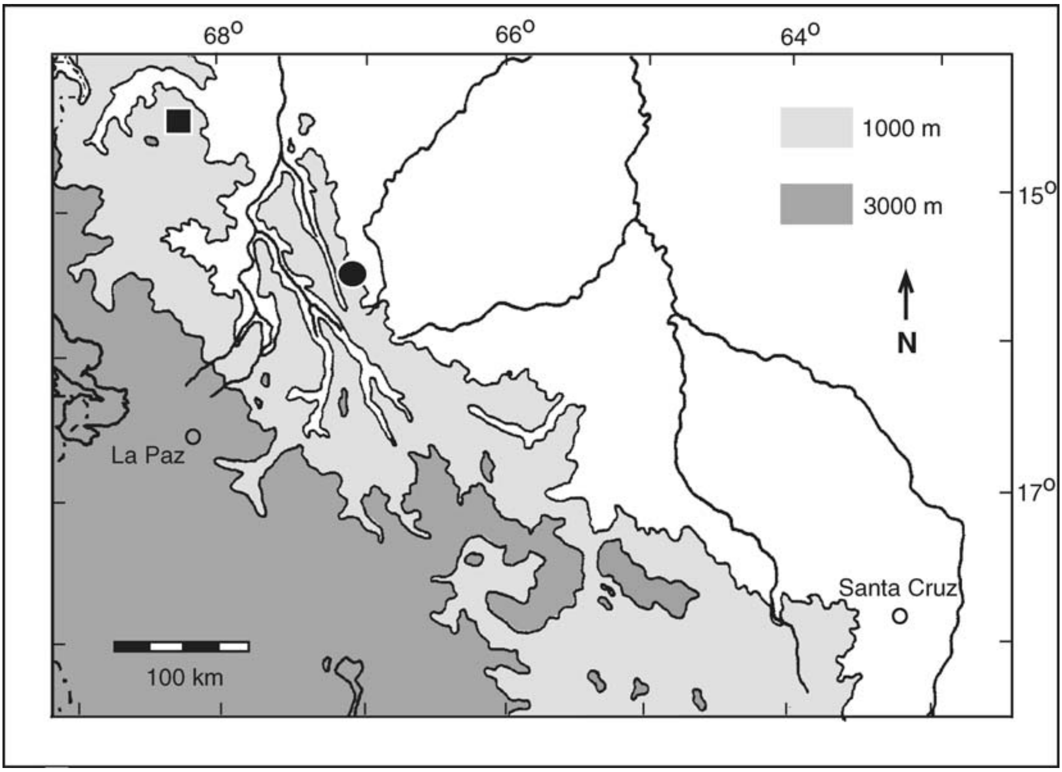

FIG. 5.—Distribution of *Eleutherodactylus madidi* sp. nov. in the Amazonian slopes of Departamento La Paz, Bolivia. The square represents the type locality.

Etymology.—The specific name refers to the Madidi National Park, where the species occurs. Madidi is one of the most important protected areas in the world in terms of biodiversity, and it holds a highly diverse amphibian fauna, which is still poorly inventoried. By naming a frog species after the park, we aim to call attention on Madidi's biological richness and the need of safeguarding this natural area for future generations.

DISCUSSION

We conclude that the new species belongs to the *Eleutherodactylus discoidalis* Group proposed by Lynch (1989); this conclusion is well supported by different morphological and call characteristics. Although some differences were found among the specimens from the two localities studied, we do not consider that they represent different species; we conclude that this represents simple interpopulational variations.

With *E. madidi*, the *E. discoidalis* Group is composed of five species sharing the putative synapomorphies proposed by Lynch (1989). The five species occur in Bolivia, one in Argentina (*E. discoidalis*) and one in Peru (*E. cruralis*). We provide an operational key for the species of the *E. discoidalis* Group, but it must be taken into account that the phylogenetic relationships and putative monophyly of this group are still obscure. Especially, the relationships between the *E. discoidalis* Group and other *Eleutherodactylus* and how the genus *Ischnocnema* relates to them, deserve future attention.

OPERATIONAL KEY TO THE SPECIES OF THE *ELEUTHERODACTYLUS DISCOIDALIS* GROUP

1. Vomerine odontophores posterior to choanae 2
   Vomerine odontophores between choanae .... *E. discoidalis*
2. Dorsal skin uniformly warty or tuberculate 3
   Dorsal skin smooth or shagreen with scattered irregular tubercles or warts 4
3. Terminal disc of fingers and toes rounded; tympanic membrane 2/5 to 1/2 of the eye diameter .... *E. madidi*
   Terminal discs of Fingers III and IV truncate; tympanic membrane evident, large, longer than 1/2 of the eye diameter .... *E. zongoensis*

4\. Tympanic membrane evident, longer than 1/2 of eye diameter; discs of Fingers III and IV two times wider than the corresponding digits; dorsum beige .... *E. ibischi*

Tympanic membrane evident, shorter than 1/2 of eye diameter; discs of Fingers III and IV at most one and a half times wider than the corresponding digits .... *E. cruralis*

Resumen

Se describe una nueva especie del grupo de *Eleutherodactylus discoidalis* de los bosques húmedos andinos del Departmento de La Paz, en el norte de Bolivia. La nueva especie se diferencia de otras especies del grupo por tener piel dorsal uniformemente rugosa, tímpano pequeño, discos digitales redondeados y ligeramente agrandados y color marrón oscuro bastante uniforme. Se proporciona una clave artificial para las especies del grupo de *E. discoidalis*.

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Associate Editor: Joseph Mendelson III

Appendix

Material Examined

Eleutherodactylus cruralis

BOLIVIA: Departamento La Paz: Boquerón, Pilón Lajas, CBG 792–93; Camino maderero El Chaval, Arroyo Mikai, Reserva Pilón Lajas, MNK-A 3759–61; Puerto Linares, 360 m, USNM 281100–30; 5 km W of San Buenaventura, USNM 280617; Departamento Cochabamba: Valle de Sajta, MNK-A 3633; Villa Tunari, MNK-A 1492; Los Guácharos MNK-A 6617–19; Entre Paractito y El Palmar, ZFMK 72541; Departamento Pando: Florida, Reserva Manuripi, MNK-A 5086; Lago Bay, Reserva Manuripi, MNK-A 6120–21; Departamento Beni: Asunción, Pilón Lajas, MNK-A 4074; Laguna Azul, Reserva Pilón Lajas, MNK-A 3975–76, 3979, 3985–87, 4003; San Luis Chico, MNK-A 4027; Serranía del Pilón, MNK-A 4182–83, 4209–13. PERU: Departamento Madre de Dios: Puerto Maldonado, 30 km SSW of Tambopata, USNM 284267, 343240, 342989–92; Colpa de Guacamayos, Zona Reservada Tambopata-Candamo, USNM 332436–37.

Eleutherodactylus discoidalis

BOLIVIA: Departamento Tarija: 12.3 km NW of Entre Ríos, on the road to Tarija, MNK-A 3877–97.

Eleutherodactylus ibischi

BOLIVIA: Departamento Santa Cruz: Km 68.5 on Santa Cruz de la Sierra-Samaipata road, 750 m elevation, CBF 3341 (holotype); Km 60 on Santa Cruz de la Sierra-Samaipata road, MNK-A 6612.

Eleutherodactylus zongoensis

BOLIVIA: Departamento La Paz: Valle de Zongo, 1250 m, CBF 2503 (holotype).

Ischnocnema quixensis

BOLIVIA: Departamento Pando, CBF 2528–29; Río Negro, MNK-A 6525–27.

Ischnocnema sanctaecrucis

BOLIVIA: Departamento Santa Cruz: El Chapé, 2060 m elevation, MNK-A 1198 (holotype); MNCN 42010–13.

Ischnocnema saxatilis

PERU: Departamento San Martín: Ponga de Shilcayo, about 4 km NNW of Tarapoto, 470 m (06° 31′ S, 76° 53′), MHNSM 8431 (paratype).

APÉNDICE VI

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Distribution and morphological variation of *Eleutherodactylus mercedesae* Lynch & McDiarmid, 1987 (Amphibia, Anura, Leptodactylidae) with first record for Peru

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Abstract

We report new distributional information for *Eleutherodactylus mercedesae* in Bolivia, and provide the first record for Peru based on an adult female. This species, previously endemic to Bolivia, now ranges across about 1000 km in cloud forests on the Amazonian slopes of the Andes from southern Peru to central Bolivia. We provide the first morphological description of females based on two specimens, compare them with the male type and paratype, add some observations to the original description, and comment on variation in the species.

Key words: *Eleutherodactylus mercedesae*, Anura, Leptodactylidae, Bolivia, Peru, distribution, variation

Resumen

Aportamos información novedosa sobre la distribución de *Eleutherodactylus mercedesae* en Bolivia y la primera cita para Perú, basada en una hembra adulta. Esta especie se consideraba hasta ahora endémica para Bolivia. Su rango conocido de distribución comprende actualmente unos 1000 km de los bosques nublados de las laderas amazónicas de los Andes, desde el sur de Perú al centro de Bolivia. Se describe por primera vez la hembra de esta especie en base a dos ejemplares, que son comparados con el holotipo y el paratipo. Añadimos algunas observaciones sobre la descripción original y la variación morfológica de la especie.

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Introduction

Eleutherodactylus mercedesae Lynch & McDiarmid, 1987 is an uncommon species inhabiting the Yungas and Humid Montane Forests of Central Bolivia (Lynch & McDiarmid 1987; De la Riva 1990). Until recently, it was exclusively known from two close localities (De la Riva 1993), but additional findings increased its known latitudinal range, first around the type locality, and subsequently further to the northwest, in Department La Paz, Bolivia (De la Riva *et al.* 2000; Köhler 2000). Thus, our information has shifted from considering this species to be restricted to the surroundings of the type locality to consider it a rare species with a distribution of 300 km wide in the Andean Cloud Forests of Bolivia (De la Riva *et al.* 2000). Despite recent intense fieldwork (Padial *et al.* 2004), including surveys of the type locality, few specimens have been collected and our knowledge of this species is still rudimentary. Nevertheless, recent collections examined (CBG and USNM) by us yielded new distributional information of this species from Bolivia and a record from Peru, where it was previously unrecorded (Lehr 2002; Frost 2004; AmphibiaWeb 2006).

Eleutherodactylus mercedesae was described from two adult males. Since then, the only additional information on the species' morphology was based on juveniles (Köhler 2000). Females of *Eleutherodactylus mercedesae* were unknown (Lynch and McDiarmid 1987). Since *Eleutherodactylus* is a large and complex frog genus, with most species in the same species group diagnosed only by subtle differences, some inter-populational variation could be misinterpreted as specific diagnostic characters if several specimens are not studied. Moreover, with scant distributional data and with long distances between collecting sites, this variation could lead to erroneous recognition of populations as different species. Therefore, we think it is important to provide any new morphological and distributional data.

Methods

Morphological characters studied mainly follow Lynch and Duellman (1997) although, following Padial & De la Riva (2005) and Padial *et al.* (2005), abbreviations and some measurements described below differ from those used by the above-mentioned authors. Measurements and their respective abbreviations are described as follows: snout-vent length, SVL; head length (from posterior margin of the lower jaw to tip of snout), HL; head width (measured at level of rictus), HW; eye length, EL; eye to nostril distance, EN; inter-narial distance, IND; eye to eye distance (distance between the anterior margins of eyes), EE; tympanic membrane height, TYH; tympanic membrane length, TYL; width of terminal disk of third finger, FIII; width of terminal disk of fourth finger, FIV; forearm length (from elbow to the proximal margin of thenar tubercle), ARM; tibia length, TL; thigh length, TH; foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL; width of terminal disk of fourth toe, TIV. We do not include values of interorbital distance (IOD) and upper eyelid width (EW). Our experience indicates that these parameters are usually of scarce utility because the preservation condition of a specimen highly influences the measurements and makes it difficult to have precise and comparable values. Specimens were measured by JMP with a digital calliper to the nearest 0.01 mm. To avoid pseudo precision we rounded all measurements following Hayek *et al.* (2001). Specimens studied are deposited in the following collections: Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural de Cochabamba, Cochabamba, Bolivia (MHNC); National Museum of Natural History, Washington DC. USA (USNM); Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK). All specimens examined are listed in the Appendix.

Results

A single adult female (USNM-346140; Figure 1) collected by A. Salas in a cloud forest at 1700 m in Department Cusco, on 22 September 1991, represents the first and only record for Peru (see appendix). New records in Bolivia are: an adult male from Serranía Bellavista in Department La Paz (CBF-3701); a juvenile from Cotapata National Park, also in Department La Paz (CBF-4120); and an adult female from Pampa Grande, Altamachi, Department Cochabamba **(**MHNC-AMS-196**)**. Thus, the known distribution of *E. mercedesae* comprises a narrow band of Montane Forest from 1400–1950 m altitude, and about 300 km in length in Bolivia, and a single record in Peru (1700 m), separated by a distance of 480 airline km from the closest Bolivian records (see Figure 2). The Peruvian record, together with recent taxonomic discoveries (e.g. Lehr *et al.* 2004), clearly shows how incomplete our knowledge on the diversity of *Eleutherodactylus* in Peru is.

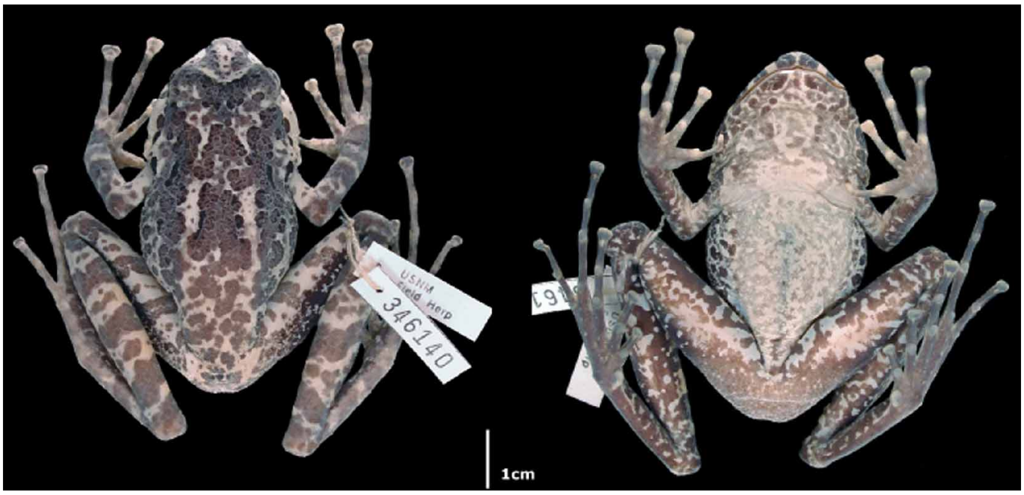

FIGURE 1. Dorsal and ventral views of adult female *Eleutherodactylus mercedesae* (USNM-346140) from Peru (SVL =62.1).

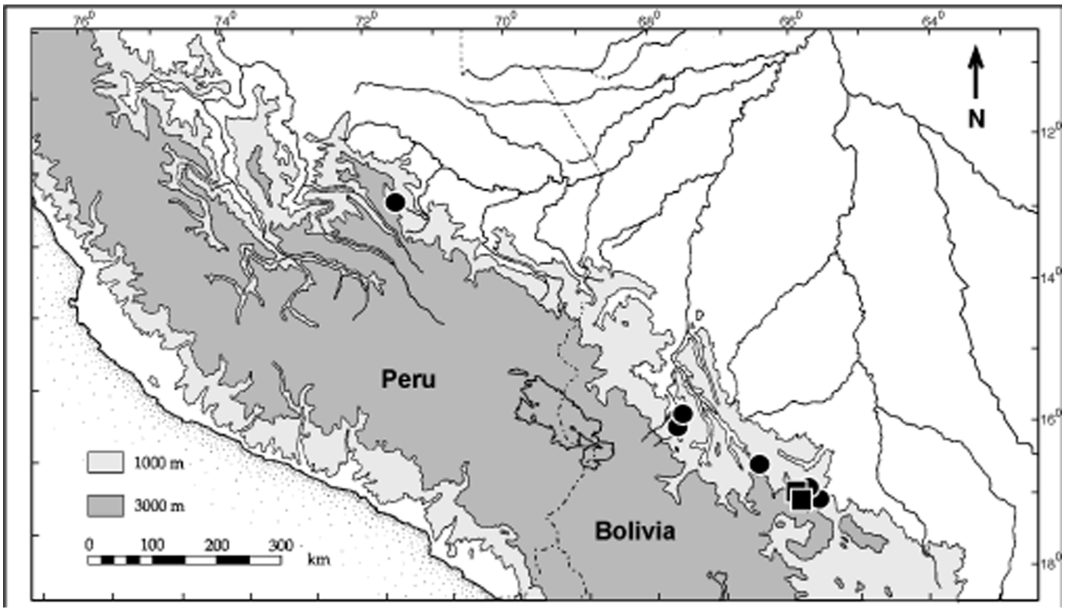

FIGURE 2. Map of Southern Peru and Northern-Central Bolivia showing the collecting sites of *Eleutherodactylus mercedesae*. Squares indicate the localities mentioned in the original description.

We herein provide information on some adult characteristics not mentioned for the types and a more detailed morphometric account (Tables 1 and 2). The description of juvenile *Eleutherodactylus mercedesae* (ZFMK-72571-3, 72597-99) by Köhler (2000) is adequate and coincident with our observations; thus, we do not describe juveniles herein.

Coloration in alcohol. Adult females are very similar to males (both types and CBF-3701) in coloration and pattern (Figure 1). The dorsum is greenish-grey to brownish-grey with cream irregular marks; the belly and throat show a cream background with moderate to intense brown mottling. All specimens have an inverted “V” or a rhomboidal cream mark on brown background on the throat (as the holotype and some specimens of *Eleutherodactylus rhabdolaemus*). Another characteristic is a butterfly-like cream mark on the scapular region (also observed in the holotype). Some black bold marks are present in both sexes, in the anterior margin of flanks, scapular region, interocular region, loreal region, subocular region, supratympanic fold, and dorsal surfaces of forearm.

Skin texture. Although in the original description the dorsal skin was described as finely granular, some specimens have a smooth skin (e.g. MHNC-AMS-196). The number of warts and granules on dorsum, head and flanks is also variable. For example, eyelid tubercles and occipital warts are absent in MHNC-AMS-196 and some of these structures have almost disappeared in the types. Dorsolateral and lateral folds are only present in the paratype.

Sexual characters. USNM-346140 (Figure 1) is a gravid female with unpigmented eggs, grouped in five round and concatenated packets in each oviduct. This morphology of oviducts has been not previously observed in any of the other Bolivian species (pers. obs.). Males have white testis, as all other Bolivian species (pers. obs.). Lynch & McDiarmid (1987) diagnosed the absence of nuptial pads in males of *E. mercedesae*, but a re-examination revealed a single, faint, low, white glandular nuptial pad on the dorsal surface of the thumb, more evident in the paratype (USNM-165753) than in the holotype (USNM-257848) and the specimen CBF-3701. The sexual dimorphism in size is evident, being females larger than males, although there are not differences in proportions (Tables 1 and 2).

TABLE 1. Measurements (in mm) and proportions of adult specimens of *Eleutherodactylus mercedesae*.

	Males			Females	
	USNM257848 (Holotype)	USNM165753 (Paratype)	CBF3701	USNM346140	MHNC-AMS-196
SVL	41.7	52.2	51.5	62.1	63.3
HL	16.1	19.8	18.2	24.6	24.5
HW	16.5	18.2	18.2	26.1	24.6
EL	5.1	6.2	5.5	7.0	5.9
EN	4.9	6.5	6.4	7.5	6.4
IND	4.0	4.7	4.4	5.7	5.5
EE	7.4	8.4	8.0	10.8	10.3
TYH	2.3	2.9	2.5	4.0	3.6
TYL	1.9	2.6	2.7	3.4	3.3
FIII	2.2	2.9	2.3	3.4	2.8
FIV	2.3	2.9	2.1	3.6	2.8
ARM	9.4	12.0	12.6	15.7	15.1
TL	28.0	35.4	35.5	43.6	42.6
TH	24.8	30.8	31.7	40.8	38.7
FL	23.5	29.3	29.4	38.9	36.5
TIV	1.8	2.2	1.8	2.6	1.9
TL/SVL	0.7	0.7	0.7	0.7	0.7
FL/SVL	0.6	0.6	0.6	0.6	0.6
HL/SVL	0.4	0.4	0.4	0.4	0.4
HW/SVL	0.4	0.3	0.4	0.4	0.4
HW/HL	1.0	0.9	1.0	1. 1	1.0
EN/EL	1.0	1.0	1.2	1.1	1.1
TYL/TYH	0.8	0.9	1.1	0.9	0.9

TABLE 2. Means and ranges of morphometric proportions of adult specimens of *Eleutherodactylus mercedesae*.

Proportions	Males (n = 3)	Females (n = 2)
TL/SVL	0.7 (0.7–0.7)	0.7 (0.7–0.7)
FL/SVL	0.6 (0.6–0.6)	0.6 (0.6–0.6)
HL/SVL	0.4 (0.4–0.4)	0.4 (0.4–0.4)
HW/SVL	0.4 (0.3–0.4)	0.4 (0.4–0.4)
HW/HL	1.0 (0.9–1.0)	1.0 (1.0–1.1)
EN/EL	1.1 (1.0–1.2)	1.1 (1.1–1.1)
TYL/TYH	0.9 (0.8–1.1)	0.9 (0.9–0.9)

Remarks. In most *Eleutherodactylus* from Bolivia, males have more rounded tympanic membranes and females more elliptical ones (JMP pers. obs.). In *E. mercedesae,* both sexes have an elliptical tympanic membrane, being the paratype the only exception. Finger lengths (3>4>1>2), toe lengths (4>3>5>2>1), and disc shapes are similar in males and females. In the female USNM-346140, low occipital “W” shaped crests are also evident, what is exclusive of this specimen. The tarsal fold is absent in MHNC-AMS-196. Finger and toe fringes are present in different degrees in all adult specimens. Development of supernumerary tubercles in the hand is also variable, being big and prominent in the holotype and less prominent in MHNC-AMS-196, which could be an artifact of preservation.

No relevant differences were found among males and females, besides from the usual sexual dimorphism present in other *Eleutherodactylus*. More relevant aspects are that both males and females are identical in the proportions (see Tables 1 and 2) and no remarkable differences were found among specimens from different localities.

The observed discontinuities in the distribution of *E. mercedesae* are surely due to the lack of adequate sampling.

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Appendix

Specimens examined

Eleutherodactylus mercedesae: BOLIVIA. **Department Cochabamba:** “Old” Chapare road, 1400–1500 m, (ZFMK-72571-73); “Old” Chapare road, 1650 m, (ZFMK-72597–99); Pampa Grande, Departmental Protected Area Altamachi, (MHNC-AMS–196); 3.3 km N of Cochabamba to Villa Tunari road on road to San Onofre, at a point 97.5 km from Cochabamba on road from Cochabamba to Villa Tunari, 1690 m, 17º10’S/65º46’W (USNM-257848, holotype); Limbo, 73.5 Km from Cochabamba to Villa Tunari, 1950 m, 17º10’S/65º48’W (USNM–165753, paratype). **Department La Paz:** Hornuni, Cotapata National Park, Prov. Nor Yungas, 16º12’50’’S/67º53’10’’W (CBF-4120); road to Coroico, Serranía Bella Vista, Province Nor Yungas, 16º14’S/67º43’W (CBF-3701). PERU. **Department Cusco:** Paucartambo, 68 km by road NE of Puente Unión on río Tachila (Bosque de las Nubes, km 150 on Paucartambo-Atalaya road), 1700 m, 13º14’13’’S/71º34’00’’W (USNM-346140).

APÉNDICE VII

Herpetologica, 63(1), 2007, 114–122

A NEW SPECIES OF THE *ELEUTHERODACTYLUS DISCOIDALIS* GROUP (ANURA: BRACHYCEPHALIDAE) FROM CLOUD FORESTS OF PERU

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Abstract: We describe a new species of the *Eleutherodactylus discoidalis* group from cloud forests of the Apurimac and Kosñipata valleys, southern Peru. The new species differs from other species of the group mainly by having a coarsely shagreen dorsum, long and slender hind legs, very long feet, Finger I equal in length to Finger II and fingertips not expanded. The species also lacks supernumerary tubercles on the feet.

Key words: Anura; Brachycephalidae; *Eleutherodactylus discoidalis* group; new species; Peru

Lynch (1976) defined the *Eleutherodactylus discoidalis* Group and recognized five species: *E. cruralis, E. discoidalis, E. elassodiscus, E. granulosus* and *E. nigrovittatus*. Subsequently, Lynch, (1989) considered *E. granulosus* to be a junior synonym of *E. cruralis*, and split the former *E. discoidalis* Group, leaving it with only the two southern species: *E. discoidalis* (Argentina and Bolivia) and *E. cruralis* (southern Peru and Bolivia) (De la Riva, 1993). Recent studies in poorly surveyed areas of Bolivia revealed three new species of *Eleutherodactylus* belonging to this group: *E. zongoensis, E. ibischi* and *E. madidi* (Padial et al., 2005; Reichle and Köhler, 1997; Reichle et al., 2001). Members of the *E. discoidalis* Group are terrestrial and inhabit different ecoregions: the Tucumanian and Yungas Forest (*E. discoidalis*), the Andean Montane Rainforest (*E. cruralis, E. madidi, E. zongoensis*), the Amazonian Lowland Rainforest (*E. cruralis*), and the Inner Andean Dry Forest (*E. ibischi* and *E. cruralis*) (De la Riva et al., 2000; Köhler, 2000; Padial et al., 2005). In Peru, only *E. cruralis* occurs, and it is found in the lowland rainforest and adjacent foothills of the Andes. Nevertheless, examination of some specimens from the Apurimac River Valley and the Kosñipata Valley (Peru) identified as *E. cruralis* in the collection of the National Museum of Natural History (Washington DC) and the American Museum of Natural History (New York), led us to think that there was one undescribed species. After the study of the latter and additional specimens recently collected in the Kosñipata Valley, and comparisons with all types of currently recognized and synonymized species of the group and most species of the putatively related genus *Oreobates* (sensu Caramaschi and Canedo, 2006), we concluded that these specimens represent an unknown species, which is described herein.

Material and Methods

For morphological and color characteristics used in the diagnosis and description, we followed Lynch (1989) and Lynch and Duellman (1997). In the Appendix, we include a list of specimens examined. We took measurements with a digital caliper to the nearest 0.01 mm, but we rounded all measurements to only one decimal to avoid pseudo-precision (Hayek et al., 2001). Measurements taken are: snout–vent length, SVL; head length (from posterior margin of lower jaw to tip of snout); head width (measured at level of rictus); eye length (measured horizontally); eye to nostril distance (from the anterior corner of the eye to the centre of the nostril); internarial distance; eye–eye distance (between the anterior margins of the eyes); tympanic membrane height; tympanic membrane length; arm length (from posterior margin of thenar tubercle to elbow); tibia length; thigh length (from vent to knee); and foot length (from proximal border of inner metatarsal

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FIG. 1.—*Eleutherodactylus lehri* sp. nov. (MHNC 4564) from Esperanza, Kosñipata Valley, Department Cusco, Peru.

tubercle to tip of fourth toe). Museum abbreviations other than cited by Leviton et al., (1985) are: Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Cochabamba, Bolivia (CBG); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNK–A [Amphibian Collection]); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Peru (MHNSM), Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru (MHNC).

SYSTEMATICS

Eleutherodactylus **lehri** *sp. nov.*

(Figs. 1, 2, 3 and 4)

Holotype.—USNM 537848, an adult female from the Apurimac River Valley, Camisea Natural Gas Pipeline, Wayrapata Camp, 2445 m asl, (12°50′10″ S, 73°29′43″ W), Department Cusco, Peru, collected by E. Solezer on 31 July 1998 (field number, F 50066).

Paratypes.—USNM 537846–57 are paratopotypes: USNM 537846–7, two subadult males, collected by H. Gutiérrez on 30 July 1998 (field number, F50062, F50065); USNM 537849, a gravid female, collected by H. Gutiérrez on 3 August 1998 (field number, F50069); USNM 537850, a subadult male, collected by A. Portilla on 5 August 1998 (field number, F50096); USNM 537851, juvenile, collected by J. Icochea on 5 August 1998 (field number, F50097); USNM 537852, a subadult male, collected by A. Portilla on 7 August 1998 (field number, F50114); USNM 537853, juvenile, collected by J. Amanzo on 9 August 1998 (field number, F50135); USNM 537854–5, subadult male and female respectively, collected by J. Icochea on 11 August 1998 (field numbers, F50139–40); USNM 537856, juvenile, collected by A. Portilla on 11 August 1998 (field number, F50143); USNM 537857, a subadult male, collected by J. Icochea on 12 August 1998 (field number, F50147). AMNH 11831, an adult male from Pillahuata between Puente Kosñipata and road, 2430 m asl, (13°09′52″S, 71°35′46″W), Department Cusco, Peru, collected by L. O. Rodríguez on 15 January 1998. MHNC 4557, 4564–7, 4583–6, 4601–2, 4682–3 and MNCN 43740–1, Esperanza, Kosñipata Valley, 2600–2800 m asl, (13°10′56″S, 71°36′14″W), Department Cusco, Peru, collected by Juan C. Chaparro and A. Mendoza on 26 January to 23 February 2003 (MHNC 4557, 4564, 4602, 4683 and MNCN 43741, adult males; MHNC 4682 and MNCN 43740, adult females; MHNC 4565, 4601, subadult females; MHNC 4566–7, 4583–6, juveniles).

Diagnosis.—A member of the *Eleutherodactylus discoidalis* Group, as defined by Lynch (1989), characterized by: (1) skin on dorsum coarsely shagreen, granules regular in size, small, round, only some of them slightly enlarged; granules on flanks slightly larger than those of dorsum; venter smooth; posterior surfaces of limbs smooth to shagreen; discoidal fold present; no dorsolateral folds; postrictal glands weak or absent; (2) tympanic membrane and annulus distinct, their length about half eye length; supratympanic fold weak, short; (3) head large, slightly longer than wide or subequal; snout round in dorsal and lateral views; canthus rostralis sinuous in dorsal view, round in profile; (4) cranial crests absent; upper eyelid covered by small granules; (5) vomerine odontophores large, situated posteromedial to choanae; (6) males with vocal slits; (7) hands with long and slender fingers, first finger about same length as second; subarticular tubercles round to conical, well developed; supernumerary tubercles low, not prominent, round to conical, smaller than subarticular tubercles; terminal discs of

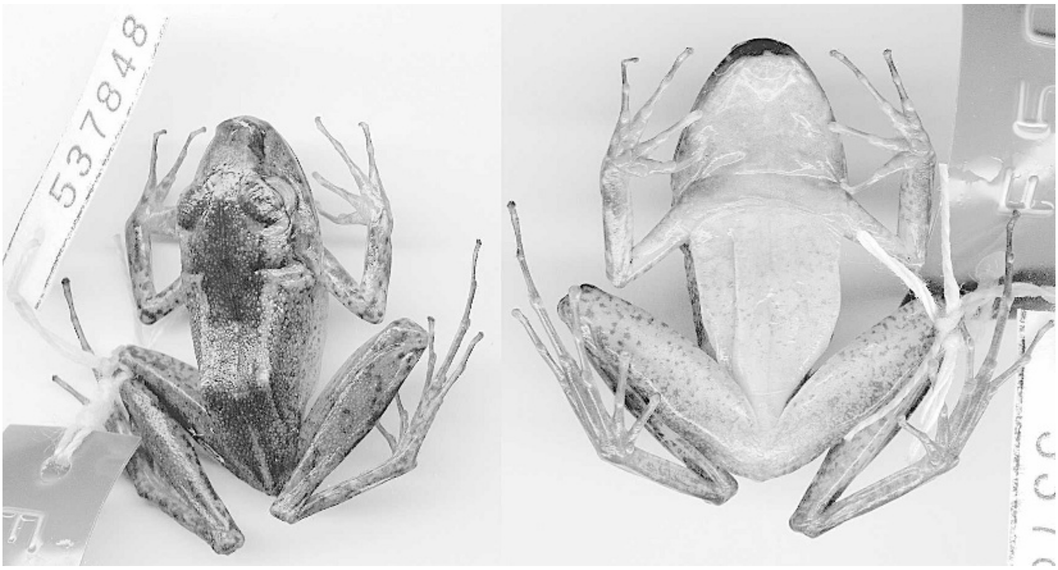

FIG. 2.—Dorsal and ventral views of the holotype of *Eleutherodactylus lehri* sp. nov. (USNM 537848) from Apurimac River Valley, Department Cusco, Peru.

fingers truncate to round, not enlarged, lacking circumferential grooves and ungual flap (Fig. 3); lateral fringes and keels on fingers absent; (8) ulnar tubercles absent; (9) no tubercles on heel and tarsus; (10) inner metatarsal tubercle ovate to round, prominent, outer smaller, round, prominent; supernumerary tubercles absent; (11) toes long and slender (foot length 60–70% SVL), lacking lateral fringes or keels (or very weak at the base of Toe III), webbing absent; fifth and third toes reaching midpoint of second subarticular tubercle of Toe IV; tips of toes moderately enlarged, rounded, with ungual flap not indented (Fig. 3); (12) dorsal coloration pale brown to dark brown or grayish brown with darker marks and bold spots, a pair of cream dorsolateral stripes and a short longitudinal sacral stripe present in some specimens; snout dark brown with darker bars; throat and chest light grey to dark brown; belly cream with brown mottling or reticulations on anterior margin.

Eleutherodactylus lehri can be distinguished from all other species of the group by its long feet, Fingers I, II, IV of the same length, absence of supernumerary tubercles on the feet, and coarsely shagreen dorsal skin. In all the species assigned to the *E. discoidalis* Group and species now placed in the genus *Oreobates* (except some individuals of *O. quixensis*), foot length ≤50% SVL, whereas in *E. lehri* foot length ≥60% SVL. Additionally, some qualitative characters distinguish this species as follows (character of the other species in parentheses). It differs from *E. cruralis* by having not expanded finger discs (truncate and enlarged on Fingers III and IV), larger females, Finger I equal to II (I > II), dorsal skin coarsely shagreen homogeneously (smooth to finely shagreen with enlarged warts), and no fringes (fringes well developed in fingers and toes). *E. lehri* can be distinguished from *E. discoidalis* as follows: vomerine odontophores posterior to choanae (between choanae), Finger I equal to II (I > II), tips of Fingers III and IV not expanded (truncate and expanded), tympanic membrane smaller or equal to half of eye diameter (larger), and dorsal skin coarsely shagreen (finely shagreen). *E. lehri* differs from *E. granulosus* by having longer feet and slender fingers and toes (shorter and more robust), low supernumerary tublercles on hands (very prominent), dorsal skin coarsely shagreen homogeneously (granular with some enlarged warts), slender extremities (robust), head slightly longer than wide or equal (wider than long), and smaller size of both males and females. *E. lehri* differs from *E. ibischi* by

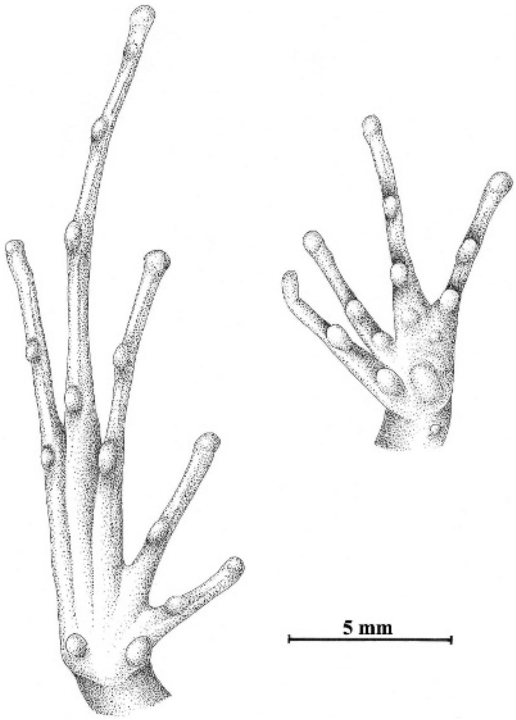

FIG. 3.—Right palm of the specimen USNM 537855 and plantar surface of the left foot of the holotype (USNM 537848) of *Eleutherodactylus lehri* sp. nov.

having tympanic membrane smaller or equal to half of eye diameter (larger), finger discs not expanded (discs on Fingers III and IV two times wider than the digits proximal to the disc), and dark brown dorsal coloration with cream stripes (light brown to beige with darker blotches). The new species can be distinguished from *E. madidi* by lacking enlarged fingertips (slightly enlarged), having Finger I equal to II (I > II), dorsal skin coarsely shagreen (homogeneously warty), and larger females. *E. lehri* can be distinguished from *E. zongoensis* by its shagreen dorsum (dorsum and extremities tuberculate in *E. zongoensis*), head longer than wide (wider than long), tympanic membrane smaller or equal to half of eye diameter (larger), labial bars present (absent), and ventral region cream with fine mottling (uniformly dark pinkish–brown).

Species of the genus *Oreobates* share many morphological characters with species of the *Eleutherodactylus discoidalis* Group (e. g., warty skin and absence of T–shaped digital tips). *E. lehri* can be distinguished from *O. saxatilis* by having dorsal skin coarsely shagreen (irregularly warty and tuberculate), Finger I equal to II (longer), and a different coloration; additionally, *E. lehri* is less robust and smaller (SVL of *O. saxatilis* to 63.0 mm). *E. lehri* has slender body and extremities than *O. sanctaecrucis*, possess shagreen skin (tuberculate in *O. sanctaecrucis*), lacks scarlet marks on dorsum (present), and lacks toe and finger fringes (present). *E. lehri* differs from *O. sanderi* by having Finger I longer than II, no finger fringes, and homogenously-sized granules on dorsum. *E. lehri* differs from *O. choristolemma* by having shagreen dorsum (tuberculate dorsal skin), smaller size, ungual flap not indented (indented on pedal digits II–V), and Finger I equal to II (longer). Moreover, all these species of *Oreobates* except *O. simmonsi* differ from *E. lehri* by having relatively shorter feet with prominent supernumerary tubercles; *O. simmonsi* has long feet. *Eleutherodactylus lehri* differs from *O. simmonsi* by having dorsal skin shagreen (skin of dorsum and limbs covered with uniformly-sized, spicule-like warts), Finger I equal to II (longer), and supratympanic fold present (absent). Additionally, *E. lehri* lacks finger and toe fringes and supernumerary tubercles on the feet.

Description of the holotype.—Head slightly longer than wide (head width/head length = 0.95); snout round in dorsal view and lateral profile; nostrils slightly protuberant, oriented laterally; canthus rostralis sinuous in dorsal view, round to slightly sharp in frontal profile; loreal region slightly concave, sloping gradually to the lips; lips not flared; upper eyelid without tubercles but covered by small granules; no cranial crests. Supratympanic fold distinct, thin; tympanic membrane and its annulus distinct; tympanic membrane nearly round, its length about half of eye length; postrictal glands absent. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, round, medial, separated by distance equal to 6–7 times diameter of choana; vomerine odontophores large, prominent, triangular in shape, situated posteromedial to choanae (posterior margin at level of choanae), their width about 1.5 times di-

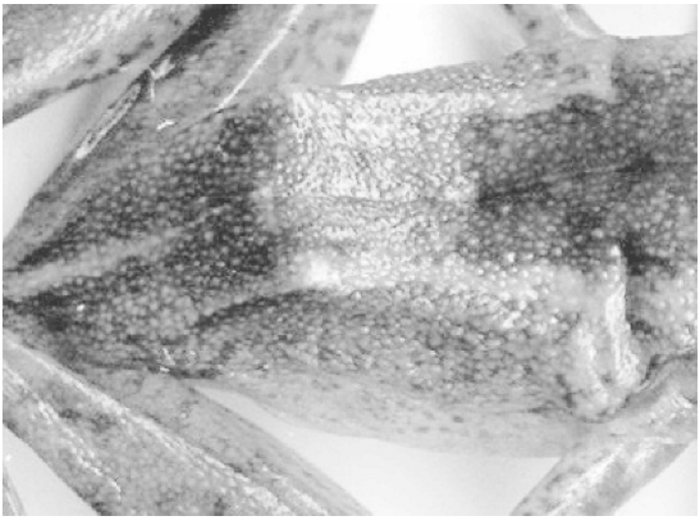

FIG. 4.—Detail of the dorsum of the holotype (USNM 537848) of *Eleutherodactylus lehri* sp. nov.

ameter of choanae, bearing 4–5 vomerine teeth; vocal slits short, placed posterolaterally. Skin of dorsal surfaces and posterior parts of hind limbs coarsely shagreen (Fig. 4); ventral surfaces smooth; no occipital folds; no dorsolateral folds; discoidal fold weak.

Ulnar tubercles or white spots present; palmar tubercle oval, flat, prominent, divided in two subunits, one larger, ovate, equal to elongate thenar tubercle, and one smaller, round, flat and prominent; supernumerary tubercles low, round, small, 4–5 on each hand; subarticular tubercles round to subconical, larger than supernumerary tubercles; finger tips truncate to round, not enlarged, of same width as corresponding digits; fingers lacking lateral fringes and keels; relative length of fingers: I = II = IV < III (Fig. 3).

Toes very long and slender (foot length 63% of SVL); heel and tarsus lacking tubercles or folds; inner metatarsal tubercle oval to round, prominent, slightly larger than outer metatarsal tubercle; metatarsal tubercle round, conical; supernumerary tubercles absent; subarticular tubercles prominent, subconical to conical, inclined; toes without lateral fringes and keels, except thin, short keel at the base of internal side of Toe III; toe tips rounded, slightly enlarged, about 1.3 times width of corresponding digit; ungual flap not indented; relative length of toes IV > III > V = II > I (Fig. 3); Toe III and V reaching penultimate subarticular tubercle of Toe IV.

Color.—In preservative, dorsal surfaces brown, with two dorsolateral light brown stripes from posterior margin of eyelid to groin; granules of dorsum cream on brown background; short cream stripe on sacrum; flanks dark brown with lighter granules; canthus dark brown, almost black; dorsal and loreal regions of snout lighter than rest of head; two subocular dark brown stripes; tympanic membrane dark brown; tympanic fold darker than membrane, almost black. Limbs light brown with irregular darker blotches, hands and feet slightly lighter; plantar surfaces dark brown with grey tubercles. Throat grayish-brown with cream mottling; belly cream with fine brown mottling; groin with reddish-brown mottling; posterior and anterior surfaces of hind limbs cream with dense brown mottling.

Measurements of the holotype.—SVL, 24.4; head length, 9.8; head width, 9.3; eye length, 3.6; eye to nostril distance, 3.3; internarial distance, 2.4; eye–eye distance, 4.1; tympanic membrane height, 2.0; tympanic membrane length, 2.0; arm length, 6.5; tibia length, 15.7; thigh length, 14.3; foot length, 15.1.

Variation.—Despite the sexual dimorphism in size (males are smaller than females), the proportions of males and females are almost

TABLE 1.—Morphometrics of *Eleutherodactylus lehri*. Mean ± standard deviation in parentheses follow ranges (in mm).

	Females (n = 4)	Males (n = 6)
SVL	31.0–39.9 (35.1 ± 3.9)	28.5–34.3 (32.1 ± 2.1)
Head length	12.8–16.0 (14.1 ± 1.5)	10.7–13.6 (12.5 ± 1.0)
Head width	12.4–15.2 (13.6 ± 1.4)	10.0–12.9 (11.9 ± 1.1)
Eye length	4.1–4.8 (4.5 ± 0.3)	4.0–5.1 (4.6 ± 0.5)
Eye–nostril	3.7–4.8 (4.2 ± 0.4)	3.0–4.1 (3.7 ± 0.4)
Inter-narial	3.3–4.4 (3.8 ± 0.5)	3.0–3.9 (3.4 ± 0.3)
Eye–eye	5.1–6.3 (5.7 ± 0.6)	3.2–5.3 (4.7 ± 0.8)
Tympanum height	2.1–2.6 (2.3 ± 0.2)	1.9–5.7 (3.1 ± 1.4)
Tympanum length	2.0–2.7 (2.3 ± 0.3)	1.9–5.7 (3.1 ± 1.4)
Arm length	8.1–8.7 (8.4 ± 0.3)	2.4–7.6 (6.1 ± 2.1)
Tibia length	18.9–25.4 (22.0 ± 3.0)	17.5–20.8 (19.7 ± 1.2)
Thigh length	18.4–21.7 (20.1 ± 1.6)	15.4–19.1 (17.9 ± 1.4)
Foot length	19.6–24.3 (21.8 ± 2.4)	18.2–20.2 (19.5 ± 0.7)
Tibia length/SVL	0.6–0.7 (0.6 ± 0.0)	0.6–0.6 (0.6 ± 0.0)
Foot length/SVL	0.6–0.7 (0.6 ± 0.0)	0.6–0.6 (0.6 ± 0.0)
Head length/SVL	0.4–0.4 (0.4 ± 0.0)	0.4–0.4 (0.4 ± 0.0)
Head width/SVL	0.4–0.4 (0.4 ± 0.0)	0.4–0.4 (0.4 ± 0.0)
Head width/Head length	1.0–1.0 (1.0 ± 0.0)	0.9–1.0 (1.0 ± 0.0)
Eye–nostril/Eye length	0.9–1.0 (0.9 ± 0.1)	0.7–0.9 (0.8 ± 0.1)
Eye diameter/ Head width	0.3–0.3 (0.3 ± 0.0)	0.3–0.4 (0.4 ± 0.0)
Tympanum length/height	0.8–1.2 (1.0 ± 0.2)	0.9–1.0 (1.0 ± 0.1)

identical (Table 1). Males bear posterolateral vocal slits but no nuptial pads. Vocal slits are present in some juveniles and subadult males, although not completely open. Skin texture is very homogeneous (as in Fig. 4), although there are slightly enlarged granules in some specimens. Some males have a more developed discoidal fold, which seems to be an artifact of preservation. A faint mid-dorsal fold is present in some individuals. Both a W-shaped occipital fold and a) (-shaped mid-dorsal fold are present in some specimens, although degree of development varies.

Dorsal coloration varies from dark brown with some irregular bold marks to light reddish-brown with irregular brown marks sometimes outlined of cream. Common dorsal marks are an occipital W-shaped mark, an arrow-shaped mid-dorsal mark and a transversal irregular ellipse in presacral region. A vertical light sacral stripe is present in all but some specimens. Some specimens bear a pair of dorsolateral cream stripes from posterior margin of eye to the level of mid-dorsum or to groin. Bold spots and lines usually outline the W-shaped occipital fold, the supratympanic fold, the ") (-shaped" mid-dorsal fold, and the border of the flanks. Labial bars also vary, with darker individuals having less conspicuous bars. The tympanic membrane and annulus are always brown. The throat pattern varies from light grey to dark brown. Some specimens have a fine light line in the middle of the dark throat. One specimen (USNM 537850) has an inverted cream "V" on brown background on the anterior part of the throat. The chest is usually mottled, with mottling diminishing to the belly. The belly varies from white to cream and usually have scarce fine brown mottling on the sides and anterior margin. Limbs are usually pigmented with transversal irregular bars, or transversal irregular ellipses. Arms are spotted with brown irregular marks. Plantar surfaces vary from dark grey to dark brown with grey or cream plantar tubercles.

In life (based on MHNC 4564 from Esperanza, Kosñipata Valley) the dorsum is pale brown with irregular dark brown or black spots and marks surrounded by beige, and some small red or orange spots. Some of the dorsolateral warts are bold, and the flanks are mostly pale brown with two or three oblique rows of dark brown spots. The lips have three bold bars separated by cream spots. The tympanic membrane is fleshy brown. The belly is white, and the throat has brown mottling. The inner surfaces of limbs are red to orange. Limbs and arms are brown with dark brown irregular bars. The iris is golden

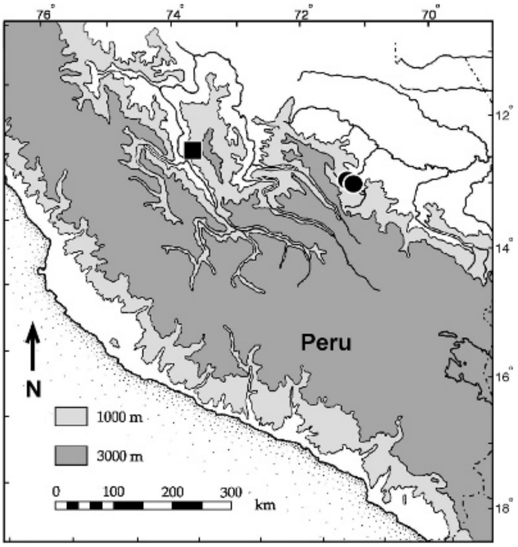

FIG. 5.—Map of southern Peru showing with a bold square the type locality of *Eleutherodactylus lehri* sp. nov. in the Apurimac River Valley and with bold circles the localities in the Kosñipata Valley.

dorsally and ventrally, with a metallic orange transversal stripe.

Distribution and ecology.—*Eleutherodactylus lehri* is known from the type locality in the Apurimac River Valley and from two localities in the Kosñipata Valley (Fig. 5). Both localities lie in cloud forests and are separated by an airline distance of approximately 200 km. Nothing is known about its natural history.

Etymology.—The name is a patronym for Edgar Lehr, German herpetologist and friend, who has contributed greatly to the knowledge of the Peruvian herpetofauna.

DISCUSSION

We conclude that the new species belongs to the *Eleutherodactylus discoidalis* Group sensu Lynch (1989). He proposed two putative synapomorphies for the two species (*E. cruralis* and *E. discoidalis*) then comprising the group: (1) conical subarticular tubercles, and (2) supernumerary plantar tubercles. In general appearance, the new species is very similar to members of the *E. discoidalis* Group although it lacks supernumerary tubercles on the feet (but they are present on the hands). Nevertheless, development of plantar tubercles varies between species. Moreover, validity of this character as a synapormophy for the group and, further, the monophyly of the group remain uncertain. With *E. lehri*, the *E. discoidalis* Group contains six species from southern Peru to northern Argentina. Five occur in Bolivia (*E. discoidalis, E. cruralis, E. ibischi, E. madidi* and *E. zongoensis*), one in Argentina (*E. discoidalis*) and two in Peru (*E. cruralis* and *E. lehri*) (Padial et al., 2005; Reichle and Köhler, 1997; Reichle et al., 2001). Lynch (1989) synonymized *E. granulosus* with *E. cruralis*, a likely unfounded taxonomic decision (J. M. Padial, personal observations). Boulenger (1903) described *E. granulosus* from Santo Domingo de Carabaya (SE Peru), a locality 200 km apart from the closest populations of *E. lehri*. Thus, there was the possibility of *E. granulosus* and *E. lehri* to be conspecific. Nevertheless, after the examination of the holotype of *E.* granulosus, we discard this possibility (see diagnosis for *E. lehri*).

Lynch (1989) considered the *E. discoidalis* Group to be more advanced than its putative sister taxa, the *E. binotatus* species group, that would be more closely related to the genus *Oreobates* (= *Ischnocnema*). Indeed, Lynch (1989) considered *Oreobates* as the putative ancestor of all *Eleutherodactylus*. Frost et al.'s (2006) phylogeny supports this scenario, since it places *O. quixensis* as basal to other *Eleutherodactylus* and *E. binotatus* as its sister group. Nevertheless, the placement of *O. quixensis* between other *Eleutherodactylus* in the phylogeny of hyloid frogs (Darst and Cannatella, 2004) lead us to think that the phylogenetic hypotheses of basal *Eleutherodactylus* are still inconclusive.

RESUMEN

Se describe una nueva especie del grupo *Eleutherodactylus discoidalis* de los bosques nublados de los valles Apurimac y Kosñipata, en el sur de Perú. La nueva especie difiere del resto principalmente por tener piel dorsal regularmente granular, extremidades traseras largas y delgadas, pies muy largos, el dedo I de la mano igual de largo que el II, las puntas de los dedos de la mano no dilatadas, y carecer de tubérculos supernumerarios en la planta de los pies.

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Appendix: Material Examined

Eleutherodactylus cruralis

BOLIVIA: Departamento La Paz: Boquerón, Pilón Lajas, CBG 792–93; Camino maderero El Chaval, Arroyo Mikai, Reserva Pilón Lajas, MNK A 3759–61; La Paz (locality in error), BM 1947.2.15.70 (holotype); Puerto Linares, 360 m, USNM 281100–30; 5 km W of San Buenaventura, USNM 280617; Departamento Cochabamba: Valle de Sajta, MNK A 3633; Villa Tunari, MNK A 1492; Los Guácharos MNK A 6617–19; Entre Paractito y El Palmar, ZFMK 72541; Departamento Pando: Florida, Reserva Manuripi, MNK A 5086; Lago Bay, Reserva Manuripi, MNK A 6120–21; Departamento Beni: Asunción, Pilón Lajas, MNK A 4074; Laguna Azul, Reserva Pilón Lajas, MNK A 3975–76, 3979, 3985–87, 4003; San Luis Chico, MNK A 4027; Serranía del Pilón, MNK A 4182–83, 4209–13. PERU: Departamento Madre de Dios: Puerto Maldonado, 30 km SSW of Tambopata, USNM 284267, 343240, 342989–92; Colpa de Guacamayos, Zona Reservada Tambopata-Candamo, USNM 332436–37. Department Puno: Santo Domingo, Carabaya, 6000 ft, BM 1947.2.15.72 (holotype of *Hylodes granulosus*).

Eleutherodactylus discoidalis

ARGENTINA: Province Tucumán: "13 km W Tucumán", Horco Molle, Sierra de San Javier, ca. 1200 m, BM

1947.2.15.63–65 (paralectotypes); Horco Molle, MCZ 35583; without locality, MCZ 117097; Province Jujuy: San Lorenzo, BM 98.7.7.19.20. BOLIVIA: Departamento Tarija: 12.3 km NW of Entre Ríos on the road to Tarija, MNK A 3877–97; 12.3 km NW of Entre Ríos on the road to Tarija, UTA 45645, 45648–50, 45652, 45658–62.

Eleutherodactylus ibischi

BOLIVIA: Departamento Santa Cruz: Km 68.5 on Santa Cruz de la Sierra-Samaipata road, 750 m elevation, CBF 3341 (holotype); Km 60 on Santa Cruz de la Sierra-Samaipata road, MNK A 6612; Samaipata, ZFMK 60402 (paratype).

Eleutherodactylus madidi

BOLIVIA: Departamento La Paz: Arroyo Huacataya, Serranía Eslabón, Área Natural de Manejo Integrado Madidi, MNK A 7856 (holotype), 7197 (paratype), MNCN 42014–15 (paratype); La Cascada, Biosphere Reserve Pilón Lajas, MNK A 4137–38 (paratypes).

Eleutherodactylus verrucosus

BRAZIL: "Byen [= city] Juiz de Fora i Minas Geraes" ZMUC 51 (R 1180) (holotype).

Eleutherodactylus zongoensis

BOLIVIA: Departamento La Paz: Valle de Zongo, 1250 m, CBF 2503 (holotype).

Oreobates quixensis

BOLIVIA: Departamento Pando, CBF 2528–29; Río Negro, MNK A 6525–27.

Oreobates sanctaecrucis

BOLIVIA: Departamento Santa Cruz: El Chapé, 2060 m elevation, MNK A 1198 (holotype), MNCN 42010–13.

Oreobates sanderi

BOLIVIA: Department La Paz: Arroyo Bilunto, Chunirumi Valley, Bilunto Mountains, Area Natural de Manejo Integrado Madidi, near Santa Cruz de Valle Ameno, CBF 5385 (holotype), MNCN 42016–7 (paratypes), CBF 5383–4 (paratypes); Bajo Hornuni, CBF 4119–22, 4218–19, 4223 (paratypes); Colonia Eduardo Avaroa, ca. 30 km north of Caranavi on the road from Caranavi to Yucumo, ZFMK 80600–1 (paratypes), MNK A 6563 (paratypes); Road from Apolo to Sarayo, MNK A 6695–6 (paratypes).

Oreobates saxatilis

PERU: Departamento San Martín: Pongo de Shilcayo, about 4 km NNW of Tarapoto, 470 m, MHNSM 8431 (paratype).

Oreobates simmonsi

ECUADOR: Morona-Santiago: Río Piuntza, 1830 m, KU 147068 (holotype).

APÉNDICE VIII

Zoological Journal of the Linnean Society (article in press)

Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement calls in the *Eleutherodactylus discoidalis* species group (Anura)

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The taxonomic status of a very poorly known group of Andean frogs (the *Eleutherodactylus discoidalis* group) is assessed through acoustic and statistical analyses of differences in temporal parameters of advertisement calls, such as number of pulses and call duration, but also in the spectral parameter of dominant frequency. Since these species are usually misidentified or ignored due to their taxonomic complexity in both ecological and biodiversity studies, we provide a bioacoustical diagnosis for each species in order to facilitate identification in the field. Differences in acoustic parameters support the specific status of *E. cruralis*, *E. discoidalis*, *E. ibischi* and *E. madidi*. The name *E. cruralis* is probably applied to three different species: the nominal form from Amazonian forests of the Andean slopes and adjacent lowlands, and two cryptic species restricted to inter-Andean dry valleys and cloud forests from central Bolivia. Moreover, the distribution of energy through the call and the aggregation of pulses seem to be useful meristic characters for detecting interspecific differences. Populations from each macrohabitat can be recognized by distinctive advertisement calls, usually corresponding to a recognized species. For the whole group, pulse rate is significantly correlated to latitude, which could indicate a speciation process along the Andes in relation to habitat changes and isolation.

ADDITIONAL KEYWORDS: advertisement call; Anura; *Eleutherodactylus discoidalis* group; geographic variation; taxonomy.

INTRODUCTION

Eleutherodactylus frogs, with nearly 500 recognized species, comprise one of the most diverse genera of terrestrial vertebrates (Frost, 2006; AmphibiaWeb, 2006). These are direct-development frogs that inhabit most tropical forests from Central America to northern Argentina (Lynch & Duellman, 1997). They are infamous among Neotropical naturalists for their incomplete and complex taxonomy and for the difficulties in their identification, both in the field and in the laboratory. This is mainly due to the high phenotypic variability within populations and scant morphological differences among species (Crawford & Smith, 2005). Given this scenario, misidentifications of *Eleutherodactylus* included in regional biodiversity lists, ecological studies, and even museum specimens, are frequent (JMP pers. obs.).

In order to facilitate taxonomic work, this large genus has been subdivided into several species groups (Lynch & Duellman, 1997). The *Eleutherodactylus discoidalis* group was defined by Lynch (1989) based on two putative morphological synapomorphies. This group is comprised of small, terrestrial frogs inhabiting the Andean slopes and adjacent lowlands from central Peru to northern Argentina (Padial, González & De la Riva, 2005). Until very recently, only two species were described and assigned to this group: *E. discoidalis* (Peracca, 1895) and *E. cruralis* (Boulenger, 1902) (Lynch, 1989; De la Riva, 1990, 1993). After intensive fieldwork in Bolivia, three new species were discovered. One species, *E. zongoensis* Reichle & Köhler, 1997, was defined on the basis of morphological characters, and two others, *E. ibischi* Reichle, Lötters & De la Riva, 2001 and *E. madidi* Padial, González & De la Riva, 2005, were distinguished mainly by advertisement call characters and subtle morphological differences (see Reichle & Köhler, 1997; De la Riva *et al.* 2000; Köhler, 2000a; Padial *et al.*, 2005 for recent accounts).

All of these species are still very poorly known. This is not surprising since most areas where these species occur are remote; they inhabit the understory of Andean tropical forests; they are very difficult to detect in the field even when they are calling because their calls are erratic (thus, obtaining recordings is also quite a difficult task); and they do not occur in high densities. Thus, *E. madidi* and *E. zongoensis* are only known from the types (six and one specimens, respectively) and *E. ibischi* from the two types and three additional specimens. *Eleutherodactylus cruralis* and *E. discoidalis* are the best-known species of the group; some data on their biology and ecology have been published (Köhler, 2000a; Lavilla *et al.*, 2000; Doan & Arizábal, 2002; Vaira, 2002; Duellman, 2005).

Lynch (1989), based on the differences in size, had already pointed out that what was then considered *E. cruralis* could probably be a composite of cryptic species (see also De la Riva *et al.*, 2000). This prediction was not unfounded, because all subsequently described species were more similar to *E. cruralis* than to *E. discoidalis*. Moreover, two new species are currently being described by us based on morphological characters only, and both of them are similar to *E. cruralis*. Based on current taxonomy, *E. cruralis* is the most broadly distributed species of the group, occupying various habitats from the lowland Amazonian forests and Andean foothills in southern Peru, to semi-deciduous and deciduous forests in central Bolivia (De la Riva *et al.*, 2000; Köhler, 2000a); *E. discoidalis* inhabits the Tucumanian-Bolivian montane forests, semideciduous forest and cloud forest of central and southern Bolivia and northern Argentina; *E. ibischi* inhabits exclusively semi-deciduous and deciduous mountain forests of the inter-Andean dry valleys in central Bolivia (Reichle, Lötters & De la Riva, 2001); *E. madidi* is known only from two localities in humid montane forests of northern Bolivia above 1000 m a.s.l. (Padial *et al.*, 2005); *E. zongoensis* is known only from Valle del Zongo in La Paz Department (Reichle & Köhler, 1997). The habitat (humid montane forest) at the type locality of *E. zongoensis* has been largely destroyed (Köhler, 2005).

Advertisement calls are important for mate recognition in anurans and hence contribute to pre-mating isolation among sympatric species (e.g. Duellman & Pyles, 1983). These calls are highly valuable for determination of species identity and solving taxonomic problems, and further allow for identification of cryptic species and hybrids (Heyer, García-López & Cardoso, 1996; Wycherley, Doran & Beebee, 2002; Angulo, Cocroft & Reichle, 2003; Gergus, Reeder & Sullivan, 2004). Indeed, in the absence of consistent morphological characters to distinguish among species, advertisement calls have proven to be of great help in detecting and describing putative new *Eleutherodactylus* (e. g. Köhler & Lötters, 1999; Köhler, 2000b; Reichle *et al.*, 2001). Within the *E. discoidalis* group, only the advertisement calls of *E. cruralis*, *E. ibischi* and *E. madidi* are known. Márquez, De la Riva & Bosch (1995) described the call of one population assigned to *E. cruralis* that was subsequently assigned to *E. ibischi* by Reichle *et al.* (2001). These authors also compared the advertisement calls of *E. cruralis* and *E. ibischi*. The call of Amazonian populations of *E. cruralis* was clearly different from that of *E. ibischi*, but certain populations from inter-Andean dry valleys, considered *E. cruralis*, showed some appreciable differences that were not analysed then. Köhler (2000a) described the call of a population from a transitional area between semi-deciduous and upper montane forest and found differences that led him to consider this population as *E.* cf. *cruralis*. He further noted some morphological differences, but data were too scarce to allow for the description of this population as a different species. The distinctive advertisement call of *E. madidi* was recently described (Padial *et al.*, 2005), and in this aspect the species was more similar to Amazonian *E. cruralis* than to *E. ibischi*.

Analyses of advertisement calls of members of the *E. discoidalis* group, including the undescribed call of *E. discoidalis*, revealed marked inter-populational differences not noted in previous studies. Since the taxonomic status of some populations is still unresolved (De la Riva *et al.*, 2000); a comparative approach should help to reassess the taxonomic status of the described species and to identify differences between populations that could indicate the existence of additional taxa and biogeographic patterns. Moreover, a comparative analysis will help to identify the most useful characters for detecting putative hidden species, and may provide other biologists with valuable information to better recognize the calls of previously described species. Therefore, we try to pinpoint quantitative and meristic characters that may allow for separation of species or populations of the same species. Finally, we discuss the current taxonomic status of the species and the consequences that could derive from this call analysis.

MATERIAL AND METHODS

The study area includes the Andean slopes (between 500 and 3000 masl) and adjacent lowlands, from southern to northern Bolivia (Fig. 1). We recorded advertisement calls along this latitudinal axis, in several habitats. See Table 1 for localities and their respective habitat association, and Köhler (2000a) for more details about the habitats.

We identified the recorded calls as advertisement calls based on the behaviour of observed frogs. Other call types are mostly the result of changes in individual motivation or interactions, while advertisement calls are usually emitted continuously under appropriate conditions with the goal of mate attraction (Duellman & Trueb, 1986). Recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone for the calls of *E. cruralis*, *E. ibischi* and *E. madidi*. The sounds were recorded on TDK SA60 cassettes, and digitized at a sampling rate of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board. *Eleutherodactylus discoidalis* was recorded with a Sharp MD-MT80 Minidisc digital recorder and a Hama 10 RMZ microphone at a sampling rate of 44.1 KHz and 32 bit resolution on a digital audio Sharp minidisc MD-R 80. The compression algorithm used for recording by earlier versions of minidisc recorders can distort sound at both frequency and intensity levels. However, this is the only recording available for *E. discoidalis*, which is described for the first time. Hence, we prefer to include this call in our analyses and assume the potential limitations. Moreover, other parameters, not related to intensity and frequency (see qualitative parameters below), are relevant for distinguishing species. All calls were edited with Audacity 1.2.6 for MacOS X (Free Software

Foundation Inc., 1991). Praat 4.5.02 for MacOS X (Boersma & Weenink, 2006) software was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through Fast Fourier Transformations (FFT) (width, 1024 points). Air temperature was measured immediately after sound recording. Recordings were deposited in Fonoteca Zoológica of Museo Nacional de Ciencias Naturales, Madrid. Museum abbreviations for voucher specimens refer to: Museo de Historia Natural Alcide d'Orbigny, Cochabamba, Bolivia (MHNAO); Centro de Estudios Tropicales, Sevilla, Spain (CET); Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Amphibian Collection of the Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNK-A, formerly NKA); and Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). Digitized calls were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (Madrid). Call vouchers are indicated in Table 2. We analysed 194 calls, of which 177 belonged to recognized species (*E. cruralis*, *E. discoidalis*, *E. ibischi* and *E. madidi*), and 8 and 9 calls from each of two populations of unknown taxonomic status (*E.* cf. *cruralis* from La Hoyada and Bellavista respectively; see Tables 1, 2); in addition, we include some information from published call descriptions (Köhler, 2000a; Reichle *et al.*, 2001) for comparisons with the results of our analysis (Table 2).

Table 1. Locality names, coordinates and habitat types for each studied species and population belonging to the *Eleutherodactylus discoidalis* group from Bolivia.

Species	Localities	Coordinates	Habitat	Habitat range (m a.s.l.)	Mean annual Temperature (ºC)	Mean annual precipitation (mm)	Arid months
E. cf. *cruralis*	Bellavista Mt.	18º11’S, 63º42’W	Inter Andean Dry Valleys	1500–3000	12–16	500-700	6–8
E. cf. *cruralis*	La Hoyada	17º54’S, 64º08’W	Upper Montane Rainforest	1500–2500	15–24	2500–6000	0–2
E. cruralis	Chapare, 500 m	17º04’S, 65º29’W	Amazonian Rainforest	100–500	25–27	1800–2200	0–3
E. cruralis	Mataracú	17º33’S, 63º52’W	Amazonian Rainforest	100–500	25–27	1800–2200	0–3
E. cruralis	Chalalán	14º25’S, 67º55’W	Amazonian Rainforest	100–500	25–27	1800–2200	0–3
E. cruralis	Rurrenabaque	14º28’S, 67º34’W	Amazonian Rainforest	100–500	25–27	1800–2200	0–3
E. discoidalis	Campos de Pinos	21º54’S, 64º31’W	Tucumanian-Bolivian Montane Forest	800–3000	13-23	1000-2000	3-5
E. ibischi	Masicurí	18º50’S, 63º35’W	Inter Andean Dry Valleys	1500–3000	12–16	500-700	6-8
E. ibischi	Samaipata	18º11’S, 63º34’W	Inter Andean Dry Valleys	1500–3000	12–16	500-700	6–8
E. madidi	Eslabón	14º20’S, 68º06’W	Upper Montane Rainforest	1500–2500	15–24	2500–6000	0–2

We analysed the following quantitative parameters: call repetition rate, number of pulses per call, call duration (ms), pulse rate within a call, fundamental frequency (Hz), dominant frequency (Hz), and frequency band (Hz); and the following qualitative parameters: presence/absence and distribution of separate pulses (in some calls one or two pulses are separated from the main group of pulses at the beginning or the end of the call), distribution of intensity (whether the maximum or minimum intensity is at the beginning or the end of the call), and presence/absence of frequency modulation. All of these call characteristics are commonly used for call descriptions and taxonomic recognition (e.g., Márquez *et al.*, 1995, Köhler, 2000a; Reichle *et al.*, 2001; Bosch & De la Riva, 2004; Padial *et al.*, 2005). Terminology in call descriptions generally follows Márquez *et al.* (1995) and Köhler (2000a). Sample sizes (Table 2) do not allow for temperature correction using regression. Results are presented in two ways: (1) description or re-description and comparison of advertisement calls based on published data and all new recordings; (2) comparison of different quantitative call parameters by stepwise discriminant analysis and scatterplots. Stepwise discriminant analyses on the mean values of the 194 calls recorded for 29 specimens were performed with JMP 5.0.1.a (SAS Institute Inc., 2002) (see Table 2 for the number of calls and individuals analysed for each species). The variables selected for this analysis were number of pulses per call, call duration (ms), and dominant frequency (Hz). Call rate and fundamental frequency were discarded because they were not available for all individuals.

RESULTS

DIAGNOSIS OF THE CALLS

In the following account we distinguish six call types, four of them matching well with the morphologically distinct nominal species and two others being different at least in some parameters (see Table 2 for quantitative data).

Eleutherodactylus cruralis *from Amazonian Lowlands (Fig. 2A)*: We considered populations from the Amazonian lowlands and Andean foothills to represent *E. cruralis*, because the type locality is supposed to be located in the Amazonian versant of Department La Paz (De la Riva, 1993). In addition, this assignment is based on the comparison of call vouchers (n=11) with the holotype of the species. The advertisement calls of these populations are composed of single, pulsed notes emitted at regular intervals, moderately modulated in amplitude and frequency, with maximum amplitude in the middle or the end of the note. The terminal pulse in some calls has more energy than the rest; no harmonic structure is present. The most distinctive feature of these calls is the presence of separated pulses of high amplitude at the end of the call. These separate pulses can be single or grouped in pairs but they are always clearly separated from the rest of pulses by a prolonged inter-pulse interval, and have higher amplitude. Despite some interpopulational variability (Table 2), calls among Amazonian lowland populations are more similar to each other compared to the two other populations assigned to *E. cruralis* (from La Hoyada and Bellavista Mts., see below). These differences can be due to different motivation of individuals at the time of recording, climatic conditions or geographic variation. The call is longer when compared to *E. ibischi* but shorter than that of *E. discoidalis* and *E. madidi.* In addition, it exhibits a very high pulse rate. The highest pulse rate of all populations (and species) corresponds to the population from Chalalán. The latter population also presents a higher dominant frequency, always above 3400 Hz. *Eleutherodactylus cruralis* calls from ground vegetation, under leaves and from low branches and stones. Calling activity is highest at sunset and at night during the rainy season.

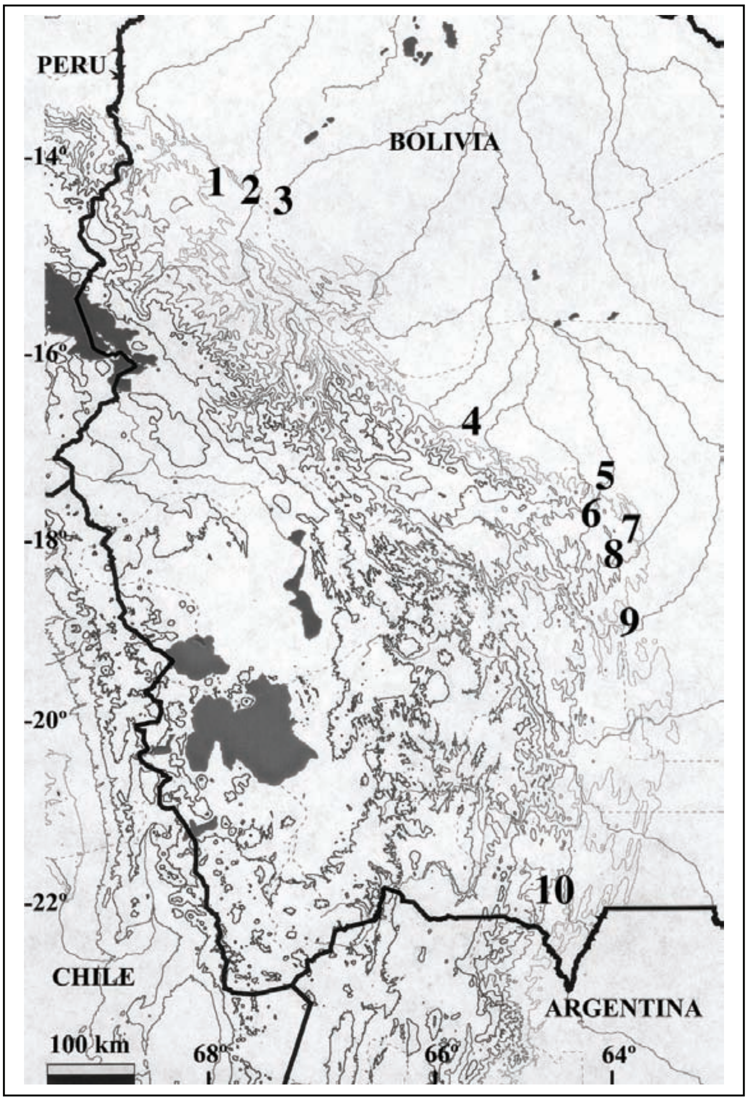

Figure 1. Map of the Andes of Bolivia showing the studied localities (see also Table 1). 1: Eslabón; 2: Chalalán; 3: Rurrenabaque; 4: Chapare 500 m; 5: Mataracú; 6: Hoyada; 7: Samaipata road; 8: Bellavista Mt.; 9: Masicurí; 10: Campos de Pinos.

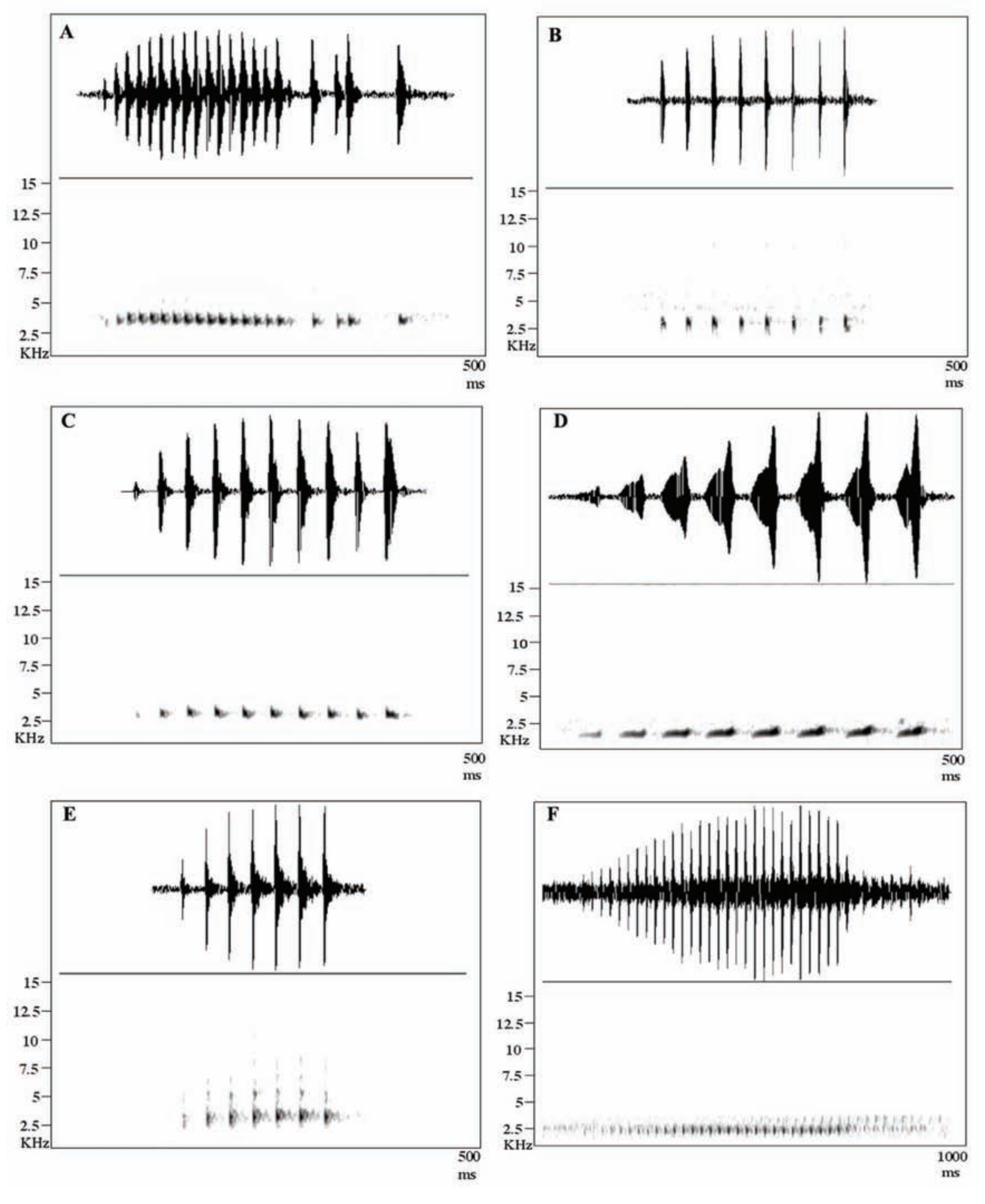

Figure 2. Oscillogram and sound spectrogram of the advertisement call of: A) *Eleutherodactylus cruralis* from Rurrenabaque, Amazonian rainforest; B) *E.* cf. *cruralis* from Bellavista Mountains; C) *E.* cf. *cruralis* from La Hoyada; D) *E. discoidalis* from Campos de Pinos; E) *E. ibischi* from Samaipata Road; F) *E. madidi* from Eslabón.

Eleutherodactylus *cf.* cruralis *from Bellavista Mountains (Fig. 2B)*: Individuals of this population emit a single, pulsed, moderately long call, slightly longer than that of some Amazonian individuals of *E. cruralis*, *E.* cf. *cruralis* from La Hoyada, and *E. ibischi*, but shorter than that of *E. discoidalis* and *E. madidi* (Table 2). It has an intermediate number of pulses, less than calls of Amazonian *E. cruralis*, *E. discoidalis, E. madidi*, and *E.* cf. *cruralis* from La Hoyada, but more than most calls of *E. ibischi*; the pulse rate is low and most energy is distributed between 2800 and 3800 Hz. The terminal pulse in some calls has more energy than the rest. Pulses are evenly distributed and there are no strong decreases in the amplitude envelope. There are relevant differences between these calls and those described by Reichle *et al.* (2001) for this area. The calls reported by Reichle *et al.* (2001) are longer, contain more pulses and have a lower dominant frequency than those recorded by us (see Table 2). This call can be heard during the night in the forest; males call from the ground or from low branches and stones.

Eleutherodactylus *cf.* cruralis *from La Hoyada (Fig. 2C)*: Köhler (2000a) illustrated and described the call of this population and we reanalysed it to compare it with new recordings. It is characterized by some amplitude modulation, with the first pulse being always relatively weak and with the last pulse separated by a longer interval; this terminal pulse has more energy than the others. Pulses are relatively evenly distributed and there are no strong decreases in the amplitude envelope. This call shows intermediate characters when compared with the Amazonian and Bellavista (see above) populations, except for the dominant frequency, which is lower in La Hoyada (Table 2). It can have the same number of pulses as the calls of *E. discoidalis*, more pulses than the call of *E. ibischi*, but less than those of Amazonian populations of *E. cruralis* and *E. madidi* (see Table 2). In addition, it has the lowest pulse rate of all known populations assigned to *E. cruralis* and the only species with a lower pulse rate is *E. discoidalis*. Köhler (2000a) noted some morphological differences in the single individual (ZFMK 72644) known from this population.

Eleutherodactylus discoidalis *(Fig. 2D)*: The call of this species is very distinctive to the human ear because it is more melodious, although the structure is conservative. The call is composed of a single, amplitude-modulated note containing 8–11 pulses with apparently no harmonics and a low pulse rate. The energy is distributed between 1706 to 3100 Hz, with a dominant frequency of 2200 Hz (see Table 2). *Eleutherodactylus discoidalis* has a call of intermediate duration, overlapping with calls of some Amazonian *E. cruralis*, while calls in *E. madidi* are longer and those of *E. ibischi* are shorter. The number of pulses overlaps with some call recordings of *E. ibischi* and *E.* cf. *cruralis* from Bellavista and La Hoyada. Pulse rate and dominant frequency are the lowest within the species group. The amplitude pattern of pulses differs from those of the other species. In *E. discoidalis*, the amplitude of each pulse increases first progressively in a bell-like shape and then increases energy abruptly to the maximum amplitude, to then fall, without modulation, back to background noise levels. In the other species of the group, the pulses show minor amplitude modulation after reaching maximum amplitude. In other words, intensity increases abruptly at the beginning of each pulse and decreases more slowly after reaching the maximum. This species was heard calling from the forest floor among herbaceous vegetation between 18:45–21:00 h in January 2005, especially after rain spells.

Eleutherodactylus ibischi *(Fig. 2E)*: Its call is short, has a reduced number of pulses, an intermediate pulse rate, and increasing amplitude to the end; a weak harmonic structure is discernible in some calls [fundamental frequency band plus dominant frequency band (see Table 2) and a third harmonic at 4500–8000 Hz]. The call energy can be distributed within a broad band, from 1400 to 4000 Hz. These characters make this call easily diagnosable. The last note is the most intense one or slightly less intense than the penultimate note. Calls may contain one or two initial pulses separated in time and amplitude. The dominant frequency is relatively low. As already noted by Reichle *et al.* (2001), *E. ibischi* can be easily distinguished from Amazonian *E. cruralis* by its call. Although the calls of some individuals overlap in call length, number of pulses and pulse rate with calls of the morphologically distinct individuals of *E.* cf. *cruralis* from Bellavista, the dominant frequency it is generally lower (Table 2 and Fig. 4B). No harmonics were detected, there are no separate pulses in the latter, and the frequency band is narrower. This call can be heard during sunset and at night in the forest. Individuals call from the ground, on stones or from low branches

Table 2. Numerical characteristics of the advertisement call of members of the *Eleutherodactylus discoidalis* group from Bolivia. The mean is followed by the standard deviation in the first parentheses and range in the second parentheses. SVL, snout–vent length (mm) of adult male specimens collected.

Species, locality, source (data from this study in blank)	**Calls analyzed (specimens)**	**Notes per call**	**Call rate**	**Call length**	**Pulses**	**Pulse rate**	**Frequency band**	**Fundamental Frequency**	**Dominant Frequency**	**ºC Air**	**SVL (mm)**	**Fonozoo**	**Vouchers**
E. cruralis Chalalán	4 (1)	1	-	227.3 (37.5) (179-260)	14.5 (1.3) (13-16)	63.4 (6.3) (58.4-72.6)	3400-4000	-	3733 (50) (3700-3800)	25	24.6	5229	MNCN 42560
E. cruralis Chapare (Reichle *et al.*, 2001)	-	1	-	352 (299-413)	15.3 (13-17)	44 (41-47)	Not given	-	3310 (3300-3330)	21.5	-	-	CBF 3347
E. cruralis Mataracú (Reichle *et al.*, 2001)	-	1	-	244 (228-272)	12.6 (12-14)	52 (51-53)	Not given	-	3410 (3390-3420)	24.5	-	37	NKA 3950, 3952, 3954, 3968
E. cruralis Rurrenabaque	53 (7)	1	9.34 (5.8-11.5)	373.1 (71.4) (210-620)	18.6 (2.5) (13-27)	50.9 (7.3) (37.5-72.4)	2500-3800	-	3090 (198) (2900-3500)	27	22.7, 25.4	5231-7	MNCN 42558-9
E. cf. *cruralis* Bellavista Mts.	9 (1)	1	6.7	212.0 (54.0) (107-316)	7.4 (1.8) (4-11)	34.7 (1.1) (33.65-37.4)	2800-3800	-	3236.4 (66.7) (3200-3400)	18	22.0	5228	MNCN 42557
E. cf. *cruralis* Bellavista Mts. (Reichle *et al.*, 2001)	-	1	-	370 (353-392)	12.3 (12-13)	33 (33-34)	Not given	-	2880 (2850-2900)	19.0	-	-	ZFMK 71997
E. cf. *cruralis* La Hoyada	8 (1)	1	8.2	331.5 (4.3) (326-338)	9.3 (0.5) (9-10)	27.9 (1.5) (26.6-30.5)	2400-3200	-	2760 (57.1) (2680-2831)	20.3	30.1	5755	ZFMK 72644
E. discoidalis Campos de Pinos	35 (10)	1	13.5 (7.6-27.8)	582 (61.7) (379-632)	8.9 (0.9) (8-11)	18,2 (1.2) (16.8-23.7)	1706-3100	-	2262 (80.9) (2114-2472)	17.4	27.9 (mean)	5749-5754	MHNC-A 1253
E. ibischi Samaipata Road (Reichle *et al.*, 2001)	10	1	-	131 (117-193)	5.3 (6-8)	41 (36-43)	-	-	2350 (2000-2500)	22	-	-	CBF 3341
E. ibischi Masicurí (Márquez *et al.*, 1995)	10	1	19.7 (3.8) (10.5-23.1)	149.2 (144.5-155.8)	6 (6-6)	40.2 (1) (38.5-41.5)	-	1599.2 (40.2) (1534.6-1655.8)	2588.7 (120.7) (2463.5-2847.1)	-	-		CET A1490
E. ibischi Samaipata Road	78 (8)	1	20.75 (16.3-24.7)	161.3 (43.6) (89-264)	6.26 (1.3) (4-9)	39.7 (4.0) (30.9-51.5)	1400-4000	1717 (184) (1200-2328)	2609 (165) (2254-2981)	22, 27	31.9, 28.9	5238-45	NKA 6612, MNCN 42959
E. madidi Eslabón	7 (1)	1-2	4.3	988.7 (50.6) (926-1075)	29.6 (1.4) (28-32)	29.9 (0.4) (29.3-30.3)	2200-2800	-	2436.39 (65.1) (2411.7-2584)	21	28.6 (mean)	5346	MNCN 42014, 42015

Eleutherodactylus madidi *(Fig. 2F)*: The call of this species is a long, multi-pulsed note (Padial *et al.*, 2005). It can be emitted as a single call, but when calling activity is higher, males usually emit two consecutive identical notes. This is the longest call among the species of the group, but the pulse rate and the dominant frequency are comparatively low (Table 2). Amplitude increases to the end of the note, but decreases in the last pulses. The single, collected specimen was calling from a natural cavity approximately 20–30 cm in diameter in the ground and at the base of large trees. The species calls during sunset and during the day.

COMPARISON OF CHARACTERS AND POPULATIONS

The stepwise discriminant analysis of the mean values of the calls of every individual for number of pulses, call length and dominant frequency separated the units considered (*E. cruralis*, *E. discoidalis*, *E.* cf. *cruralis* from Bellavista and La Hoyada, *E. ibischi* and *E. madidi*; see Fig. 3). All taxa were correctly predicted by the variables selected (p<0.001 in all cases, n=29, n-1 degrees of freedom). There were no cases of misclassifications.

Using number of pulses vs. call length (Fig. 4A), two parameters that are significantly correlated, we can separate almost all species in the scatterplot (r=0.62, p<0.001, n=29, n-1 degrees of freedom), but there is some overlap between *E. ibischi* and *E.* cf. *cruralis* from Bellavista and La Hoyada. However, these can be distinguished by qualitative parameters and dominant frequency. There are call length overlaps between La Hoyada

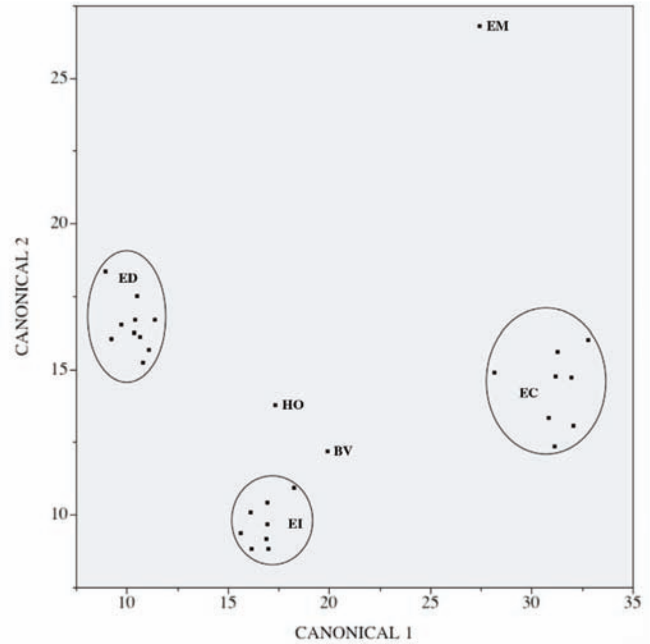

Figure 3. Discriminant function analyses of the number of pulses, call length and dominant frequency of species belonging to the *Eleutherodactylus discoidalis* group. Ellipses only intend to facilitate the observation of groups. BV, *E.* cf. *cruralis* from Bellavista Mountains; EC, *E. cruralis*; ED, *E. discoidalis*; EI, *E. ibischi*; HO, *E.* cf. *cruralis* from La Hoyada; EM, *E. madidi*.

population and some Amazonian *E. cruralis,* but they can be distinguished by the combination of pulse rate and call length. Amazonian *E. cruralis*, *E. discoidalis* and *E. madidi* are clearly distinguished by their call length and/or number of pulses. Comparing dominant frequency combined with number of pulses is useful for distinguishing *E. madidi* from Amazonian *E. cruralis*, and *E. ibischi* from *E.* cf. *cruralis* from Bellavista, but there is some degree of overlap between *E. ibischi*, *E.* cf. *cruralis* from La Hoyada and *E. discoidalis* (Fig. 4B). In the comparison of dominant frequency with call length (Fig. 4C), the overlap increases and only *E. madidi* remains completely isolated. Nevertheless, Bellavista and La Hoyada populations remain segregated between them and from *E. ibischi* and *E. discoidalis,* and the latter two species are also separated from each other. There is great overlap among Amazonian *E. cruralis* and those from Bellavista and La Hoyada. The dominant frequency of some calls of *E. ibischi* and *E. discoidalis* overlaps with that of *E. cruralis* (Table 2).

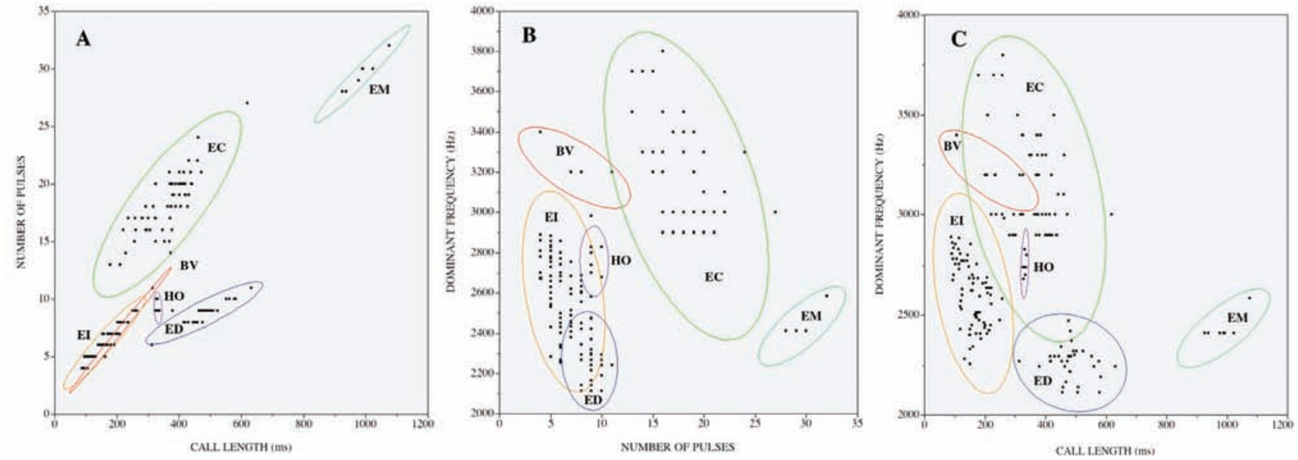

Figure 4. Scatterplot for: A) number of pulses and call length; B) dominant frequency and number of pulses; C) dominant frequency and call length of species and populations of the *Eleutherodactylus discoidalis* group. Lines correspond to normally distributed probability ellipses (0.99, n=194, n-1 degrees of freedom). BV, *E.* cf. *cruralis* from Bellavista Mountains; EC, *E. cruralis*; ED, *E. discoidalis*; EI, *E. ibischi*; HO, *E.* cf. *cruralis* from La Hoyada; EM, *E. madidi*.

Call rate was not included in the discriminant analyses or scatterplots because the calls of these species are emitted in an erratic fashion, i.e. in most cases, these frogs do not emit calls consecutively for a long period. Usually, they emit one vocalization only and do not emit another one in any predictable time. The highest call rate is for *E. ibischi,* which only overlaps partially with *E. discoidalis*. But the call rate of the latter ranges from 7.6 to 27.8 calls/min (Table 2), overlapping with all other species analysed. The lowest call rate is for a single individual of *E. madidi,* which only emits 4.3 calls/min. This species has the most erratic calling behaviour among all species of the group, and on occasions has been observed to emit only one vocalization in lapses of about 10 minutes. The call rate of *E. cruralis* overlaps with that of Bellavista and La Hoyada.

DISCUSSION

Amazonian populations of *E. cruralis* seem to represent a species with slightly variable advertisement calls (mainly in call length and number of pulses). Whether this variability is triggered by current environmental and/or behavioural factors, or is due to historical constraints, remains unknown. Variation in certain call parameters is related to observable factors. For example, dominant frequency is correlated with size (Márquez *et al.*, 1995) and pulse rate is correlated with body temperature (e.g., Gergus *et al.*, 2004). Furthermore, we found some differences between Amazonian *E. cruralis* and two populations previously considered as belonging to this species (see Köhler, 2000a; Reichle *et al.*, 2001): Bellavista and La Hoyada. Hence, the name *E. cruralis* could currently include three species, the nominal and two cryptic species inhabiting the inter-Andean dry valleys (Bellavista Mountains) and adjacent cloud forests (La Hoyada). Similar differences in the advertisement call, together with morphological traits, served for recognition of *E. ibischi* (Reichle *et al.*, 2001). The single specimen from La Hoyada shows some morphological differences compared to Amazonian *E. cruralis* (Köhler, 2000a), whereas specimens from Bellavista are morphologically similar to Amazonian *E. cruralis.* However, morphological distinctiveness is not a *sine qua non* condition for species recognition (Sáez & Lozano, 2005). Therefore, this population could represent a cryptic species within the *E. discoidalis* group, which seem to be morphologically conservative.

The calls of *E.* cf. *cruralis* from Bellavista and La Hoyada are most similar in structure to the call of *E. ibischi*. Moreover, *E. ibischi* and the population from Bellavista occur in very close sympatry. However, the call of *E. ibischi* has harmonics, a different dominant frequency, and tends to be modulated in amplitude and shorter. Furthermore, in this case, morphological differences allow unequivocal separation. Thus, although there is a high overlap in some quantitative parameters between *E. ibischi* and *E.* cf. *cruralis* from Bellavista, both seem to represent different lineages. Temporal quantitative parameters are responsible for most differences observed between these populations, but spectral characters, such as distribution of intensity in the call, and the presence of separated pulses, can also be relevant for distinguishing species.

Temporal call parameters that support the possible cryptic status of Bellavista and La Hoyada populations (i.e., number of pulses and pulse rate) are highly species-specific within the group. Moreover, there is evidence that suggests that pulse rate is anatomically constrained (Gerhardt, 2001) and is considered to be the most important call character involved in conspecific mate recognition in some species (i. e. Littlejohn, 1971; Gerhardt, 1994; Gergus, Sullivan & Malmos, 1997). However, although differences seem to be consistent, due to the low number of calls and specimens studied, we refrain to describe the populations as a new species until work in progress integrating bioacoustics, morphological and molecular analyses improve the knowledge of their taxonomic status.

The generic assignation of *Oreobates sanctaecrucis* was discussed by Harvey and Keck (1995) and Köhler (2000a). They pointed out that this species could also be a member of the *E. discoidalis* group. The call of *O. sanctaecrucis* was described and illustrated by Reichle (1999) and Köhler (2000a) as a very short, pulsed note with very high pulse rate and low dominant frequency, with a harmonic structure, and slight frequency and amplitude modulation. The structure of the call of *O. sanctaecrucis* is similar to the species studied herein. Since advertisement calls are mostly related to taxonomic position (Bosch & De la Riva, 2004) and could have some phylogenetic signal (Bosch & Boyero, 2003), the similarity between the advertisement calls of this species and those of the members of the *E. discoidalis* group could be used to reassess the generic placement of *O. sanctaecrucis*. Nevertheless, we have found similarities in call structure and quantitative parameters with other leptodactylid frogs inhabiting the central Bolivian mountain forests, such as *Phyllonastes* and a species of the *Eleutherodactylus unistrigatus* group (Köhler, 2000c). Thus, the generic assignation of this species should be reassessed using other methods (i.e. molecular phylogenetics).

All marked differences found between calls can be associated to different eco-regions. Thus, Amazonian forests, humid montane forests, cloud forests, Tucumanian-Bolivian montane forests and inter-Andean dry forests harbour populations with distinctive advertisement calls. This association seems to be related to another interesting feature of the geographic pattern in call variation. Latitude of populations is inversely and significantly correlated with number of pulses for each population ($r=-0.72$, $p<0.005$, $n=12$, $n-1$ degrees of freedom) and less significantly with pulse rate ($r=-$

0.63, p<0.05, n=12, n-1 degrees of freedom). In other words, the number of pulses and pulse rate tend to decrease to the south and increase to the north along an axis that crosses all aforementioned habitats of the Andes. A similar pattern that seems to be related to a speciation process has been found for European frogs of the genus *Rana* (Wycherley *et al.*, 2002). Two processes, drift and selective pressures, together or independently, may be responsible for the observed pattern. Vicariant events would have favoured genetic drift, which could have led to the observed patterns of interpopulational differences. Additionally, environmental selection may also have favoured divergence under different climatic and structural conditions of habitats. Wycherley *et al.* (2002) argued that genetic drift could be more relevant for causing divergence than natural selection. On the other hand, Bosch & De la Riva (2004) noted that pulse rate and call length are strongly related to taxonomic position (evolutionary constraints) and not to the environment, which suggests that the evolution of these characters is more related to the mechanisms of pre-zygotic reproductive isolation than to adaptive changes to the environment. Ryan, Cocroft & Wilczynski (1990) attributed call differences in temporal parameters between subspecies to environmental selection.

In our case, call differences could reflect the consequences of isolation together with adaptation to particular habitats, with the subsequent impossibility to colonize adjacent habitats after some time of isolation (in the sense of Wiens [2004]). Because each ecoregion shows specific climatic conditions and a distinctive type of call, associated to it, environmental selection cannot be ruled out as an evolutionary force driving differences in advertisement calls in the *E. discoidalis* group. Bosch & De la Riva (2004) suggest that large-scale habitat characteristics (ecoregions) seem not to be related to call differences, but microhabitat appears to have at least some influence in frequency modulation. Nevertheless, among members of the *E. discoidalis* group, microhabitat use is very similar, if not the same. Hence, differential selective pressures imposed by the microhabitat do not appear to be likely at this scale, although an experimental approach could help to shed more light on this matter.

To explain the observed patterns of call variation along the Andes, two scenarios can be considered. If the species of the *E. discoidalis* group are derived from an ancestral stock of species closely related to the *E. binotatus* group (as hypothesized by Lynch, 1989), the colonization of the Andes by ancestors of the *E. discoidalis* group could have taken place in southern or central Bolivia. That would be supported by the presence of *E. heterodactylus* (putatively related to the *E. binotatus* group) in Cerrado mountain ranges of Bolivia (Padial & De la Riva, 2005). This is an intermediate region between the area of occurence of the *E. binotatus* group (central and eastern Brazil) and the Andes. Moreover, the call of *E. heterodactylus* has a single pulsed call with harmonic structure (Padial & De la Riva, 2005), very similar to that of *E. ibischi* (which inhabits a similar habitat). Colonization and divergence could have followed a latitudinal axis along the Andes, as revealed by call variation, reaching central Peru to the north. A similar scenario has been proposed for the speciation of high Andean *Proctoporus* (Reptilia, Gymnophthalmidae) (Doan, 2003). In this case, the most basal species diverged first, and the subsequent branching pattern followed a latitudinal axis to the north, coinciding in a south to north direction with the uplift pattern of the Andes. However, the north is richer in *Eleutherodactylus* species and may be hypothesized as the centre of origin, where members of the *E. discoidalis* group may have emerged from a common ancestor either with *Oreobates* (formerly *Ischnocnema*) or northern members of the *E. binotatus* group (Lynch, 1989). Unfortunately, *Eleutherodactylus* systematics is so incomplete (Frost *et al.*, 2006) that none of these hypotheses can be tested to date.

Despite the limitations in sample size, this study reveals substantial differences in advertisement calls among recently described species in the *E. discoidalis* group. We hope these results will help facilitate future work on a group of frogs difficult to find and identify, and hence are very poorly known and often overlooked in ecological and biodiversity studies of their habitats. Since our results contribute definitively towards the assessment and identification of the specific status of the taxa involved (*E. cruralis*, *E. discoidalis*, *E. ibischi* and *E. madidi*), we also hope that similar studies will expand to other taxonomically complex groups of *Eleutherodactylus*. Moreover, other call parameters not considered herein, as pulse duty cycle and its associated variables (pulse duration and pulse period) may be useful for distinguishing species of *Eleutherodactylus* as demonstrated for other Neotropical frogs (Angulo *et al.*, 2003), and should be included in future research on *Eleutherodactylus* bioacoustics.

Given the current status of the taxonomy and phylogenetics of the *Eleutherodactylus* species groups, more behavioural, molecular, biogeographical and morphological data are needed to achieve a better understanding of pattern and potential processes. Moreover, given the results presented herein, where all the species in the studied group have a similar call structure, it would be interesting to test if the calls contain some phylogenetic signal relevant to the group level. Analyses of the advertisement calls of populations occupying distinctive habitats indicate that more species could be hidden under the name of widespread species. Hence, priority should be given to populations of uncertain taxonomic status that occupy habitats different from those typical of the species to which they are currently assigned.

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APÉNDICE IX

Zoological Journal of the Linnean Society (accepted-a)

Systematics of *Oreobates* and the *Eleutherodactylus discoidalis* species group (Amphibia, Anura) based on two mtDNA genes and external morphology

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We present morphological and molecular (mtDNA) evidence supporting the validity and monophyly of the genus *Oreobates*. This genus also includes members of the former *Eleutherodactylus discoidalis* species group plus *E. heterodactylus*. The presence of prominent conical subarticular tubercles and prominent supernumerary tubercles associated with the axis of fingers and toes, the presence of glandular axillary pads, and the absence of vocal sacs are proposed as morphological synapomorphies. Species of this taxon form a well-supported crown clade in a phylogeny including members of the genera *Craugastor* and *Eleutherodactylus* (sensu lato). The sister taxon to *Oreobates* is the *E. martinicensis* series; *Oreobates* does not appear to be closely related to the *E. binotatus* series or to members of the *E. dolops* and *E. nigrovittatus* species groups as previously hypothesized. The taxonomic status of all species of *Oreobates* is reassessed. *Hylodes philippi* and *H. verrucosus* are removed from the synonymy of *O. quixensis*. We redescribe *O. cruralis* on the basis of the holotype and new material from Bolivia and Peru and restrict its distribution to the humid forests of the lowlands and adjacent foothills of the Andes from southern Peru to central Bolivia. *Oreobates granulosus* is rediscovered, redescribed, and resurrected on the basis of the examination of the holotype and additional material from Peru. Phylogenetic analyses of partial 16S mtDNA are used to test the independence of lineages (species). The fourteen species of *Oreobates* are distributed from southern Ecuador to northern Argentina.

ADDITIONAL KEYWORDS: *Eleutherodactylus discoidalis* group – mtDNA – *Oreobates* – phylogenetics – taxonomy

INTRODUCTION

Research on systematics of Neotropical amphibians experienced a boost in recent decades (Glaw & Köhler, 1998). Nevertheless, many taxonomic problems remain to be solved. New species are being described each year and many phylogenetic hypotheses are under review (Padial & De la Riva, 2006). Moreover, the degree of exploration is still incomplete for lineages, areas (Kress *et al.*, 1998), and DNA sequences (Vences & Köhler, 2006). Among brachycephalids, *"Eleutherodactylus"* are famous for their incomplete and complex taxonomy together with the difficulties in their identification. This is probably due at least in part to rarity of many species, which leads to low sampling for species descriptions. In order to facilitate taxonomic work, this large genus has been subdivided into several subgenera, series and species groups (Lynch & Duellman, 1997). More recently, it has been split in several genera (Crawford & Smith, 2005; Frost *et al.*, 2006). However, the taxonomic status and monophyly of most groups and taxa now included in the family Brachycephalidae have not been assessed. Examples of this include the *Eleutherodactylus discoidalis* species group and the genus *Oreobates*.

Jiménez de la Espada (1872) described the genus *Oreobates* and the species *O. quixensis* from the Ecuadorian Amazon. Subsequently, Lynch (1971) and Lynch & Schwartz (1971) considered *Oreobates* a synonym of *Ischnocnema*, a genus described by Reinhardt & Lütken (1862) to accommodate *Leiuperus verrucosus* Reinhardt & Lütken, 1862 from Minas Gerais (southeastern Brazil). Lynch & Schwartz (1971) also placed two *Hylodes* (=*Eleutherodactylus*) species illustrated by Jiménez de la Espada (1875) as synonyms of *I. quixensis*. The types of *Oreobates* and one of the *Hylodes* were considered lost and *I. verrucosa* was only known from the holotype, a badly preserved and broken juvenile specimen. Given this lack of relevant material for study, further taxonomic reassessment has not been possible. Therefore, subsequent authors that discovered new *Ischnocnema* species in the Andes (Lynch, 1974; Duellman, 1990; Harvey & Keck, 1995; Harvey & Sheehy, 2005; Padial *et al.*, 2005a) followed Lynch & Schwarz's arrangement (1971). However, Caramaschi & Canedo (2006) rediscovered *I. verrucosa* and placed *Ischnocnema* in the synonym of *Eleutherodactylus* Duméril & Bibron, 1841. They also resurrected the genus *Oreobates* for *O. quixensis* and the Andean species then assigned to *Ischnocnema*.

The *Eleutherodactylus discoidalis* group was proposed by Lynch (1976), who recognized five species: *E. cruralis* (Boulenger, 1902), *E. discoidalis* (Peracca, 1895), *E. elassodiscus* Lynch, 1973, *E. granulosus* (Boulenger, 1903) and *E. nigrovittatus* (Anderson, 1945). He defined this group on the basis of a unique combination of traits and treated it as monophyletic. Additionally, he noted some heterogeneity within the group as evidenced by the presence of pointed digital tips in *E. elassodiscus* and *E. nigrovittatus* in contrast to the rounded tips in the three southern species (*E. cruralis*, *E. discoidalis* and *E. granulosus*). He also pointed out that the southern species resembled the Brazilian species *E. octavioi* of the *E. binotatus* species group in having large outer metatarsal tubercles. Later, Lynch (1989) considered *E. granulosus* a junior synonym of *E. cruralis*, and split the former *E. discoidalis* group, leaving it with only the two southern species: *E. discoidalis* and *E. cruralis*. *Ischnocnema* was then considered phylogenetically more primitive or ancestral to *Eleutherodactylus*, and those species placed in the *E. binotatus* group were proposed as the species most closely related to *Ischnocnema* and ancestral to the *E. discoidalis* group (Lynch, 1989). He also proposed that a complex of species, the *E. fitzingeri* group of Lynch (1976) and Lynch & Myers (1983) [now divided into the genus *Craugastor* and the *E. conspicillatus* group (Crawford & Smith, 2005; Lynch, 1986)] would be advanced in relation to (and/or derived from) the frogs identified as members of the *E. discoidalis* group. Further, Lynch (1989) also rejected Savage's (1987) hypothesis of relationships that considered *Ischnocnema* and the Mexican genus *Tomodactylus* as sister groups.

More recently, molecular phylogenetic analyses placed *I. quixensis* among *Eleutherodactylus* and as sister group of the *E. binotatus* species group (Frost *et al.*, 2006); however, due to the limited taxon sampling the only supported conclusion was the non-monophyly of *Eleutherodactylus* (see also Darst & Cannatella, 2004). Hence, the taxonomic status and phylogenetic relationships of *Ischnocnema* could not be assessed, other than saying that *I. quixensis* was close to what we call *Eleutherodactylus* [a position that was already held by Jiménez de la Espada (1872, 1875)]. With the resurrection of *Oreobates* and its restriction to the Andean and Amazonian species, the hypotheses of relationships are still more uncertain. If *Iscnocnema verrucosa* is an "*Eleutherodactylus*" and *Oreobates* a valid taxon, which one among the several "*Eleutherodactylus*" groups is the sister clade of *Oreobates*? Moreover, is *Oreobates* monophyletic? Furthermore, if we assume no relationships of *Oreobates* with southeastern Brazilian *Eleutherodactylus* (*E. binotatus* series), a position held by Caramaschi & Canedo (2006), then we have to look for putatively related groups in the Andes or the Amazon. The *E. discoidalis* species group seems to be the best candidate. It shares with *Oreobates* many external morphological features, among them, those proposed by Lynch (1989) as synapomorphies for this group. Moreover, Padial *et al.* (2005a) already pointed out the difficulties of assigning some specimens from the Andes either to what was then considered *Ischnocnema* or to the *E. discoidalis* group. Further, some characteristics of the advertisement call of *O. sanctaecrucis* were considered similar, but putatively primitive, to those calls characteristics of the *E. discoidalis* group (Padial *et al.* in press). Finally, a species of the Brazilian shield recently rediscovered, *E. heterodactylus*, shows morphological characters of both the *E. binotatus* and the *E. discoidalis* groups (Padial & De la Riva, 2005; Padial *et al.* in press), and its phylogenetic relationships deserve a detailed study.

None of the previous hypotheses have been tested to date. Hence, the goal of our study is to answer the following questions: (1) are the *Eleutherodactylus discoidalis* group and *Oreobates* monophyletic? (2) What is their phylogenetic relatationship? (3) Do former members of the *E. discoidalis* group belong to this group? (4) What is the current species diversity of those taxa? (5) To which group does *E. heterodactylus* belong? To answer these questions we apply an integrative taxonomic approach sensu Dayrat (2005), Will *et al.* (2005) and Padial & De la Riva (2006). We include molecular phylogenetics (using mtDNA) and classical taxonomic analyses. We also consider Padial *et al.*'s (in press) analyses on advertisement calls as additional evidence to test our hypothesis. Finally, we provide a taxonomic account with remarks and redescriptions for several species whose taxonomic status is unclear or still very poorly known.

MATERIAL AND METHODS

EXTERNAL MORPHOLOGY

We followed Lynch (1989) and Lynch & Duellman (1997) for morphological and colour characteristics used in the diagnosis and description. A single person (JMP) took measurements with a digital calliper to the nearest 0.01mm, but to avoid pseudo-precision (Hayek *et al.*, 2001), we rounded all measurements to only one decimal. Abbreviations are as follows: snout–vent length, SVL; head length (from posterior margin of lower jaw to tip of snout), HL; head width (measured at level of rictus), HW; eye length (measured horizontally), EL; eye to nostril distance, EN; internarial distance, IND; eye-eye distance, EE; tympanic membrane height, TYH; tympanic membrane length, TYL; arm length (from posterior margin of thenar tubercle to distal point of elbow), FA; tibia length, TL; thigh length, TH (from vent to knee); foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL. We do not include values of interorbital distance (IOD) and upper eyelid width (EW). Our experience indicates that these parameters are usually of little utility because the preservation condition of specimens highly influences the measurements and makes it difficult to have precise and comparable values. Colour characteristics were noted in life and in alcohol. We determined age and sexual condition by dissection or observation of external secondary sexual characters. The condition of the trigeminal nerve (see Lynch, 1986) was determined through dissection of the skin above the tympanic area and through a horizontal cut of the mandibular joint. Museum abbreviations other than cited by Leviton *et al.* (1985) are: Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Cochabamba, Bolivia (CBG); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA [Amphibian Collection], formerly NKA); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Peru (MHNSM), Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru (MHNC). In the Appendix, we include a list of specimens examined.

MOLECULAR PROCEDURES AND PHYLOGENETIC ANALYSES

Origin of sequences, collection numbers and localities for each terminal and corresponding amplified fragment are detailed in Table 1. Following recent hypothesis of hyloid relationships (Darst & Cannatella, 2004; Frost *et al.*, 2006), we selected *Cryptobranchus* sp., *Rhinella amboroensis* (see Chaparro *et al.*, 2007), *Leptodactylus griseigularis* and *L. rhodonotus* as outgroup taxa. Sequences not amplified by us were downloaded from NCBI database (http://www.ncbi.nlm.nih.gov/) or provided by Matt Heinicke and Blair Hedges (*Eleutherodactylus dolops* and *E. elassodiscus*). We used the standard phenol-chloroform extraction protocol (Sambrook *et al.*, 1989) with minor changes to isolate genomic DNA. Fragments of approximately 591 bp from the mitochondrial gene 16S and approximately 350 bp of cytb were amplified using previously described PCR conditions (Hillis *et al.*, 1996), using the universal primers 16Sar-5’ and 16Sbr-3’ and cytb Z15-5’ and cytbB2-3’, respectively (Hillis *et al.*, 1996; Goebel *et al.*, 1999). Amplification PCR products were purified and sequenced by SECUGEN S.A (Madrid, Spain) in an ABI PRISM 3700 (Applied Biosystems) instrument. Sequences were edited in Sequencher 4.6 (Gen Codes Corporation © 1991–2006). Alignments were performed using the program CLUSTAL X 1.83.1 (Thompson *et al.*, 1997) under default parameters. Ambiguously aligned regions were removed from the analysis (see below). For Bayesian phylogenetic analyses (Rannala & Yang, 1996) we used MrBayes version 3.2.1 (Huelsenbeck & Ronquist, 2001). The majority rule consensus tree was produced from four Metropolis-coupled Monte Carlo Markov chains (MCMC; Yang & Rannala, 1997); each run used one cold chain (the head chain) and three heated chains (scout chains). The analysis was run simultaneously for ten million generations. Chain swapping and parameters update rates were monitored at the beginning and the end of the runned analyses to ensure that tree searches were being improved. Trees were sampled every 1000 generations. Burn-in was evaluated by examination of the standard deviation of split frequencies (> 0.01) to determine at what point the values had reached stationarity. Stationarity was reached after the first 1.000.000 generations and, hence, the first 1000 from 10001 concensus trees were discarded. Following Crawford & Smith (2005), default priors and conditions were used in all cases. Maximum Parsimony analyses (MP) analyses performed in PAUP* 4.0b10 (Swofford, 1998) using heuristic searches under parsimony (all characters weigthed equally) with TBR branch swapping and 1000 random addition sequence replicate with no limit imposed to the number of tree searched. In order to obtain estimates of clade support, non-parametric bootstrapping was performed with heuristic searches of 1000 replicate datasets and 100 random addition sequences per dataset with no limit imposed to the number of tree searched. Gaps were treated as fifth base in both parsimony and bayesian analyses (Crawford & Smitth, 2005).

Phylogenetic analyses are presented in two ways. First, the parsimony and Bayesian analyses of a 16S plus cytb, with data-partitioned dataset, includes 756 equal-weight characters (427 constant, 85 parsimony-uninformative and 224 parsimony-informative, gaps considered as “fifth-base”) of 14 taxa including outgroups. *Leptodactylus griseigularis* and *L. rhodonotus* were used as outgroups for this analysis. We removed 38 and 12 basepairs corresponding to ambiguously aligned regions of the 16S dataset. Second, MP and Bayesian analyses of the single 16S dataset, with larger taxon samplig, that includes 479 equal-weight characters (222 constant, 55 parsimony-uninformative and 192 parsimony-informative, gaps considered as “fifth-base”) of 32 taxa (four outgroups: *Cryptobatrachus* sp., *Rhinella amboroensis*, *Leptodactylus griseigularis* and *L. rhodonotus*). Uncorrected pair-wise distances for members of *Oreobates* were calculated in PAUP* 4.0b10 from this later dataset after removing ambiguous regions.

Table 1. Localities, voucher information, and GenBank accession numbers for sequences and specimens used in this study. Numbers in parentheses correspond to sequences used in previous studies: (1) Darst and Cannatella (2004); (2) Faivovich *et al.* (2005); (3) Frost *et al.* (2006). Abbreviations [other than cited in the text and in Leviton *et al.* (1985)]: CFBH: Celio F. B. Haddad specimen collection; ICNMNH: Instituto de Ciencias Naturales, Bogota, Colombia; JAC: Jonathan A. Campbell´s field series; JCC: Juan C. Chaparro´s field series; JDL: John D. Lynch's field series; SIUC: Southern Illinois University at Carbondale (USA).

Species	*Locality*	**Museum no.**	**MNCN DNA**	**16S**	**Cytb**
Craugastor					
C. augusti	México: Sonora: Alamos.	UAZ unnumbered	-	DQ283271 (3)	-
C. fitzingeri	Costa Rica: Limón: Estación Experimental La Lola	-	-	AY326001	-
C. rhodophis	México: Oaxaca: El Mirador, Municipio Santa María Chilchotla.	JAC 22721	-	DQ283317 (3)	-
Eleutherodactylus					
E. platydactylus	Peru: Cusco: Pantiacolla	JCC (PL025A)	20549	…	…
E. binotatus	Brazil: São Paulo: Parque Estadual da Serra do Mar, Núcleo Santa Virginia, São Luis do Paraitinga.	CFBH 5813	-	DQ283092 (3)	-
E. chloronotus	Ecuador: Napo: 3.5 km E Santa Bárbara.	KU 202325	-	AY326007 (1)	-
E. dolops	Colombia: Caquetá: municipio Florencia, vereda Santa Elena, 26.5 km (by road) arriba de Florencia ("poste 0"), 940 m	ICNMNH 23809	-	…	-
E. duellmani	Ecuador: Carchi: ~5 km W La Gruel; 2340 m.	KU 202404	-	AY326003 (1)	-
E. elassodiscus	Ecuador: Napo: Cuyujua.	KU 177282	-	…	-
E. fenestratus	Bolivia: Cochabamba: Los Guácharos	MNK A 6631	4088	…	…
E. thymelensis	Ecuador: Carchi; 12 km W Tufino; 3520 m.	KU 202519	-	AY326009 (1)	-
E. platydactylus	Bolivia: La Paz: Serranía Bellavista	MNCN-43224	3818	…	…
E. samaipatae	Bolivia: Santa Cruz: Quebrada a 6 Km de la Angostura en dirección a Samaipata	MNCN-42987	3899	…	…
E. supernatis	Ecuador: Napo; 3.5 km E Santa Barbara	KU 202432	-	AY326005 (1)	-
Oreobates					
O. choristolemma	Bolivia: La Paz: Boquerón	CBG 765, 768	5715, 5745	…	-
O. cruralis	Bolivia: Santa Cruz: Camino a Bella Vista.	MNK A7171	6098	…	…
O. discoidalis	Bolivia: Tarija: Serranía Aguarague.	MNK A 7247	6123	…	
O. discoidalis	Bolivia: Tarija: Entre Ríos, Chiquiacá.	MNCN-43133	6134	…	…
O. granulosus	Peru: Puno: Santo Domingo, Carabaya	MHNC 3396	20550	…	-
O. heterodactylus	Bolivia: Santa Cruz: Cerro del Arco, Serranía de Santiago.	MNK A7177	6018	…	…
O. heterodactylus	Bolivia: Santa Cruz: Cerro del Arco, Serranía de Santiago.	MNK A7175	6061	…	…
O. lehri	Peru: Cusco: Cosñipata Valley	MHNC 3236	20551	…	-
O. madidi	Bolivia: La Paz: Serranía Eslabón.	MNK A7856	5999	…	…
O. quixensis	Bolivia: Pando: San Sebastián.	MNCN-43147	6216	…	…
O. sanctaecrucis	Bolivia: Cochabamba: Chaquisacha.	CBG 153	5719	…	-
O. sanderi	Bolivia: La Paz: Santa Cruz de Valle Ameno	MNCN-42017	3723	…	…
Outgroups					
Leptodactylus griseigularis	Bolivia: la Paz: Serranía Bellavista	4118	3815	…	…
L. rhodonotus	Bolivia: La Paz: Serranía Bellavista	-	3836	…	…
Rhinella amboroensis	Bolivia: Santa Cruz: San Juandel Portrero	MNK-A 5302	…	DQ283386 (3)	
Cryptobatrachus sp.	Colombia: Santander: 7 km by road SW San Gil	JDL 14865		AY326050 (1)	

RESULTS

Inferred phylogenies and nodal support of Bayesian partitioned analyses of partial 16S and cytb support the monophyly of *Oreobates* (bpp = 100) and a group including the *Eleutherodactylus conspicillatus* and *E. martinicensis* series (sensu Lynch & Duellman, 1997) (bpp = 100; Fig. 1). Maximum Parsimony partitioned analysis of partial 16S and cytb resulted in two equally most-parsimonious trees, also with statistical support for both clades (bss= 98 and 85, for *Oreobates* and *Eleutherodactylus* respectively; Fig. 1). Bayesian analyses of partial 16S alone (with larger taxon sampling) are concordant in their support for the monophyly (bpp ≥0.95) of the following main clades: *Oreobates*, *E. conspicillatus* series plus *E. martinicensis* series, *Craugastor* and *E. dolops* plus *E. nigrovittatus* species groups. Maximum parsimony analysis for the same dataset of partial 16S resulted in three equally most parsimonious trees with statistical support for the monophyly of *Oreobates*, *Craugastor* and the *E. dolops* group (Fig. 2). Within the clade *Oreobates*, there are two evident and well-supported subclades (bpp>0.98). One clade contains *E. cruralis*, *E. madidi*, *E. discoidalis*, and *E. heterodactylus*,

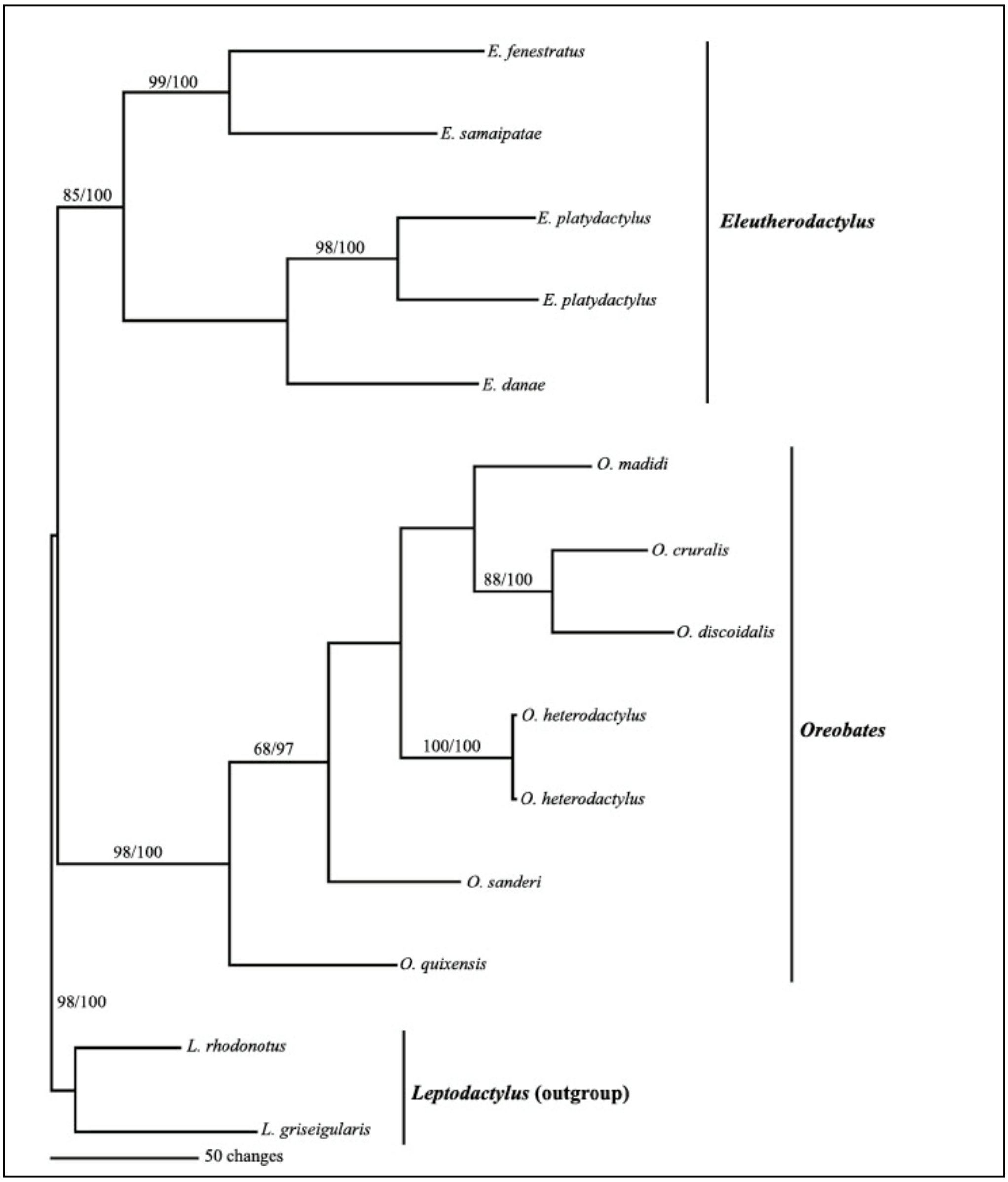

Figure 1. Majority rule concensus tree based on Maximum Parsimony (MP) and Bayesian phylogenetic analyses of combined data from the partial cytb (ca. 350 bp) and 16S (ca. 590 bp) mtDNA. Numbers above branches indicate boostrap support (≥50%) for the MP topology followed by Bayesian posterior probabilities for the Bayesian topology (≥95).

and the other contains *O. choristolemma*, *O. sanctaecrucis*, *O. sanderi* and *O. granulosus* (Fig. 2). *Oreobates quixensis* and *O. lehri* are the sister group of these two subclades in the Bayesian and MP analyses (Figs. 1–2). The sister group of *Oreobates* is the subgenus *Eleutherodactylus* (including the *E. conspicillatus* and *E. martinicensis* series), and the genus *Eleutherodactylus* turns out to be non-monophyletic (Fig. 2). The clade that includes species assigned to the Middle American genus *Craugastor* (Crawford & Smith, 2005; Frost *et al.*, 2006) has the *E. binotatus* series as sister group, with low boostrap support (bss=88%) and no Bayesian support. The *E. dolops* and *E. nigrovittatus* species groups are not closely related to *Oreobates*. However, it must be stressed that our taxon and character sampling for all these groups except our focal groups (*Oreobates* and the *E. discoidalis* group) are incomplete. What is intended herein is to answer the five questions listed above.

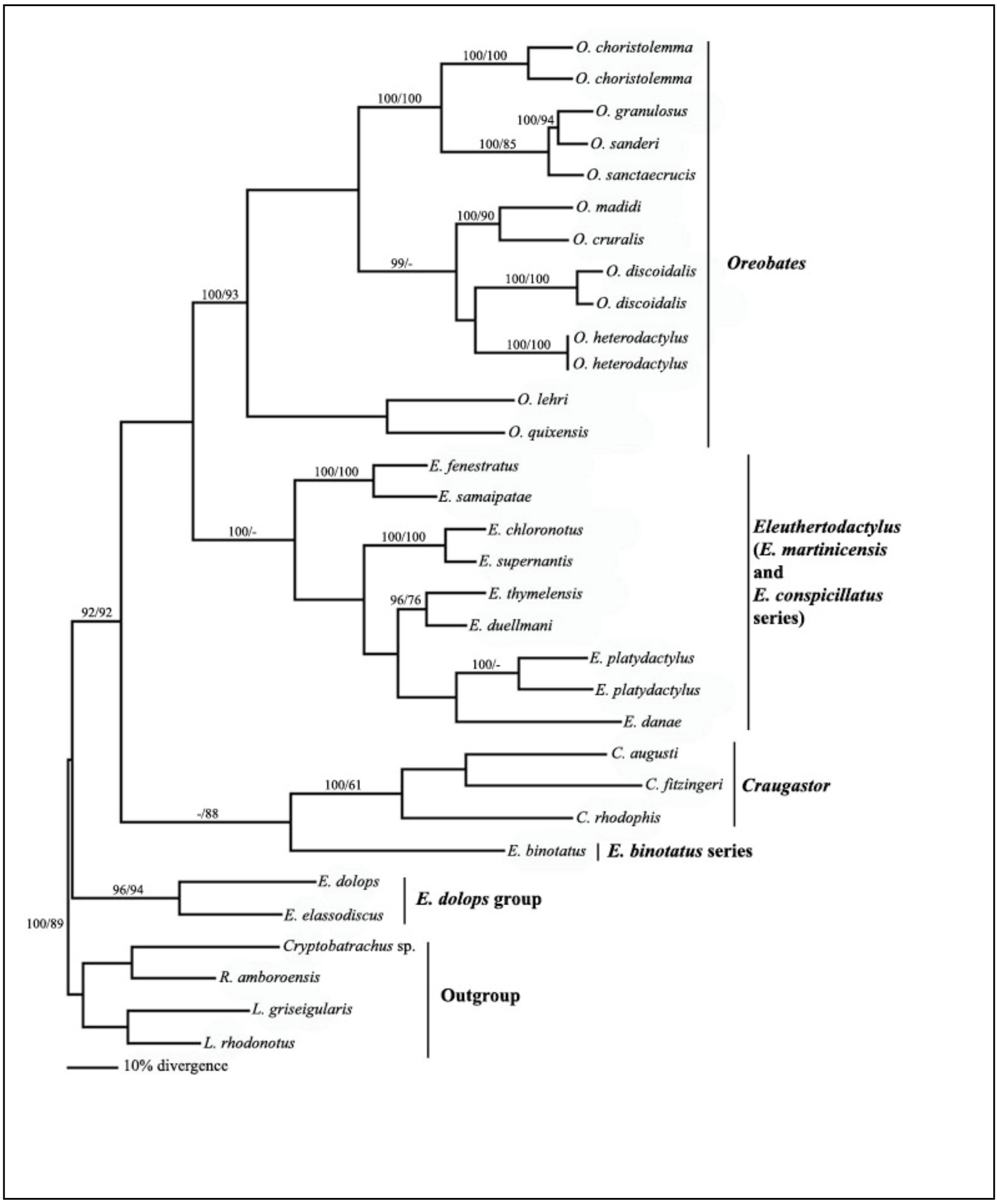

Figure 2. Majority rule concensus tree based on Bayesian phylogenetic analyses of partial 16S (ca. 590 bp) mtDNA of some members of the genera *Oreobates*, *Eleutherodactylus* and *Craugastor*. Numbers above branches are Bayesian posterior probabilities followed by boostrap support for maximum parsimony topology. Values lower than 0.90 Bayesian posterior probability or 60 for boostrap are not depicted.

The clade *Oreobates* includes species assigned by Lynch (1989) and by us (Padial *et al.*, 2005b; Padial *et al.*, 2007; Padial *et al.* in press) to the *Eleutherodactylus discoidalis* group and to members of the genus *Oreobates* (sensu Caramaschi & Canedo, 2006). Also, it includes *E. heterodactylus* (Miranda-Ribeiro, 1937), which had already been suggested to belong in the *E. discoidalis* group (Padial & De la Riva, 2005; Padial *et al.* in press). Hence, members of the *E. discoidalis* species group are now included in the genus *Oreobates*. Following the Phylocode (Cantino & de Queiroz, 2004), the genus *Oreobates* can be node–based defined as the least inclusive crown clade including *O. discoidalis*, *O. lehri*, *O. quixensis*, and *O. sanctaecrucis*. Additionally, differences in the 560 bp fragment of the 16S mitochondrial DNA also support the specific status of species assigned to the former *E. discoidalis* group and to *Oreobates* (Table 2), although the value for the pair *O. granulosus*-*O. sanderi* is moderate to low (2.8%).

Table 2. Percentage of divergence based on uncorrected p-distances in partial 16S mtDNA (ca. 560 bp) between several members of *Oreobates*.

	choristolemma	*cruralis*	*discoidalis*	*granulosus*	*heterodactylus*	*lehri*	*madidi*	*quixensis*	*sanctaecrucis*
cruralis	9.2								
discoidalis	10.9	9.8							
granulosus	4.7	9.6	13.0						
heterodactylus	10.2	8.3	9.8	9.5					
lehri	11.0	11.2	10.8	11.2	11.7				
madidi	9.6	6.4	9.6	10.2	9.6	11.1			
quixensis	10.4	11.3	11.2	11.1	11.4	10.9	12.1		
sanctaecrucis	3.7	8.5	11.9	3.5	9.3	10.8	9.4	10.9	
sanderi	5.0	9.4	12.4	2.8	10.1	11.7	10.2	11.3	3.2

Regarding external morphology, we propose the presence of enlarged conical plantar supernumerary and subarticular tubercles, together with a smooth texture of the skin covering plantar surfaces, as a synapomorphy for *Oreobates*. Two other putative synapomorphies are the presence of axillary glandular pads and the absence of a vocal sac in males. Harvey & Sheehy (2005) first described the presence of axillary glandular pads for *O. choristolemma*. We additionally found this character in *O. cruralis*, *O. discoidalis*, *O. granulosus*, *O. heterodactylus*, *O. ibischi*, *O. madidi*, O. *sanctaecrucis, O. sanderi*, and *O. lehri* (Padial *et al.*, 2007). We were not able to look for this character in *O. simmonsi*, *O. saxatilis* or *O. zongoensis*.

SYSTEMATICS

OREOBATES JIMÉNEZ DE LA ESPADA 1872, *An. Soc. Esp. Hist. Nat.*, 1: 86.

Ichnocnema—Lynch & Schwarz (1971)
Oreobates—Caramaschi & Canedo (2006)

Type species: *Oreobates quixensis* Jiménez de la Espada 1872, *An. Soc. Esp. Hist. Nat.*, 1: 87. Lectotype: MNCN 1708 (formerly 330).

The genus *Oreobates* includes small to medium-sized frogs (SVL of males 20–44 mm, of females 25–55 mm) with the following characters: snout short; sexual dimorphism in size; cranial crests absent; body robust; limbs moderately long; skin of venter smooth; skin on dorsal surfaces from smooth to tuberculate; skin of plantar surfaces smooth (not considering plantar tubercles); axillary and/or inguinal glandular pads present; discoidal fold conspicuous; dorsolateral folds rudimentary or absent; males with faint or absent nuptial pads, vocal slits present, vocal sac absent; tympanic membrane and annulus conspicuous; Finger I longer or equal than II; finger tips usually rounded with reduced, or absent, disc structure, when present only on Finger III and IV, and always with incomplete circumferential grooves and poorly defined ungual flap; supernumerary and subarticular tubercles present, prominent, subconical to conical on smooth plantar surface; Toe V equal or slightly shorter than Toe III, not reaching distal subarticular tubercle of Toe IV [condition B sensu Lynch & Duellman (1997)]; toes lacking discs; webbing absent; no tubercles on heel or tarsus; subarticular tubercles prominent, conical, supernumerary tubercles from absent or low and round to prominent and conical; dorsal colouration overall brown with an occipital W-shaped dark mark, a X-shaped middorsal dark mark and a broad and oblique dark band at the anterior margin of the flanks; vomerine odontophores short, prominent, almost at the level of choanae or between them; mandibular ramus of the trigeminal nerve passing lateral to the *m. adductor mandibulae externus* (S condition sensu Lynch, 1986); reproductive mode by terrestrial eggs with direct-development (mode 17 of Duellman & Trueb, 1986); advertisement call consisting of a single click or a series of clicks (4–32) and with low dominant frequency (2000–3800 Hz) (Padial *et al.*, in press).

The genus *Oreobates* includes fourteen species: *O. choristolemma*, *O. cruralis*, *O. discoidalis*, *O. granulosus*, *O. heterodactylus*, *O. ibischi*, *O. lehri*, *O. madidi*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*, *O. saxatilis*, *O. simmonsi*, and *O. zongoensis*.

Figure 3. Photographs of adult specimens of *Oreobates*. A, *O. choristolemma* from Altamachi, Department Cochabamba, Bolivia; B, *O. cruralis* from road to Bellavista, Department Santa Cruz, Bolivia; C, *O. cruralis* from Chalalán, Department La Paz, Bolivia; D, *O. discoidalis* Serranía Aguaragüe, Department Tarija, Bolivia; E, *O. granulosus* (female) from Santo Domingo, Department Puno, Peru; F, *O. granulosus* (male) from Santo Domingo, Department Puno, Peru; G, *O. heterodactylus* from Serranía de Santiago, Department Santa Cruz, Bolivia; H, *O. ibischi* from Masicurí, Department Santa Cruz, Bolivia; I, *O. lehri* from Cosñipata Valley, Department Cusco, Peru; J, *O. madidi* from Serranía Eslabón, Department La Paz, Bolivia; K, *O. quixensis* from Amacayacu, Department Amazonas, Colombia; L, *O. sanctaecrucis* from El Chapé, Department Santa Cruz, Bolivia; M, *O. sanderi* from Santa Cruz de Valle Ameno, Department La Paz, Bolivia; N, *O. saxatilis* from Ponga de Shilcayo, Department San Martin, Peru; O, *O. zongoensis* from Valle de Zongo, Department La Paz, Bolivia.

SPECIES ACCOUNTS

OREOBATES CHORISTOLEMMA (Harvey & Sheehy, 2005)

Ischnocnema choristolemma Harvey & Sheehy, 2005
(Fig. 3A)

Oreobates choristolemma Harvey & Sheehy, 2005, *Herpetologica*, 61 (3): 269. Holotype: CBF 5611. Type locality: "Serranía de Bellavista, Caranavi Province, La Paz Department, Bolivia, ca. 1000 m" (Fig. 4), coordinates: approx. 15º40'S, 67º30'W.

Remarks: The original description and diagnosis for this species are accurate (Harvey & Sheehy, 2005). Morphological differences with the two redescribed species (*O. cruralis* and *O. granulosus*) are listed in their respective diagnosis (see below); for differences with *O. lehri* see Padial *et al.* (2007) see the operational key below. This species was only known from the holotype and paratypes collected in the Yungas de la Paz, Bolivia. No additional specimens were known prior to this study. Although we were not able to review the types, the precise original description allowed us to identify some specimens from Bolivia (see Appendix) as *O. choristolemma*. The specimens studied by us show three main diagnostic characters: the presence of axillary glandular pads, enlarged discs on Finger III and IV and notched ungual flap (Harvey & Seehy, 2005). However, axillary, inguinal and sacral glandular pads are present in other species of the genus (see above). Nevertheless, the presence of an indented (notched) ungual flap is exclusive for this species (among members of this genus). The specimen ZFMK 72569, from Chapare Province, Departamento Cochabamba, Bolivia, represents the southernmost record of this species, and increases its distribution area by about 500 km to the south. This specimen is an adult female (SVL=41.0) with enlarged and indented fingers III and IV and without axillary glands. Harvey and Sheehy (2005) did not report any condition of nuptial excrescences because the type series is composed of an adult female and a subadult male. The specimen CBG 767, adult male (SVL=28.7), has posterolateral vocal slits and a single, white, glandular, non-spinous nuptial pad on the dorsal surface of each thumb. All but one specimen (CBG 765) of the series CBG 765–768, from Yungas de La Paz, show axillary glands. All of these specimens have moderately enlarged discs on Finger III and IV with notched ungual flaps. All specimens examined by us lack toe or finger fringes.

Table 3. Measurements and proportions of three species of *Oreobates* redescribed herein (mean ± standard deviation in parentheses follow ranges). For abbreviations, see text.

	O. cruralis		*O. discoidalis*		*O. granulosus*	
	males (n=44)	females (n=34)	males (n=8)	females (n=23)	males (n=6)	females (n=6)
SVL	20.3–30.2(24.8±2.2)	24.9–33.6(29.3±2.0)	25.6–30.4(27.9±1.4)	29.5–39.7(34.7±2.3)	22.6–28.5(26.6±2.1)	34.4–39.5(36.9±1.7)
HL	7.6–11.5(9.4±0.7)	8.9–11.8(10.7±0.7)	9.2–11.3(10.3±0.7)	11.3–14.5(12.5±0.7)	9.0–11.1(10.4±0.8)	13.0–17.5(14.8±1.5)
HW	7.3–10.6(8.7±0.7)	9.1–11.6(10.1±0.6)	8.4–11.0(9.6±0.8)	10.7–13.9(11.8±0.8)	9.1–11.4(10.4±0.8)	13.3–15.2(14.4±0.8)
EL	2.9–4.6(3.6±0.4)	2.9–4.7(4.0±0.4)	3.4–4.2(3.7±0.3)	3.7–5.5(4.4±0.4)	3.2–4.6(3.8±0.5)	4.1–5.2(4.5±0.5)
EN	2.0–3.6(2.8±0.3)	2.4–3.7(3.2±0.3)	2.7–3.6(3.3±0.3)	3.1–5.0(3.9±0.4)	2.8–3.7(3.1±0.3)	3.8–4.7(4.3±0.4)
IND	1.8–3.1(2.4±0.3)	2.2–3.3(2.7±0.2)	2.2–2.8(2.5±0.2)	2.6–3.6(3.1±0.2)	2.4–3.1(2.7±0.3)	3.2–3.9(3.6±0.3)
EE	2.9–5.1(4.4±0.4)	4.4–6.2(5.0±0.4)	4.2–5.3(4.7±0.3)	5.1–6.5(5.7±0.4)	4.1–5.2(4.8±0.4)	5.6–6.5(6.0±0.4)
TYH	1.5–2.3(1.9±0.2)	0.7–2.6(2.1±0.4)	1.5–2.3(2.0±0.3)	2.0–2.9(2.6±0.2)	1.5–2.1(1.9±0.3)	2.4–2.8(2.6±0.2)
TYL	1.4–2.2(1.7±0.2)	0.7–2.5(2.0±0.3)	1.5–2.3(2.0±0.3)	1.8–2.9(2.3±0.3)	1.5–2.1(1.9±0.2)	2.2–2.6(2.4±0.2)
FA	4.8–6.7(5.6±0.5)	5.3–15.2(6.6±1.6)	5.5–7.4(6.3±0.6)	6.5–9.0(7.6±0.7)	5.4–7.4(6.2±0.7)	7.8–9.2(8.4±0.7)
TL	2.2–15.5(13.1±2.6)	11.3–17.8(15.1±1.3)	13.4–15.1(14.2±0.5)	15.5–20.3(17.8±1.0)	12.3–16.1(14.4±1.4)	19.0–21.1(19.8±0.8)
TH	9.0–14.2(12.5±1.1)	12.3–17.4(14.2±1.2)	12.2–14.8(13.3±1.0)	14.7–19.0(16.8±1.1)	11.8–15.3(14.0±1.2)	16.8–19.4(18.0±1.0)
FL	10.5–15.3(12.9±1.1)	1.0–17.7(14.1±2.7)	12.5–14.8(13.4±0.8)	14.4–20.5(17.2±1.4)	12.3–16.4(14.6±1.4)	18.9–20.8(19.9±0.8)
TL/SVL	0.3–0.6(0.5±0.1)	0.3–0.6(0.5±0.0)	0.5–0.5(0.5±0.0)	0.5–0.6(0.5±0.0)	0.5–0.6(0.5±0.0)	0.5–0.6(0.5±0.0)
FL/SVL	0.5–0.6(0.5±0.0)	0.5–0.6(0.5±0.0)	0.4–0.5(0.5±0.0)	0.4–0.6(0.5±0.0)	0.5–0.6(0.6±0.0)	0.5–0.6(0.5±0.0)
HL/SVL	0.3–0.4(0.4±0.0)	0.3–0.4(0.4±0.0)	0.4–0.4(0.4±0.0)	0.3–0.4(0.4±0.0)	0.4–0.4(0.4±0.0)	0.4–0.5(0.4±0.1)
HW/SVL	0.3–0.4(0.4±0.0)	0.3–0.4(0.3±0.0)	0.3–0.4(0.3±0.0)	0.3–0.4(0.3±0.0)	0.4–0.4(0.4±0.0)	0.4–0.4(0.4±0.0)
HW/HL	0.8–1.1(0.9±0.1)	0.8–1.1(0.9±0.0)	0.9–1.0(0.9±0.0)	0.9–1.0(0.9±0.0)	0.9–1.1(1.0±0.1)	0.8–1.1(1.0±0.1)
EN/EL	0.6–1.0(0.8±0.1)	0.6–1.1(0.8±0.1)	0.8–1.0(0.9±0.1)	0.7–1.1(0.9±0.1)	0.7–1.0(0.8±0.1)	0.9–1.1(1.0±0.1)
EL/HW	0.4–0.5(0.4±0.0)	0.3–0.5(0.4±0.0)	0.4–0.4(0.4±0.0)	0.3–0.4(0.4±0.0)	0.3–0.4(0.4±0.0)	0.3–0.3(0.3±0.0)
TYL/TYH	0.7–1.3(0.9±0.1)	0.5–1.2(0.9±0.1)	0.9–1.0(1.0±0.0)	0.7–1.1(0.9±0.1)	0.9–1.1(1.0±0.1)	0.9–1.0(0.9±0.0)

Distribution: this species inhabits the humid forest of the Andean foothills from 1000–1500 m asl, from Yungas de Cochabamba to Yungas de La Paz, central Bolivia (Figs. 4–5).

OREOBATES CRURALIS (BOULENGER, 1902) NEW COMBINATION

Hylodes cruralis Boulenger, 1902
Eleutherodactylus cruralis—Stejneger (1904)
(Figs. 3 B–C, 6 A–B)

Hylodes cruralis Boulenger, 1902, *Ann. Mag. Nat. Hist.,* Ser. 7, 10: 396. Holotype: BM 1947.2.15.70 (formerly 1901.8.2.44). Type locality: "La Paz, Bolivia, 4000 m." (Fig. 4).

Lynch (1989) redescribed this species after examining the holotype. He also studied numerous specimens from Bolivia and Peru deposited in several collections. He confronted two problems. One, the type locality of *Hylodes cruralis*, was La Paz, Bolivia, 4000 m (type collected by P. O. Simmons). Lynch considered this locality to be in error and subsequently concurred with by De la Riva (1990), De la Riva

(1993), and De la Riva *et al.* (2000). The second problem was the great variability in size of adults. Most of these specimens were in very poor preservation condition and, therefore, many subtle morphological characters were difficult or impossible to observe or identify with confidence. Moreover, the specimens studied by Lynch came from very different altitudes and habitats. For example, the largest sample available to him was the series AMNH 6060–73, and the locality of procedence “Juliaca” was also in error, since it lies in the dry altiplano of southern Peru.

De la Riva *et al.* (2000) previously stated that what was considered *E. cruralis* could be in reality a composite of species. Some species described recently were similar in external appearance to *E. cruralis* (*O. ibischi*, *O. madidi*) and differences in advertisement calls were pivotal for assessing their distinctness (Reichle *et al.*, 2001; Padial *et al.*, 2005, Padial *et al.* in press). We studied 181 specimens of *E. cruralis* from Bolivia and Peru collected by us as well as those deposited in various collections, including the holotype and almost all specimens studied by Lynch (1989). After recognizing *O. granulosus* (see below), *O. ibischi*, O. *madidi*, *O. sanderi*, and *O. lehri*, *O. cruralis* can be defined with confidence. The redescription of *E. cruralis* by Lynch (1989) is not only based on the holotype but on a series of specimens. Furthermore, in our point of view, the series examined by him contain three different species (*O. cruralis*, *O. granulosus* and *O. sanderi*). Hence, in order to avoid confusion we provide a thorough diagnosis of *O, cruralis* and a redescription based exclusively on the holotype. The study of the intraspecific variation is based on additional specimens listed in the Appendix.

Diagnosis: A small *Oreobates* (SVL of adults 20.3–33.6) characterized as follows: (1) skin on dorsum coarsely shagreened without keratinized granules, texture composed of small, round, low, flat warts regular in size, only some of them slightly enlarged; warts on flanks slightly larger than those of dorsum; a pair of incomplete dorsolateral folds composed by enlarged warts; venter smooth; posterior surfaces of limbs smooth; discoidal fold present; postrictal glands present; (2) tympanic membrane and annulus distinct, about half eye length; supratympanic fold weak, short; (3) head large, slightly longer than wide; snout round in dorsal and lateral views; canthus rostralis sinuous in dorsal view, round in profile; (4) cranial crests absent; upper eyelid covered by small granules; (5) vomerine odontophores large, situated posteromedial to choanae; (6) males with vocal slits and no nuptial pads; (7) hands with long and slender fingers, first finger longer than second; subarticular tubercles large, prominent, conical; supernumerary tubercles large, prominent, round to conical, smaller than subarticular tubercles; tips of fingers III and IV truncate, slightly enlarged, lacking circumferential grooves and ungual flap; lateral fringes and keels on fingers from moderate to absent; (8) ulnar tubercles absent; (9) no tubercles on heel and tarsus; (10) inner metatarsal tubercle ovate to round, prominent; outer metatarsal tubercle smaller, round, prominent; supernumerary tubercles absent; (11) toes long and slender (foot length 50% SVL), lateral fringes weak or absent, webbing absent; fifth and third toes reaching midpoint of second subarticular tubercle of Toe IV; tips of toes moderately enlarged, rounded, ungual flap not indented; (12) axillary gland present; (13) dorsal colouration pale brown to dark brown or greyish-brown with W-shaped occipital and mid-dorsal X-shaped dark marks or a pair of cream dorsolateral stripes and a short longitudinal sacral stripe; throat and chest light grey to dark brown; belly cream with brown mottling or reticulations on anterior margin.

Oreobates cruralis can be distinguished from other *Oreobates* (characters of other species in parentheses) as follows: from *O. choristolemma* by lacking keratinized granules on dorsum, smaller size (SVL of the single known adult female 46.4) (table 3) and lacking indented ungual flap on finger discs; from *O. discoidalis* by having warty dorsal skin (finely shagreened with few enlarged warts in some specimens); moderately enlarged and truncate tips on fingers III and IV (enlarged and ovate); vomerine odontophores posteromedial to choanae (between choanae); canthus rostralis sinuous in dorsal view and round in lateral profile (straight and sharp); differences in advertisement call and habitat (Padial *et al.*, in press). From *O. granulosus* by having slightly enlarged and truncate tips on fingers III and IV (rounded, not enlarged); dorsal skin coarsely shagreened, composed of round low warts, without keratinized granules on dorsum (dorsal skin with low, round, non-pungent keratinized granules and warts); smaller SVL of adult females, 24.9–33.6 mm, (SVL 34.4–39.5) and head longer than wide (equal) (Table 3); by having numerous, conical and prominent supernumerary tubercles on feet (supernumerary tubercles low, few, round). From *O. heterodactylus* by having coarsely shagreened dorsal skin with enlarged warts (smooth); slightly enlarged and truncate tips of fingers III and IV (very enlarged and ovate); numerous, conical and prominent supernumerary tubercles on feet (supernumerary tubercles low, few, round); advertisement call and habitat (Padial *et al.*, in press). From *O. ibischi* by having tympanum length half or less than half of eye-length (more than half of eye-length); coarsely shagreened dorsal skin with enlarged warts (smooth to finely shagreened with some enlarged warts); slightly enlarged and truncate tips on fingers III and IV (enlarged and ovate, finger tips two times wider than the digit); numerous, conical and prominent supernumerary tubercles on feet (supernumerary tubercles low, scarce, round); head longer than wide (wider than long); differences in advertisement call and habitat (Padial *et al.*, in press). From *O. lehri* by having slightly enlarged and truncate tips on fingers III and IV (finger tips not expanded); first finger longer than second (Finger I equal to II); smaller size of adult males and females (SVL, 31.0–39.9 mm), (Table 3); shorter feet, FL/SVL=50% (60%); numerous conical and prominent supernumerary tubercles on feet (supernumerary tubercles low, few, round). From *O. madidi* by having dorsal skin with small, round, uniform warts with sparse enlarged warts (homogeneously warty, larger warts*),* slightly enlarged and truncate tips on fingers III and IV (rounded finger tips); advertisement call (Padial *et al.*, 2005; Padial *et al.* in press). From *O. quixensis* by smaller size, mean SVL of males and females 24.8 and 29.3 respectively, [mean SVL of adult males 39.0 (n=9), that of adult females 50.4 (n=14)] (Table 3);

coarsely shagreened dorsal skin with enlarged warts (dorsal skin coarsely tuberculate, with enlarged and prominent warts and granules, some of them keratinized); slightly enlarged and truncate tips on fingers III and IV (rounded finger tips). From *O. sanctaecrucis* by smaller size, mean SVL of males and females 24.8 and 29.3 respectively, [mean SVL of adult males 35.2 (n=3), that of adult females 46.0 (n=3)] (Table 3); coarsely shagreen dorsal skin with enlarged warts (dorsal skin coarsely tuberculate, with enlarged and prominent warts and granules, some of them keratinized); slightly enlarged and truncate disc on fingers III and IV (rounded finger tips). From *O. sanderi* it differs by smaller size [mean SVL of adult males 29.2 (n=6), mean SVL of adult females 36.5 (n=4)] (Table 3); head longer than wide (wider than long); coarsely shagreened dorsal skin with enlarged warts (dorsal skin covered by sparse keratinized granules and some warts); slightly enlarged and truncate tips on fingers III and IV (rounded finger tips). From *O. saxatilis* by smaller size, mean SVL of females 29.3 (49.0 and 43.7, holotype and paratype respectively) (Table 3); coarsely shagreened dorsal skin with enlarged warts (dorsal skin coarsely tuberculate, with enlarged and prominent warts and granules, some of them keratinized); slightly enlarged and truncate disc on fingers III and IV (rounded finger tips). From *O. simmonsi* by having coarsely shagreened dorsal skin with enlarged warts (densely granular with round, pungent, keratinized granules); slightly enlarged and truncate tips on fingers III and IV (rounded finger tips); ulnar tubercles absent (abundant, round, small). From *E. zongoensis* by having coarsely shagreened dorsal skin with enlarged warts (densely granular with round, pungent, keratinized granules); slightly enlarged and truncate tips on Finger III and IV (rounded finger tips); head longer than wide (wider than long).

Description of the holotype: An adult female (small ovarian eggs) with head slightly longer than wide; snout round in dorsal view and round to subacuminate in lateral profile; nostrils slightly protuberant, oriented dorsolaterally; canthus rostralis straight in dorsal view, round in frontal profile; loreal region slightly concave, sloping gradually to the lips; lips not flared; upper eyelid without tubercles but covered by small warts; no cranial crests. Supratympanic fold distinct, thin, short; tympanic membrane and its annulus distinct; tympanic membrane slightly ovate vertically, its length about 2/5 of eye length; two postrictal glands. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, round, lateral, separated by distance equal to four times the diameter of a choana; the specimen has one discernible vomerine odontophore, situated posteromedial to and far from the choanae. Skin texture of dorsal surfaces and posterior parts of hind limbs composed of small, low, round warts; ventral surfaces smooth; a W-shaped occipital fold; no dorsolateral folds; discoidal fold evident.

Ulnar tubercles absent; palmar tubercle bifid, flat, prominent; thenar tubercle ovate, prominent, 2/3 the size of palmar tubercle; supernumerary tubercles large, round, prominent, smaller than subarticular tubercles; subarticular tubercles large, prominent, subconical; tips of fingers I and II round, not enlarged, those of fingers III and IV moderately enlarged, truncate; basal lateral fringes on fingers I–III; relative length of fingers: II<I≤IV<III.

Toes long and slender (foot length 50% of SVL); heel and tarsus lacking tubercles or folds; inner metatarsal tubercle round, prominent, slightly larger than outer metatarsal tubercle; outer metatarsal tubercle round, conical; six supernumerary tubercles, small, round to elongate; subarticular tubercles prominent, subconical to conical; toes with basal lateral fringes; toes I and II with rounded, not expanded tips, toes III–V with slightly enlarged and truncate tips; ungual flap not indented; relative length of toes I<II<V<III<IV; toes III and V reaching penultimate subarticular tubercle of Toe IV.

Dorsal surfaces reddish brown, with darker marks including a W-shaped occipital dark brown mark, an interocular bar, two subocular dark brown stripes, and a supratympanic stripe. Arms and hind-limbs light brown with transverse brown stripes; concealed surfaces of hind-limbs brown; flanks beige with dark brown spots that merge in an oblique wide band posterior to the arm insertion; ventrally cream with fine grey mottling on throat, chest and anterior margin of belly.

Measurements of the holotype: SVL, 27.4; HL, 8.9; HW, 9.2; EL, 4.1; EN, 3.2; IND, 2.4; EE, 4.4; TYH, 1.7; TYL, 1.4; FA, 5.6; TL, 14.6; TH, 14.1;FL, 14.0.

Variation: The most variable characters of *O. cruralis* are adult size and colouration. These two characters can lead to confusion with other species. Although we have removed several taxa (with different adult sizes) from what was thought to be "*O. cruralis*" (see remarks section below and on *O. granulosus*), the variation in size of this species is still considerably high compared to other members of this genus. However, this is the species for which the largest data set has been analysed and, hence, such observed variability may be influenced by sample size. Adult males of *O. cruralis* range from 20.3 mm to 30.2 mm and females from 24.9 to 33.6. The sizes males and females overlap broadly. Another very relevant character is skin texture, but this character is easily diagnosable under stereomicroscope. The dorsal skin texture of this species varies from scarcely warty (almost smooth) to densely and homogeneously warty with few or abundant enlarged warts. We consider the skin texture to be warty, although it has been considered as rugose (Boulenger, 1902), shagreened or smooth to finely areolate (Padial *et al.*, 2005a, b). The relevant distinction is that the dorsal skin of this species lacks granules (i.e. hard, keratinized structures, usually rounded or conical) or tubercles (enlarged, prominent, conical warts). All dorsal warts are always flat, low and constitute soft structures. As in *O. granulosus* and *O. sanderi*, there are two colour morphs. The most commom morph consists of a pale to dark brown or reddish-brown dorsum with pale and dark irregular flecks, a W-shaped mark on occipital region, and a)(-shaped mark on middorsum, one or two broad dark oblique bands on flanks, and dark interocular, labial and transversal bars on extremities. This pattern occurs in 60 of 91 (66%) specimens for which colour pattern was noted. Another 26 specimens (29%) show the following colour pattern: a pale to dark brown or reddish-brown dorsum outlined by a pair of pale dorsolateral bands, a sacral stripe and two wide dark brown stripes on flanks, one anterior to groin and one posterior to arm insertion, transverse bars on extremities less evident. The intensity and tonalities of both patterns varies. A third, rare colour pattern, shared only with *E. madidi*, is the presence of a thin, white (in alcohol, yellow in life) middorsal stripe from snout to vent. This pattern was observed in five specimens, one female and four males. In all three morphs, the ventral pattern is similar: overall cream with fine mottling on throat and chest, mottling varying in density, intensity and colour, from grey to dark brown. Other variable characters are as follows. Although breeding males of *O. cruralis* do not have nuptial pads, a non-swollen white region can be observed on the dorsal surface of thumbs of some individuals. Axillary glands can be present or absent or present on only one side; finger fringes can be present (weak) or absent; a faint thin middorsal fold is shown in some specimens; the number of supernumerary tubercles on a single toe varies from 2–4, and from weak to prominent; the degree of enlargement of finger tips of fingers III and IV varies, although this variation seems to be related to fixation. The most divergent specimens studied by us are those from La Hoyada [MNK A 5577 and ZFMK 72644], which are overall dark grayish-brown and have conspicuous finger and toe fringes. Specimens from the lowlands of Peru (KU207749, 215461–2) seem to have slightly indented ungual flaps on thefingers. Specimens from higher altitudes are more affected by parasitic subdermal mites (see Wohltmann *et al.* 2006) that may lead one to think they have unusual skin structures.

Remarks: The specimens AMNH 91579, 153046, 153085, 153086, and KU 173230-32 were identified in collections as *O. cruralis*, but they are neither *O. cruralis* or any other known species of *Oreobates*. The specimens AMNH 91579, KU 173230–32 and MZUM 64120, 135341, identified by Lynch (1989) as *O. cruralis* do not correspond with any known species of *Oreobates*. The specimen KU 182814 illustrated as *O. cruralis* [see Fig. 3 in Harvey & Keck (1995)] is an adult female of *O. discoidalis*. The comparison between *O. discoidalis* and *O. cruralis* provided by Cei (1987) includes some observations that do not correspond with characters of *O. cruralis*. The broad range of adult size and intraspecific genetic distances, the broad altitudinal gradient occupied by this species (including several life zones) and the broad latitudinal distribution, together with some differences in advertisement calls (Padial *et al.*, in press), suggest that the name *O. cruralis* perhaps is still being applied to more than one species. The best candidates to be recognized as new species are those populations from humid montane forests and cloud forests in the departments of Cochabamba and Santa Cruz, Bolivia, but our data are still inconclusive.

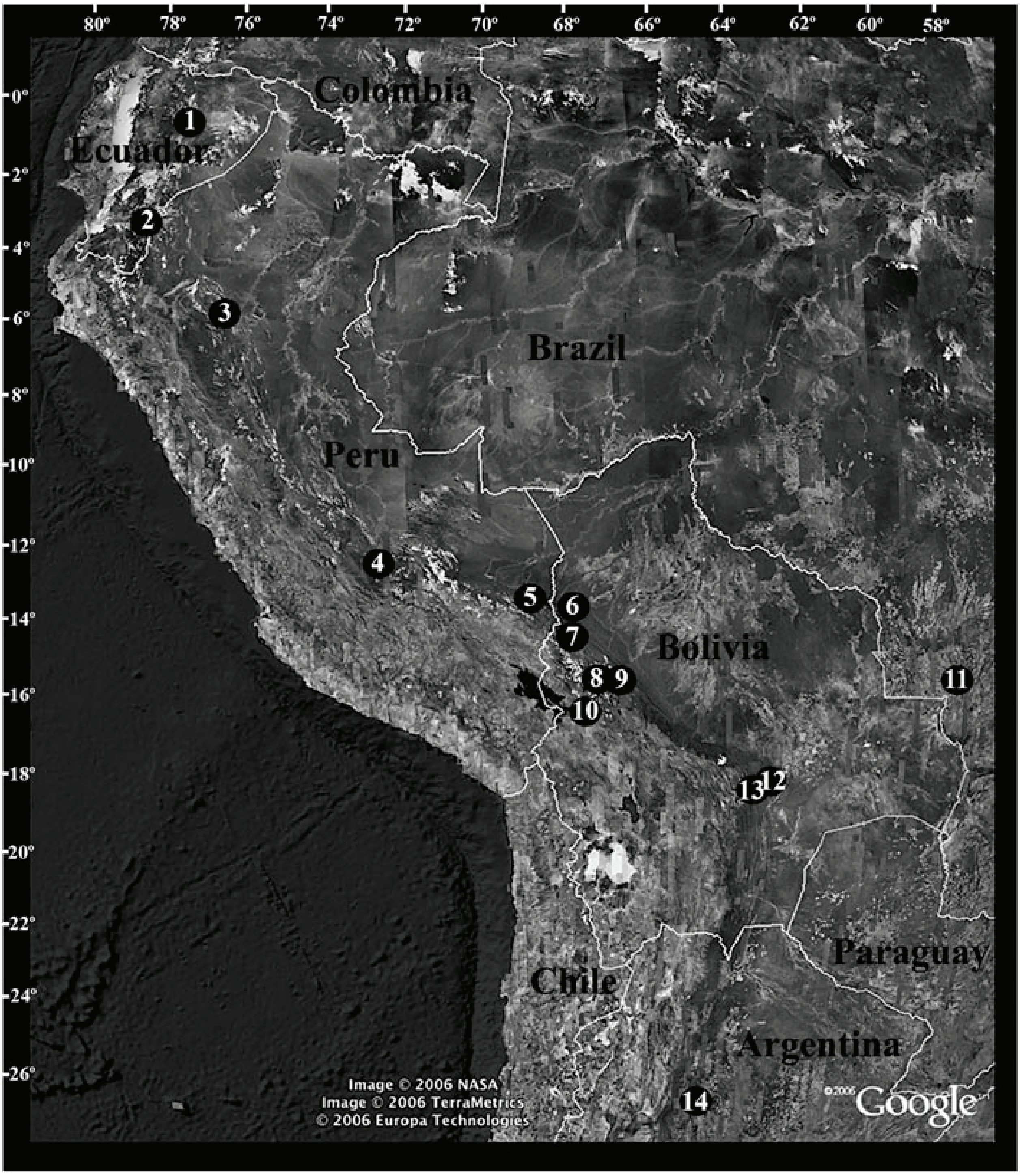

Figure 4. Type localities of members of *Oreobates*. 1) *O. quixensis*: San José de Moti, Prov. Napo, Ecuador; 2) *O. simmonsi*: Río Piuntza 1830 m, Cordillera del Condor, Prov. Morona-Santiago, Ecuador; 3) *O. saxatilis*: Ponga de Shilcayo 470 m, Dept. San Martín, Peru; 4) *O. lehri*: Apurimac River Valley 2445 m, Dept. Cusco, Peru; 5) *O. granulosus*: Santo Domingo, Carabaya, Dept. Puno, Peru, 1800 m; 6) *O. madidi*: Arroyo Huacataya, Serranía Eslabón, 1500 m, Dept. La Paz, Bolivia; 7) *O. sanderi*: Arroyo Bilunto, Chunirumi Valley 1800 m, near Santa Cruz de Valle Ameno, Dept. La Paz, Bolivia; 8) *O. zongoensis*: Valle de Zongo, 1250 m, Dept. La Paz, Bolivia; 9) *O. choristolemma*: Serranía de Bellavista ca. 1000 m, Dept. La Paz, Bolivia; 10) *O. cruralis*: Dept. La Paz, Bolivia, 4000 m (in error); 11) *O. heterodactylus*: gruta Facendinha, State Mato-Grosso, Brazil; 12) *O. ibischi*: km 68.5 on Santa Cruz de la Sierra-Samaipata road ca. 750 m, Dept. Santa Cruz, Bolivia; 13) *O. sanctaecrucis*: El Chapé, Dept. Santa Cruz, Bolivia, 2060 m; 14) *O. discoidalis*: Tucumán, Prov. Tucumán, Argentina.

Distribution: this species inhabit the lowland rainforests, humid forest and cloud forests of the Andean foothills from 200 to 2000 m asl, from Department Cusco in southern Peru to Departament Santa Cruz in central Bolivia (Figs. 4–5). This species also reaches the semideciduous forests of the inter-Andean valleys of central Bolivia. Cei (1987) tentatively cited *O. cruralis* for Argentina without locality, but Lavilla & Cei (2001) discarded this possibility. Köhler (2000), Reichle *et al.* (2001), and Padial *et al.* (in press) described its advertisement call. Some data on its biology and ecology can be found in Köhler (2000), Doan & Arizábal (2002) and Duellman (2005).

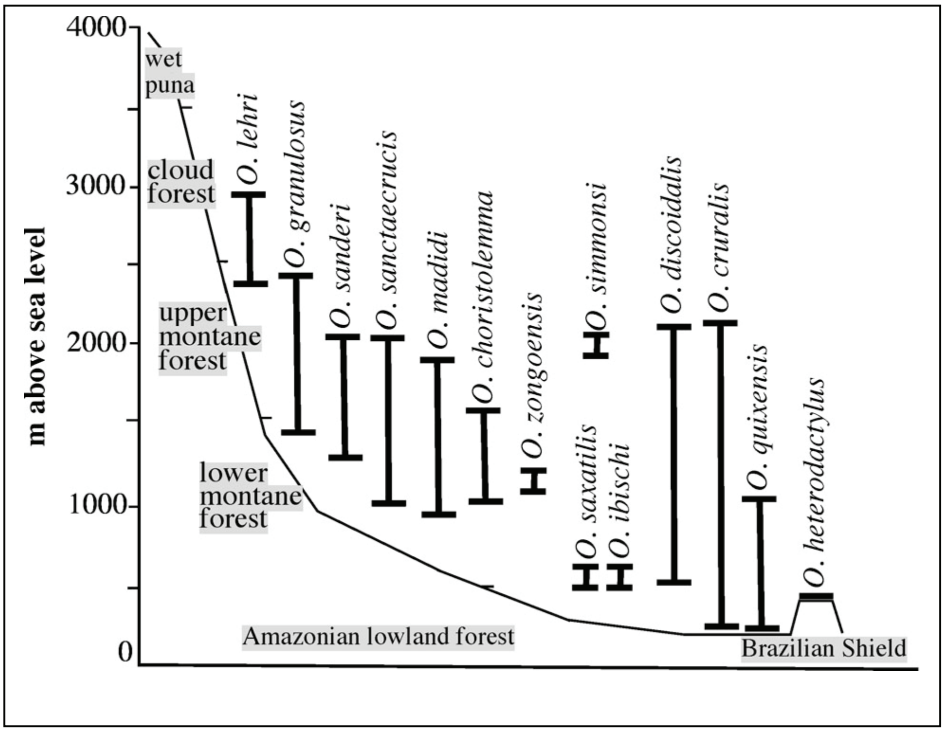

Figure 5. Altitudinal distribution across habitat typess of members of the genus *Oreobates*.

OREOBATES DISCOIDALIS (PERACCA, 1895), NEW COMBINATION

Hylodes discoidalis Peracca, 1895
Eleutherodactylus discoidalis—Stejneger (1904)
(Figs. 3D, 6E–F)

Hylodes discoidalis Peracca, 1895, *Boll. Mus. Zool. Anat. Comp. Univ. Torino*, 10: 24. Lectotype: MZUT An 427.1, designation by Gavetti and Andreone, 1993, Cat. Mus. Reg. Sci. Nat., Torino, 10: 129. Type locality: "Tucumán (Argentina)" (Fig. 4), coordinates: approx. 26º50'S, 65º13'W.

Lynch (1989) redescribed this species based on type material, but neither Peracca (1895) nor Lynch designated one of the syntypes as a lectotype. Lynch´s (1989) redescription is based on the syntypes BM. 1947.2.15.63–65 and several syntypes deposited in the MZUT, Torino, Italy. The lectotype was designated by Gavetti & Andreone (1993) from the MZUT series. Although they mention that the lectotype was in good state of preservation, the plate provided evidence that the specimen is not very well preserved. Moreover, the description of the lectotype is rather incomplete (for example it does not include data on skin texture, tubercles on plantar surfaces or degree of development of finger and toe tips). The BM series studied by us is better preserved and, in order to avoid future misidentification, we herein describe the best-preserved specimen of this series (BM 1947.2.15.63) (Fig. 6E–F), which is in general very similar to the lectotype.

Diagnosis: a medium-sized *Oreobates* (SVL of adults 25.6–39.7 mm) characterized as follows: (1) skin of dorsum finely shagreened or smooth, with low warts; posterior surfaces of limbs smooth; discoidal fold present; no dorsolateral folds; large postrictal glands; (2) tympanic membrane and annulus distinct, its length longer than half eye length; supratympanic fold prominent; (3) head longer than wide; snout long, subacuminate in dorsal view, round in lateral profile; canthus rostralis straight in dorsal view, sharp in profile; (4) cranial crests absent; eyelid tubercles absent; (5) vomerine odontophores medial to choanae; (6) males with vocal slits, nuptial pads absent; (7) hands with long and slender fingers, first finger longer than second; subarticular tubercles large, prominent, conical; supernumerary tubercles large, conical, smaller than subarticular tubercles; terminal tips of fingers III and IV truncate or ovate, enlarged, circumferential grooves weak or absent, ungual flap not indented; lateral fringes and keels on fingers absent; (8) ulnar tubercles absent; (9) no tubercles on heel or tarsus; (10) inner metatarsal tubercle ovate, prominent; outer metatarsal tubercle smaller, round, prominent; supernumerary tubercles inconspicuous; (11) toes long and slender (foot length 40–60% SVL, see Table 3), lateral fringes absent, webbing present, rudimentary; (12) axillary, sacral and inguinal glands present; (13) dorsal colouration pale brown, greyish-brown, reddish-brown or dark brown with cream flecks, sometimes with W-shaped occipital and/or middorsal X-shaped dark marks; belly cream with brown mottling or reticulations on anterior margin.

Oreobates discoidalis can be distinguished from *O. choristolemma*, *O. cruralis*, *O. granulosus*, *O. lehri*, O. *madidi*, *O. quixensis*, *O. sanctaecrucis*, *O. sanderi*, *O. saxatilis*, *O. simmonsi*, and *O. zongoensis* by having dorsal skin smooth to finely shagreened and enlarged and ovate finger tips on fingers III and IV. These two characters are shared with *O. heterodactylus* and *O. ibischi*. Nevertheless, *O. heterodactylus* exhibits a pattern of dorsal colouration consisting of an arrow-shaped and X-shaped dark dorsal marks and it occurs on the Brazilian shield; *O. ibischi* has moderately enlarged and truncate finger tips, low warts on dorsal skin and head wider than long. These three species can be further distinguished by differences in advertisement call (see Padial & De la Riva, 2005; Padial *et al.* in press).

Description of BM. 1947.2.15.63: An adult female (SVL 39.9 mm), with head slightly longer than wide; snout subacuminate in dorsal view and round in lateral profile; nostrils slightly protuberant, oriented dorsolaterally; canthus rostralis straight in dorsal view, sharp in frontal profile; loreal region flat; lips not flared; upper eyelid without tubercles or warts; no cranial crests. Supratympanic fold prominent; tympanic membrane and annulus distinct; tympanic membrane large, round, longer than half eye length; two postrictal glands on each side of the head. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, round, lateral, separated by distance equal to seven times the diameter of a choana; vomerine odontophores ovate, medial to choanae, slightly separated from each other but far from choanae. Skin texture of dorsal surfaces smooth to finely shagreened; ventral surfaces smooth; no occipital or dorsolateral folds; discoidal fold conspicuous.

Ulnar tubercles absent; palmar tubercle round, flat, prominent; thenar tubercle ovate, prominent, 2/3 the size of palmar tubercle; supernumerary tubercles large, conical, prominent, smaller than subarticular tubercles; subarticular tubercles large, prominent, conical; tips of fingers I and II round, not enlarged, those of fingers III and IV very large, truncate; lateral fringes absent; relative length of fingers: II<I≤IV<III.

Toes moderately long (foot ≤ 50% of SVL); heel and tarsus lacking tubercles or folds; inner metatarsal tubercle ovate, prominent, slightly larger than outer metatarsal tubercle; outer metatarsal tubercle large, round, conical; three inconspicuous supernumerary tubercles, small, round; subarticular tubercles prominent, conical; toes with faint basal membrane between toes II–III; toes I, II and V with rounded, not expanded tips, toes III–IV with slightly enlarged and truncate tips; ungual flap not indented; relative length of toes I<II<V<III<IV; toes III and V reaching penultimate subarticular tubercle of Toe IV.

Colour in preservative: Dorsal surfaces reddish-brown, with many irregular dark and light (cream, grey, brown) marks. Tympanic fold almost black; subocular bars dark brown; flanks as dorsum but with a dark irregular brown oblique wide band posterior to the arm insertion; ventrally cream with intense reddish-brown mottling on throat, chest, anterior margin of belly, and proximate surfaces of flanks.

Measurements of BM. 1947.2.15.63: SVL, 39.1; HL, 13.3; HW, 13.0; EL, 5.0; EN, 4.7; IND, 3.1; EE, 6.4; TYH, 2.9; TYL, 2.9; FA, 8.7; TL, 18.3; TH, 17.5;FL, 17.9.

Remarks: In the label of the jar containing *O. discoidalis* specimens catalogued as BM.98.7.7.1920 there is a hand-written note with the word "Types". Nevertheless, these specimens are not types. They were collected by Dr. Borelli at San Lorenzo, Province Jujuy, Argentina, and probably sent to the BM by

Peracca with the types [Peracca (1895, 1897) studied Borelli's collections]. San Lorenzo also is the locality of the two syntypes catalogued as NMW 16510 (Häupl *et al.*, 1994). The locality "Tucumán" of the lectotype designated by Gavetti & Andreone (1993) is quite ambiguous, since it may refer to Province Tucumán or to San Miguel de Tucumán (popularly known as Tucumán). We suspect that these specimens may originate from near the town of San Miguel de Tucumán, probably from the Sierra de San Javier, as do the paralectotypes BM 1947.2.15.63–65. The specimen KU 182814 [see Fig. 3 on Harvey & Keck (1995)] reported as *O. cruralis* is an adult female of *O. discoidalis*. Cei (1980) provided a basic but accurate description of this species and an inaccurate illustration of the plantar surfaces. He also provides an illustration of the pectoral girdle. Padial *et al.* (in press) first described the advertisement call. Alcaide de Pucci *et al.* (1992) and De Mopty *et al.* (1992) provide information about the lingual structures in relation to feeding habits. Lavilla & Cei (2001) consider this species a poorly known taxon.

Distribution: In Argentina, *O. discoidalis* occurs in subtropical forest from Province Jujui to Province Tucumán, (Cei, 1980). De la Riva *et al.* (2000) and Köhler (2000) cite this species for Tucumanian-Bolivian montane forests and cloud forests from southern Bolivia (Departamento Tarija) to cloud forest of Parque Nacional Amboro (Departamento Santa Cruz) in central Bolivia (Figs. 4–5).

OREOBATES GRANULOSUS (BOULENGER, 1903), NEW COMBINATION

Hylodes granulosus Boulenger, 1903
Eleutherodactylus granulosus—Stejneger (1904)
(Figs. 3E–F, 6G–H)

Hylodes granulosus Boulenger, 1903, *Ann. Mag. Nat. Hist., Ser.* 7, 12: 553. Holotype: BM 1947.2.15.72 (formerly 1902.11.28.15). Type locality: "Santo Domingo, Carabaya, S. E. Peru, 6000 feet" (Fig. 4), coordinates: 13º49'59.6"S, 69º38'31.8"W.

Lynch (1989) synynomized *Eleutherodactylus granulosus* with *E. cruralis*, probably influenced by his broad concept of *E. cruralis* that included specimens from very different sizes, altitudes, and habitats. Indeed, the series studied by him included several species: *O. cruralis*, *O. granulosus* and *O. sanderi*. After studying the holotype of both *O. cruralis* and *O. granulosus* and additional specimens from around the type locality of *O. granulosus* (deposited in public collections), we concluded that *O. granulosus* is a valid species (see diagnosis and redescription of the holotype below). Moreover, recently collected specimens of *O. granulosus* by one of the authors (JC) at the type locality (Santo Domingo, Cordillera de

Carabaya, Provincia Sandia, Departamento Puno, Peru) confirm this position. This represents a remarkable discovery, since no specimen of this species had been found since Mr. Ockenden's expedition at the end of the 19th century.

Diagnosis: A medium-sized *Oreobates* (SVL of adults 22.6–36.9) characterized as follows: (1) skin of dorsum granular, with round keratinized granules and small, sparse, low, flat warts, only some of them slightly enlarged; warts on flanks slightly larger than those of dorsum; occipital W-shaped fold and/or)(-shaped fold on middorsum; venter smooth; posterior surfaces of limbs smooth, groin areolate; discoidal fold present; no dorsolateral folds; large postrictal glands; (2) tympanic membrane and annulus distinct, both with length about half eye length; supratympanic fold weak, short; (3) head large, as wide as long; snout short, round in dorsal and lateral views; canthus rostralis slightly convex or sinuous in dorsal view, round in profile; (4) cranial crests absent; upper eyelid covered by small warts; (5) vomerine odontophores large, oblique, almost in contact, situated posteromedial to choanae; (6) males with vocal slits and nuptial pads; (7) hands with long and slender fingers, first finger equal or slightly shorter than second; subarticular tubercles large, prominent, round to conical; supernumerary tubercles large or small, round to conical, smaller than subarticular tubercles; tips of fingers III and IV round, slightly enlarged, lacking circumferential grooves and ungual flap; lateral fringes and keels on fingers absent; (8) ulnar tubercles absent; (9) no tubercles on heel or tarsus; (10) inner metatarsal tubercle ovate, prominent; outer metatarsal tubercle smaller, round, prominent; supernumerary tubercles inconspicuous, small or absent; (11) toes long and slender (foot length 50–60% SVL), lateral fringes weak or absent, webbing absent; Toe V reaching the distal margin of the second subarticular tubercle of Toe IV, and Toe III reaching the proximal margin; tips of toes moderately enlarged, rounded, with ungual flap not indented; (12) axillary, sacral and inguinal glands present; (13) dorsal colouration pale brown to dark brown with cream flecks, sometimes with W-shaped occipital and/or middorsal X-shaped dark marks or a pair of cream dorsolateral stripes and a short longitudinal sacral stripe; throat and chest cream with fine brown mottling; belly cream with brown mottling or reticulations on anterior margin.

Oreobates granulosus can be distinguished from other *Oreobates* (characters of other species in parentheses) as follows: from *O. choristolemma* by smaller size of females [SVL of the only known adult females 46.4 (holotype) and 41.0 (ZFMK 72569)] (Table 3) and lacking indented ungual flap on discs. From *O. discoidalis* by having warty and granular dorsal skin (finely shagreened with few enlarged warts in some specimens); rounded tips of fingers III and IV (enlarged and ovate); vomerine odontophores posteromedial to choanae (between choanae); canthus rostralis convex or sinuous in dorsal view and round in lateral profile (straight and sharp). From *O. cruralis* by having slightly rounded, not enlarged finger tips (enlarged and truncate tips on fingers III and IV); dorsal skin with low, round, non-pungent keratinized granules and warts (dorsal skin coarsely shagreened, composed of round low warts, without keratinized granules on dorsum); larger size, mean SVL of adult females 36.9 mm (mean SVL of adult females 29.3 mm) and head as long as wide (head longer than wide) (Table 3); inconspicuous supernumerary tubercles, low, few?, round (numerous, conical and prominent supernumerary tubercles on feet). From *O. heterodactylus* by having dorsal skin with low, round, non-pungent keratinized granules and warts (smooth); rounded, slightly developed tips of fingers III and IV (enlarged and ovate). From *O. ibischi* by having tympanum length half or less than half of eye-length (more than half of eye-length); granular and warty dorsal skin (smooth to finely shagreened with some enlarged warts); rounded, slightly developed tips of fingers III and IV (enlarged and ovate discs two times wider than the digit); head as long as wide (wider than long). From *O. lehri* by having dorsal skin with low, round, non-pungent keratinized granules and warts (homogeneously warty); by smaller size, mean SVL of adult males, 26. 6 mm (mean SVL of adult males 32.1 mm) (Table 3); shorter feet, FL/SVL=50–60% (60–70%). From *O. madidi* by having dorsal skin with low, round, non-pungent keratinized granules and warts (homogeneously warty); head as wide as long (longer than wide); shorter feet, FL/SVL=50–60% (50%). From *O. quixensis* differs by smaller size, mean SVL of males and females 26.6 and 36.9 respectively [mean SVL of adult males 39.0 (n=9), mean SVL of adult females 50.4 (n=14)] (Table 3); dorsal skin with low, round, non-pungent keratinized granules and warts (dorsal skin coarsely tuberculate, with enlarged and prominent warts and granules, some of them keratinized); inconspicuous supernumerary tubercles on feet, low, scarce, round (numerous, conical and prominent supernumerary tubercles on feet). From *O. sanctaecrucis* by smaller size of adult females, 36.9 mm [mean SVL of adult females 46.0 (n=3)] (Table 3); dorsal colouration without scarlet flecks; supernumerary tubercles inconspicuous, scarce (numerous supernumerary tubercles, prominent); longer feet, FL/SVL 50–60% (50%). From *O. sanderi* by having supernumerary tubercles inconspicuous, scarce (numerous supernumerary tubercles, prominent); longer feet, FL/SVL=50–60% (FL/SVL=50%); head as long as wide (wider than long). From *O. saxatilis* by its smaller size, mean SVL of females 36.9 (adult females, 49.0 and 43.7, holotype and paratype respectively) (Table 3); dorsal skin with low, round, non-pungent keratinized granules and warts (dorsal skin coarsely tuberculate, with enlarged and prominent warts and granules, some of them keratinized); head as long as wide (wider than long). From *O. simmonsi* by having dorsal skin with low, round, non-pungent keratinized granules and warts (densely granular, round, pungent, keratinized granules); ulnar tubercles absent (abundant, round, small, pungent); head as long as wide (longer than wide). From *E. zongoensis* by having dorsal skin with low, round, non-pungent keratinized granules and warts (densely granular, round, pungent keratinized granules); slightly enlarged and truncate discs on fingers III and IV (rounded finger tips); head as long as wide (wider than long).

Description of the holotype: An adult male with vocal slits and single faint white, glandular, non spinous nuptial pad on each thumb; head as long as wide; snout round in dorsal view and lateral profile; nostrils small, oriented dorsolaterally; canthus rostralis slightly convex in dorsal view, round in frontal profile; loreal region slightly concave, sloping gradually to the lips; lips not flared; upper eyelid without tubercles but covered by small granules; no cranial crests. Supratympanic fold indistinct; tympanic membrane and annulus distinct; tympanic membrane nearly round, its length about half of eye length; 2–3 postrictal glands. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, round, anterolateral, separated by distance equal to 5 times the diameter of a choana; vomerine odontophores larger than choanae, oblique, large, prominent, almost in contact, situated posteromedial to choanae, bearing vomerine teeth; vocal slits short, placed posterolaterally. Skin of dorsal surfaces and posterior parts of hind limbs granular, with round, low, keratinized granules and sparse low, irregular warts; ventral surfaces smooth; W-shaped occipital fold; no dorsolateral folds; a thin middorsal fold; discoidal fold evident, almost reaching the groin.

Ulnar tubercles absent; palmar tubercle round, large, flat, thenar tubercle ovate, large, prominent, size around 2/3 of the size of palmar tubercle; supernumerary tubercles low, round, small; subarticular tubercles round, larger than supernumerary tubercles; finger tips round, slightly enlarged, but almost the same width as corresponding digits; fingers lacking lateral fringes and keels; relative length of fingers: I<IV<II<III.

Toes long and slender (foot length 54% of SVL); heel and tarsus lacking tubercles or folds; inner metatarsal tubercle oval to round, prominent, slightly larger than outer metatarsal tubercle; metatarsal tubercle round, conical; supernumerary tubercles small, elongate, inconspicuous; subarticular tubercles prominent, conical, elongate; toes apparently without lateral fringes and keels; toe tips rounded, slightly enlarged; ungual flap not indented; relative length of toes: I<II<V<III<IV.

Colour: In preservative, dorsal colouration pale brown to dark brown with cream flecks, a W-shaped occipital dark mark, a pair of faint cream dorsolateral stripes from posterior margin of eyes to occipital region, and a white spot on the middle of occipital region; head brown with cream flecks, two dark subocular bars; throat and chest cream with fine brown mottling; belly cream with brown mottling or reticulations on anterior margin; flanks cream with fine brown mottling, coalescing in an oblique, broad band posterior to arm insertion; extremities pale brown with diffuse reddish-brown transverse bars.

Measurements of the holotype: SVL, 27.6; HL, 10.67; HW, 10.5; EL, 4.6; EN, 3.2; IND, 3.1; EE, 5.2; TYH, 2.1; TYL, 2.1; FA, 6.5; TL, 14.5; TH, 14.4;FL, 14.9.

Variation: There is scarce variation in qualitative characters other than colour pattern. Juveniles are almost identical to adults. There is marked sexual dimorphism in size (females are larger than males), and males have longer feet and larger EL in relation to HW and EN (see Table 3). There are two basic colour morphs. One is the colour pattern of the holotype, consisting of a pale to dark brown dorsum with pale and dark irregular flecks and a W-shaped dark brown or cream mark on occipital region, and a)(-shaped dark or pale mark on middorsum, a pale sacral stripe, and dark transverse bars on extremities (Fig. 6G). The alternative colour pattern (present on five of 26 specimens) consists of a dark brown dorsum delimited by a pair of pale dorsolateral bands, a sacral stripe and two wide dark brown stripes on flanks, one anterior to groin and one posterior to arm insertion, and transverse bars on extremities less evident than in the first colour pattern. Both colour patterns are shared by *O. cruralis*, *O. lehri*, and *O. sanderi*. The intensity of brown mottling on throat and chest also varies. Glandular pads are present on axillary, groin, and sacral regions, but sometimes they are completely absent or absent from only one side. The adult breeding males USNM 299011–12 present all glandular pads: on axillary, groin, sacral regions and thumb. It is likely that the development of these glandular pads occurs only during the breeding season. In life, the dorsal texture and folds are also similar to preserved specimens. For example, the specimen MHNC 5328 shows incomplete dorsolateral folds composed of warts, an occipital W-shaped fold, a) (-middorsal fold and three enlarged round warts on the dorsal surface of the snout.

Description of colour patterns in life are based on the specimens MHNC 5328, 5335 (Figs. 3E–F). The adult female MHNC 5328 showed the following colouration. Head greenish-brown, two subocular and one labial irregular dark brown bars, canthus and loreal region with the same colouration; tympanic fold dark brown; lower lip barred with broad dark brown bars separated by light yellow stripes; postrictal glands orange; tympanic membrane purplish-brown, annulus greenish-brown; an interocular dark-brown bar. Dorsum reddish-brown with greenish-brown tonalities; some scattered red and orange warts on sacral and occipital regions; dark brown W-shaped occipital mark interiorly outlined by orange tones; middorsum with an irregular X-shaped dark brown mark; sacral region with a transverse dark-brown mark that continues toward the groin forming two oblique bands in posterior margin of flanks; flanks light orange to pinkish-cream with some dark brown flecks; a broad irregular, oblique band on the anterior and posterior margins of each flank; groin orange. Arms greenish-brown with irregular, transverse bands; arm insertion orange; limbs greenish-brown with well defined transverse dark brown bands and orange granules; plantar surfaces dark brown with grey tubercles. Throat purple with irregular yellow spots; chest purple; belly yellowish; ventral and posterior surfaces of limbs orange. Iris colouration composed of metallic green, yellow and orange tones, and black reticulations. Pupil horizontal, black, with a vertical black stripe ventrally. The adult male MHNC 5335 showed the following colouration. Head brown with two subocular and one labial irregular dark brown bars, canthus and loreal region with the same colouration; tympanic fold black; lower lip barred with broad dark brown bars separated by light yellow stripes; postrictal glands orange; tympanic membrane purplish-brown, annulus brown; an interocular dark-brown bar outlined anteriorly with two cream spots. Dorsum dark reddish-brown with some scattered orange warts on sacral and occipital regions; dark brown W-shaped occipital mark interiorly outlined by orange; a pair of bold black spots in dorsolateral region; sacral region with a thin transverse black stripe, that continues toward the groin forming two oblique bands on posterior margin of flanks; flanks brown with some light flecks; groin orange. Arms and limbs brown with diffuse transverse bars and orange warts. Throat brown with white spots; belly yellow with white spots on dark brown groin anterolaterally; ventral and posterior surfaces of limbs fleshy-orange. Iris bronze with black reticulations. Pupil horizontal, black, with a vertical black stripe ventrally.

Remarks: The original description by Boulenger (1903) is short but accurate. Although he overlooked the difference in relation to the degree of development of supernumerary tubercles between *Oreobates granulosus* and *O. cruralis*, he did note the pivotal difference in skin texture. He mentioned “skin slightly rugose” (Boulenger, 1902, p. 580) for *O. cruralis* and “granulate with small warts” for *O. granulosus* (Boulenger, 1903, p. 553). This species, that was known from the holotype only, is now known from 25 additional specimens (see Appendix). BM 1905.5.31.21 corresponds to seven specimens (two small juveniles, two young females, a subadult female and two adult males) collected at Limbani, Carabaya, Departament Puno, Peru, by Ockenden. Boulenger identified them as *O. granulosus*, but he did not include them as types. BM 1905.5.31.14.20 includes four specimens (two juveniles, a subadult male and a subadult female) from Agualani, Carabaya, Departament Puno, Peru. BM 1907.5.7.17–18 correspond to an adult female and a subadult female respectively. Both specimens were collected very close to the type locality by Ockenden. A series of specimens (USNM 299006–299012) collected by John Cadle at Ollachea, 1800 m, Departamento Puno, Peru, are also *O. granulosus*. From the series of specimens AMNH 6060–6073 identified as *O. cruralis* we were able to study the specimens 6060–6064. These include four specimens of *O. granulosus* and one of *O. sanderi* from Departamento Puno, collected by H. Keays. The specimens MHNC 5328 and 5335 (Figs. 3E–3F) were recently by one of us (JCC) at Santo Domingo, Distrito de Limbani, Provincia de Sandia, Departamento de Puno, Peru (13º49'59.6"S, 69º38'31.8"W) at 1400–1500 m asl. The specimen MHNC 5328 (field number 3396) is an adult female collected on 11 November 2006 along the Santo Domingo stream, 1400 m asl, on a rock 1.5 m above ground. The specimen MHNC5335 (field number 3403) is an adult male collected on 11 November 2006 on the main path to Santo Domingo, 1500 m a.s.l.; it was calling close to a small waterfall.

Distribution: This species inhabits humid montane forests and cloud forests of the eastern versant of Cordillera Carabaya, Puno, Peru (Figs. 4–5). It is known from four localities between 1400–2000 m asl. The most distant localities are Limbani and Ollachea, separated by approx. 200 km straight line distance.

OREOBATES HETERODACTYLUS (MIRANDA-RIBEIRO, 1937), NEW COMBINATION

Teletrema heterodactylum Miranda-Ribeiro, 1937
Eleutherodactylus heterodactylus—Myers (1962)
(Fig. 3G)

Teletrema heterodactylum Miranda-Ribeiro, 1937, *O Campo*, May 1937: 67. Lectotype: MN 106A, by Miranda-Ribeiro, 1955, Arquivos do Museu Nacional, Río de Janeiro 42: 411. Type locality: "gruta dita Facendinha", Matto-Grosso, Cáceres, Brazil (Fig. 4), coordinates: 16º00'S, 57º36'W.

Remarks: For a recent redescription and diagnosis of this species see Padial & De la Riva (2005). For distinguishing this species from other *Oreobates* see operational key below. Padial & De la Riva (2005) and Padial *et al.* (in press), based on the similarity of external morphology and advertisement calls, already noted that this species could belong to the *Eleutherodactylus discoidalis* group.

Distribution: It is only known from three localities in the semideciduous forest of the Precambrian Brazilian shield of western Brazil and eastern Bolivia (Heyer & Muñoz, 1999; Padial & De la Riva, 2005) (see Figs. 4–5).

OREOBATES IBISCHI (REICHLE, LÖTTERS, & DE LA RIVA, 2001), NEW COMBINATION

Eleutherodactylus ibischi Reichle, Lötters, & De la Riva, 2001
(Fig. 3H)

Eleutherodactylus ibischi Reichle, Lötters, & De la Riva, 2001, *J. Herpetol.,* 35: 21. Holotype: CBF 3341, by original designation. Type locality: "km 68.5 on Santa Cruz de la Sierra-Samaipata road, close to the village of Bermejo, Provincia Florida, Departamento Santa Cruz, Bolivia", coordinates: 18º11'S, 63º34'W, approximately 750 m above sea level (Fig. 4).

Remarks: The original description and diagnosis are accurate (Reichle *et al.* 2001). Nevertheless, the authors mention that the dorsal skin is finely tuberculate, but following Lynch & Duellman (1997) terminology the dorsal skin of this species is finely shagreenedwith some low, inconspicuous warts. Moreover, Reichle et al. (2001) mention the absence of nuptial pads in males of *O. ibischi,* but re-examination of the adult holotype revealed single, white, glandular nuptial pads on the dorsal surface of each thumb. They distinguished this species from *O. discoidalis* on the basis of enlarged tips of fingers III and IV, but this character is present in both species (*O. discoidalis* has more developed finger tips). In contrast, the presence of nuptial pads was mentioned for *O. discoidalis*, but we were not able to detect this structure in any adult male of *O. discoidalis.* Moreover, a diagnostic character provided by Reichle et al. (2001) that allows for separation of *O. cruralis* from *O. ibischi* (TYL>1/2EL) is not applicable for distinguishing *O. ibischi* from *O. discoidalis*, because both share a large tympanic membrane. However, *O. ibischi* can be easily distinguished from the most similar species (*O. discoidalis, O. heterodactylus* and *O. cruralis*) by having a head wider than long. Moreover, *O. discoidalis* and *O. heterodactylus* have sharp, distinct canthus rostralis in frontal profile, while the canthus rostralis is round and indistinct in *O. ibischi*. For additional differences with other members of *Oreobates* see operational key below. The advertisement call of *O. ibischi* allows an unequivocal identification (Reichle *et al.* 2001; Padial *et al.* in press).

Distribution: Only known from the semideciduous forests of the type locality and a nearby locality in Central Bolivia (see Appendix and Figs. 4–5). It is expected to occur in the semideciduous forests of Department Santa Cruz to Northern Argentina.

OREOBATES LEHRI (PADIAL, CHAPARRO & DE LA RIVA, 2007), NEW COMBINATION

Eleutherodactylus lehri Padial, Chaparro & De la Riva, 2007
(Fig. 3I)

Eleutherodactylus lehri Padial, Chaparro & De la Riva 2007, *Herpetologica*, 63: 115. Holotype: USNM 537848. Type locality: “Apurimac River Valley, Camisea Natural Gas Pipeline, Wayrapata Camp, 2445 m asl, (12º 50’ 10’’ S, 73º 29’ 43’’ W), Department Cusco, Peru” (Fig. 4).

Remarks: The original description and diagnosis are accurate (Padial *et al.* 2007). For additional differences with other members of *Oreobates* see operational key below.

Distribution: *O. lehri* is known from the type locality (Fig. 4) and from two localities in the Cosñipata Valley, Cusco, Peru. Both localities are situated in cloud forests (Fig. 5) and are separated by an airline distance of approximately 200 km. Nothing is known about its natural history.

OREOBATES MADIDI (PADIAL, GONZÁLES & DE LA RIVA, 2005), NEW COMBINATION

Eleutherodactylus madidi Padial, Gonzáles & De la Riva, 2005
(Fig. 3J)

Eleutherodactylus madidi Padial, Gonzáles & De la Riva, 2005, *Herpetologica*, 61: 319. Holotype: MNK-A 7856. Type locality: "Arroyo Huacataya, Serranía Eslabón, Área Natural de Manejo Integrado Madidi, Provincia Franz Tamayo, Departamento La Paz, Bolivia (14 20´ 12 S, 68 05´ 57 W), ca. 1500 m asl." (Fig. 4).

Remarks: The original description and diagnosis are accurate (Padial *et al.* 2005). For additional differences with other members of *Oreobates* see operational key below. Its advertisement call allows for an unequivocal identification of live male frogs (Padial *et al.* 2005; Padial *et al.* in press).

Distribution: O. madidi inhabits the humid montane forests of the Andean slopes of northern Bolivia at elevations of 900–1500 m (Figs. 4–5). The species is only known from La Cascada and Serranía Eslabón, two localities in the Departmento La Paz, separated by an straight line distance of 150 km.

OREOBATES QUIXENSIS JIMÉNEZ DE LA ESPADA, 1872

Hylodes verrucosus Jiménez de la Espada, 1872
Hylodes philippi Jiménez de la Espada, 1875
Borborocoetes quixensis—Boulenger (1882)
Leptodactylus tuberculosus Andersson, 1945
Eupsophus quixensis—Peters (1955)
Eleutherodactylus philippi—Gorham (1966)
Eleutherodactylus verrucosus—Gorham (1966)
Ischnocnema quixensis—Lynch & Schwartz (1971)
Oreobates quixensis—Caramaschi & Canedo (2006)
(Figs. 3K, 6I–J)

Oreobates quixensis Jiménez de la Espada, 1872, *An. Soc. Esp. Hist. Nat.,* 1: 87. Types: not given in the original descriptions. Type locality: San José de Moti, Province Napo, Ecuador (Fig. 4).

Remarks: The original description by Jimenez de la Espada (1872) is very succinct and the redescription by the same author is very accurate (Jimenez de la Espada, 1875). Several authors have diagnosed this species subsequently (e. g. Lynch & Schwartz, 1971; Duellman, 1978; Rodríguez & Duellman, 1994). For additional differences with other members of *Oreobates* see operational key below.

Lynch & Schwartz (1971) placed *Oreobates quixensis* in the genus *Ischnocnema* and considered *Hylodes philippi* Jiménez de la Espada, 1875 and *Hylodes verrucosus* Jiménez de la Espada, 1875 junior synonyms of *O. quixensis*. These two *Hylodes* (*=Eleutherodactylus*) were not described but only illustrated (Jiménez de la Espada, 1975). Based on the illustrations, Lynch & Schwartz (1971) considered that the variability of *O. quixensis* included what Jiménez de la Espada described as *H. verrucosus* and *H. philippi*. Moreover, since Duellman was not able to examine the types of *O. quixensis* and *O. verrucosus* in the MNCN, Lynch & Schwartz (1971, p. 107) considered all the types lost. This status prevailed until this paper (see Frost, 2006). The type locality of *O. quixensis* was considered "la provincia de Quijos" by Lynch & Schwarz (1971) following the original description. The type locality of *H. verrucosus* was considered unknown. For *H. philippi* they provided the correct former holotype number (MNCN 202) and the corresponding locality of origin, San José de Motí, Provincia Napo, Ecuador. Although Jiménez de la Espada never mentioned catalogue numbers in his publications, the types are preserved in the amphibian collection of the Museo Nacional de Ciencias Naturales de Madrid [see González (2006) for a detailed catalogue of the Espada's collection at the MNCN]. Our examination of the types revealed significant differences between the two *Hylodes* taxa and *O. quixensis*. For example, both *H. verrucosus* and *H. philippi* lack supernumerary plantar tubercles, a character illustrated in detail by Jimenez de la Espada (1975) for *O. quixensis*. Moreover, none of the latter species belongs to the genus *Oreobates* and both belong to the *Eleutherodactylus dolops* species group (sensu Lynch, 1989). The taxonomic status of these two species will be discussed elsewhere.

Figure 6. Type specimens of some members of *Oreobates*. A–B, holotype of *O. cruralis* (BM 1947.2.15.70); C–D, holotype of *O. simmonsi* (KU 147068); E–F, paralectotype of *O. discoidalis* (BM 1947.2.15.63); G–H, holotype of *O. granulosus* (BM 1947.2.15.72); I–J, lectotype of *O. quixensis* (MNCN 1708).

Jiménez de la Espada (1875) mentioned 11 specimens of *Oreobates quixensis* on which he based a more accurate description. In MNCN there are 14 specimens collected and identified by Jiménez de la Espada. One comes from San José de Moti, Municipio San José de Mote (or Nuevo), Province Napo; the other 13 specimens come from Municipio Archidona de Quijos, Province Napo, Ecuador. The specimen MNCN 1708 (formerly 330), an adult female from San José de Moti, corresponds to the specimen measured by Jiménez de la Espada (1875, p. 104). In the reverse of the original catalogue card corresponding to this specimen there is the inscription “Cotipo” (=cotype); at the back, there is the inscription “genotipo” (= type of the genus). Moreover, the card states that this specimen was illustrated in Jiménez de la Espada (1875). The original catalogue number 1154 corresponds to 13 specimens (MNCN 1709–21) from Archidona de Quijos, Napo, Ecuador. Nevertheless, Jiménez de la Espada (1875) only mentioned 11 specimens for this locality. González (2006) mentioned that two of these specimens could correspond to *Hylodes verrucosus* or *Hylodes philippi*. However we find them all assignable to *O. quixensis*.

We consider the specimen MNCN 1708 (formerly 330), an adult female from San José de Moti (from Municipio San José de Mote (or Nuevo), Province Napo, Ecuador, collected in June 1885 by Marcos Jiménez de la Espada the lectotype of *Oreobates quixensis* (Figs. 6I–J), and the specimens MNCN 1709–21 (formerly 1154) from Archidona, Ecuador, collected by Marcos Jiménez de la Espada on 4 May 1865 the paralectotypes of *O. quixensis.* The lectotype corresponds with the specimen measured and illustrated by Jiménez de la Espada (1875).

Distribution: Upper Amazon Basin in Colombia, Ecuador, Peru, Bolivia (Department of Pando), and western Brazil, up to 1000 m (Frost, 2006). The advertisement call was described by Duellman (1978) and Heyer & Gascon (1995); both of which also provide natural history information.

OREOBATES SANCTAECRUCIS (HARVEY & KECK, 1995)

Ischnocnema sanctaecrucis Harvey & Keck, 1995
Oreobates sanctaecrucis—Caramaschi & Canedo (2006)
(Fig. 3L)

Ischnocnema sanctaecrucis Harvey & Keck, 1995, *Herpetologica*, 51: 57. Holotype: MNK A 1198, by original designation. Type locality: "El Chapé, Provincia Florida, Departamento de Santa Cruz, Bolivia, 2060 m” (Fig. 4), coordinates: 18º03'34"S, 63º54'36"W.

Remarks: The original description and diagnosis are accurate (Harvey & Keck, 1995). For distinguishing this species from other members of *Oreobates* see operational key below. Its advertisement call allows for an unequivocal identification of active male frogs (Reichle, 1999; Köhler, 2000).

Distribution: O. sanctaecrucis inhabits the humid montane forests and cloud forests of the Andean slopes of central Bolivia, in Departments Cochabamba and Santa Cruz, at elevations of 1000–2100 m (Figs. 4–5).

OREOBATES SANDERI (PADIAL, REICHLE, & DE LA RIVA, 2005)

Ischnocnema sanderi Padial, Reichle, & De la Riva, 2005
Oreobates sanderi—Caramaschi & Canedo (2006)
(Fig. 3N)

Ischnocnema sanderi Padial, Reichle, & De la Riva, 2005, *J. Herpetol.*, 39: 187. Holotype: CBF 5385. Type locality: "Arroyo Bilunto (14 38′ 24 S, 68 31′ 45 W), Chunirumi Valley, Bilunto Mountains, Área Natural de Manejo Integrado Madidi, near Santa Cruz de Valle Ameno, Province Franz Tamayo, Departament La Paz, Bolivia, 1800 m" (Fig. 4).

Remarks: The original description and diagnosis are accurate (Padial *et al.*, 2005). For distinguishing this species from other members of *Oreobates* see operational key below. Its advertisement call is unknown.

Distribution: O. sanderi inhabits the humid montane forests and cloud forests of the Andean slopes of northern Bolivia, in Department La Paz, at elevations of 1300–2000 m (Figs. 4–5). Two specimens from Department Puno, Peru, one from Abra de Maruncuna, 10 km SW of San Juan del Oro, 1650 m (KU 206101), and one from Juliaca (AMNH 6063, locality in error since Juliaca lies in the dry Altiplano. This specimen was probably send to Juliaca from somewhereelse in the humid montane forests of Departamento Puno, Peru), are tentatively assigned to this species.

OREOBATES SAXATILIS (DUELLMAN, 1990)

Ischnocnema saxatilis Duellman, 1990
Oreobates saxatilis—Caramaschi & Canedo (2006)

(Fig. 3N)

Ischnocnema saxatilis Duellman, 1990, *Occas. Pap. Mus. Nat. Hist. Univ. Kansas*, 138: 1. Holotype: KU 212556. Type locality: "Ponga de Shilcayo, about 4 km NNW of Tarapoto, 470 m (6°31´S, 76°53´W), Provincia de San Martín, Departamento de San Martín, Peru" (Fig. 4).

Remarks: The original description and diagnosis are accurate (Duellman, 1990). For distinguishing this species from other members of *Oreobates* see operational key below. Its advertisement call is unknown; nothing is known about the natural history of this species.

Distribution: Known only from the type locality (Fig. 4).

OREOBATES SIMMONSI (LYNCH, 1974)

Ischnocnema simmonsi Lynch, 1974
Oreobates simmonsi—Caramaschi & Canedo (2006)
(Figs. 6C–D)

Ischnocnema simmonsi Lynch, 1974, *J. Herpetol.*, 8: 85. Holotype: KU 147068, by original designation. Type locality: "Río Piuntza, Cordillera del Cóndor, Morona-Santiago Prov., Ecuador, 1830 m" (Fig. 4).

Remarks: The original description and diagnosis are accurate. It is based on two specimens (Lynch, 1974). For distinguishing this species from other members of *Oreobates* see operational key below. Its advertisement call is unknown. Nothing is known about the natural history of this species. It is only known from the types.

Distribution: only known from the type locality (Fig. 4).

OREOBATES ZONGOENSIS (REICHLE & KÖHLER, 1997), NEW COMBINATION

Eleutherodactylus zongoensis Reichle & Köhler, 1997
(Fig. 3Q)

Eleutherodactylus zongoensis Reichle & Köhler, 1997, *Amphibia-Reptilia*, 18: 33. Holotype: CBF 2503. Type locality: "Valle del Zongo (16°03′31 S, 68°00′72W), 1250 m above sea level, Departamento La Paz, Bolivia" (Fig. 4).

Remarks: The original description and diagnosis are accurate although based on a single specimen although based on a single specimen (Reichle & Köhler, 1997). For distinguishing this species from other members of *Oreobates* see operational key below. Its advertisement call is unknown; nothing is known about the natural history of this species. It is only known from the holotype.

Distribution: Known only from the type locality (Fig. 4).

DISCUSSION

HYPOTHESES OF RELATIONHIPS

Phylogenetic analyses of mtDNA support the hypothesis of monophyly for the genus *Oreobates*. Monophyly is also supported by the presence of three putative morphological synapomorphies. This genus now comprises all members of the former *Eleutherodactylus discoidalis* group (sensu Padial *et al.*, 2007) and *Oreobates* (sensu Caramaschi & Canedo, 2006). The structure of the advertisement calls in *Oreobates* also suggests close relationship (Padial *et al.*, in press). The sister group of *Oreobates* is a well-supported clade including the *E. conspicillatus* and *E. martinicensis* series of the subgenus *Eleutherodactylus* (Figs. 1–2). However, since *E. binotatus*, *E. dolops*, and *E. elassodiscus* (representing the *E. binotatus*, *E. dolops*, and *E. nigrovittatus* species groups of the subgenus *Eleutherodactylus*) do not cluster with the *E. martinicensis* series (which includes the type species of *Eleutherodactylus*), we consider *Eleutherodactylus* non-monophyletic. This conclusion is also supported by Darst & Cannatella (2004) and Frost *et al.*'s (2006) molecular phylogenies. The genus *Eleutherodactylus* should probably be restricted to the *E. martinicensis* and *E. conspicillatus* series. Our data also support the hypothesis of monophyly of *Craugastor* (Crawford & Smith, 2005), but *E. binotatus* clusters with no statistical support with this taxon. Hence, either the *E. binotatus* series is the sister taxon of *Craugastor* or *Craugastor* includes members of this series. However, our limited dataset and scarce taxon sampling for these groups do not allow for definitive conclusions.

The most important synapomorphy suggested by us for *Oreobates* was already proposed by Lynch (1989) for two species (*O. cruralis* and *O. discoidalis*): presence of conical subarticular and supernumerary plantar tubercles. Jiménez de la Espada (1875) also described this character for *O. quixensis*. This character is present in all other species assigned to *Oreobates* by Caramaschi & Canedo

(2006). Lynch (1989) compared the skull morphology of several putatively related species, but he only selected this synapomorphy to split the former *E. discoidalis* species group (which by that time included species without conical subarticular tubercles and without supernumerary tubercles). The two other remaining groups (*E. dolops* and *E. nigrovittatus* species groups) were considered very closely related to each other but not to the *E. discoidalis* group. This hypothesis is corroborated by our mtDNA analyses (Fig. 2). Although the presence of conical subarticular and supernumerary plantar tubercles may be considered of doubtful phylogenetic utility because of its potential adaptive function, and hence more likely convergent evolution (Henning, 1979), the degree of development does not seem to be directly related to habitat use. For example, it could be hypothesized that those species with more terrestrial habits or those that live on hard substrates may show the most enlarged tubercles. Nevertheless, species auch as *O. cruralis*, *O. discoidalis*, *O. heterodactylus* and *O. ibischi* usually perch on bushes or are found on leaves, yet they present different degrees of development in plantar tubercles. In two terrestrial species, *O. granulosus* and *O. lehri*, the supernumerary tubercles of feet are reduced in number and size. On the other hand, the degree of development of external finger discs seems to be associated to the arboreal life of these frogs. For example, terrestrial and rock-dwelling species as *O. saxatilis*, *O. quixensis* or *O. granulosus* have lesss developed finger disks.

As stated above, Lynch (1971) and Lynch & Schwarz (1971) considered *Oreobates* a junior synonym of *Ischnocnema*. More recently, Caramaschi & Canedo (2006) resurrected *Oreobates* and placed *Ischnocnema* in the synonymy of *Eleutherodactylus*. Both Lynch (1971) and Caramaschi & Canedo (2006) used some osteological differences to support their positions, but these characters were neither proposed nor tested as synapomorphies. The most significant difference mentioned by Caramaschi & Canedo (2006) to synonymize *Ischnocnema* was the presence of "T-shaped terminal phalanges". Jiménez de la Espada (1872, 1875) also described the osteology of *O. quixensis* and stated that the moderate bifurcation of the terminal phalanges was an intermediate character between *Hylorhina sylvatica* and *Hylodes* (=*Eleutherodactylus*). Nevertheless, members of what we consider *Oreobates* have very well bifurcated T-shaped terminal phalanges (e. g., *O. discoidalis* and *O. heterodactylus*).

The presence of conical supernumerary and subarticular tubercles probably influenced the synonymization of *Oreobates* with *Ischnocnema* and led to the hypothesis of relationship proposed by Lynch (1989), in which *Ischnocnema* was the ancestor of members of the *E. discoidalis* group. Indeed, *E. verrucosus* (formerly *Ischnocnema verrucosa*) or *E. octavioi* (of the *E. binotatus* Series), from southeastern Brazil, also show prominent conical subarticular tubercles. Nevertheless, the development of plantar tubercles in the latter species seems to be associated to skin texture. These species have general granular skin texture that extends to the plantar surfaces, which contain some scattered enlarged granules similar to supernumerary tubercles. In the genus *Oreobates* the supernumerary tubercles are strictly associated with the axis of the phalanges while in *E. verrucosus* the plantar tubercles are randomly distributed on the plantar surface. Furthermore, while in *Oreobates* each finger or toe bears 1–3 supernumerary tubercles, in *E. verrucosus* there are only 1–3 for the whole plantar surface. Unfortunately, we were not able to test the relationships of *Oreobates* with *E. verrucosus* from southeastern Brazil in our phylogeny. Nevertheless, no synapomorphy is shared by these species and *Oreobates* to suggest close relationship. Indeed, the degree of development of the terminal phalanges seems to be associated with the degree of development of disc structures. The development of these structures is also variable within *Oreobates*, which suggests its limited utility for inferring phylogenetic relationships. Hence, until hypotheses of relationship can be empirically tested, we agree with the biogeographical argument of Caramaschi & Canedo (2006) that considers doubtful the relationship of *Oreobates* (Andean and Amazonian species) with *E. verrucosus* (southeastern Brazil).

Another hypothesis of relationship proposed by Lynch (1989) suggests that members of the *E. binotatus* Series are intermediate between *Ischnocnema* and members of the *E. discoidalis* group, and that the latter would be annectant to the *E. fitzingeri* group (now *Craugastor*). However, although our molecular phylogeny is insufficient to address this problem, it does not show any close phylogenetic relationship of *E. binotatus* or *Craugastor* with the *Oreobates* clade. Examination of Lynch's (1971, 1989) illustrations of the skulls of *O. quixensis* and *O. discoidalis* reveals a morphology of the vomers similar to that of members of the *E. binotatus* Series and *Euhyas*. This would be the only possibility to suggest close relationships, but, as Lynch (2001) stated, this character seems to be non-homologous for both *Euhyas* and *E. binotatus*. We extend this assumption to *Oreobates*. Thus, there is no putative synapomorphy to support this hypothesis.

HYPOTHESES OF ORIGIN

Combining our information about distribution, habitat use, and phylogenetic relationships we can hypothesize about the origin of the taxa under study (Graham *et al.* 2004). The highest diversity and endemicity of *Oreobates* is reached in the Eastern Cordillera of the Central Andes (sensu Gregory-Wodzicki, 2000). This diversity seems to be related to vicariant events as a result of Andean orogeny (Gregory-Wodzicki, 2000; Lynch & Duellman, 1997; Doan, 2003), and/or habitat fragmentation promoted by climatic changes (Weir, 2006; Hughes & Eastwood, 2006). Only three species are found in the lowlands (*O. quixensis*, *O. cruralis* and *O. heterodactylus*). *Oreobates quixensis* occurs in the Upper Amazon Basin and invades the Andean foothills, where it reaches altitudes of 1000 masl.. (Frost, 2006). *Oreobates cruralis* also inhabits the humid forests of the Andean foothills up to 2000 masl.. Some members of the group, such as *O. ibischi* and *O. discoidalis*, occupy the semideciduous forests of the inter-Andean dry valleys from Central Bolivia to northern Argentina, and *O. cruralis* reach the northern limit of this habitat (close to the Amazonian forests).

Padial *et al.* (in press) found that differences in calls of some *Oreobates* were related to distribution in a certain habitat and suggested that these differences could reflect consequences of isolation together with adaptation to a particular environment. Moreover, they found a geographic pattern in call variation along the latitudinal axis of the Andes, and proposed two possible scenarios for explaining these patterns. In the first, the colonization of the Andes by ancestors of the *E. discoidalis* group could have taken place in southern or central Bolivia, with subsequent expansion to the north (Peru) and to the south (northern Argentina). This hypothesis requires the *E. discoidalis* group to be related to the *E. binotatus* group (as hypothesized by Lynch, 1989). Since the *E. binotatus* series is not closely related to *Oreobates* (Fig. 2), we reject this hypothesis.

In the second hypothesis, members of the *E. discoidalis* group would have emerged from a common ancestor of *O. quixensis* or a northern member of the *E. binotatus* series (Lynch, 1989). Our data discard the possibility of the *E. binotatus* series being related to *Oreobates*, but rather suggest that *O. quixensis* could be the sister species to the rest of *Oreobates* (Figs. 1–2). In this case, the origin of *Oreobates* would be the Upper Amazon Basin or the Andean foothills. Indeed, members of *Oreobates* are direct-developing frogs that require moist conditions for reproduction and the greatest species diversity in the genus occurs in humid forests of the Andean slopes from 1000–2000 m a.s.l. (Fig. 5). Those species inhabiting drier forests always seem to be restricted to humid microhabitats, such as for example the vegetation close to streams or caves. Moreover, there are two well-supported clades within *Oreobates* (Fig. 2), one including former members of *Oreobates* (robust frogs with marked granular skin and very short, weakly pulsed calls) and another including former members of the *E. discoidalis* group (small and slender frogs with warty or shagreened dorsal skin and longer calls with higher numbers of pulses). These facts suggest two events of diversification, one involving more robust *Oreobates* (i. e., *O. choristolemma*, *O. granulosus*, *O. sanctaecrucis*, *O. sanderi*, and *O. saxatilis*) in the Andean hills close to the Upper Amazon Basin with a subsequent colonization to the south, and another diversification of smaller *Oreobates* (*O. discoidalis*, *O. cruralis*, *O. ibischi*, and *O. madidi*) in the Andes of from Bolivia to Argentina.

The most isolated species, from other members of *Oreobates*, is *O. heterodactylus*, which occurs in two isolated mountain ranges of the Precambrian Brazilian shield (Heyer & Muñoz, 1999; Padial & De la Riva, 2005). This species inhabits relictual humid forests in small canyons and hills of isolated rocky outcrops. The distribution of this species is interesting because this area lacks any other brachycephalid species. This area lays inbetween the range of Andean and eastern Brazilian taxa. The occurrence of *E. heterodactylus* in this area probably made difficult the correct assignment of this species to any species group. For example, Lynch and Myers (1983) placed this species in the former *E. fitzingeri* group, while

Lynch & Duellman, (1997) included it in the *E. binotatus* group from the Atlantic Forests, something that Heyer & Muñoz (1999) considered as not making zoogeographic sense. Padial & De la Riva (2005) pointed out that *O. heterodactylus* resembles species of the *E. binotatus* (Duellman & Lynch, 1997) and *E. discoidalis* (sensu Lynch, 1989) species groups. Subsequently, Padial *et al.* (in press) suggested putative relationships with members of the *E. discoidalis* group based on similarities in advertisement calls. However, according to our analyses *O. heterodactylus* clearly belongs to *Oreobates*. Moreover, our phylogeny supports a close relationship of *O. heterodactylus* with the clade including putatively advanced *Oreobates* in the Andes of Bolivia and Argentina. Indeed, *O. discoidalis*, *O. ibischi* and *O. heterodactylus* are very similar in morphology and share a similar habitat (semi-deciduous forests). Therefore, the distribution pattern of these species indicates a broader distribution of this *Oreobates* in the lowlands during more humid periods and evidences a biogeographical connection between the Andes and the Precambrian shield (see also Pennington *et al.* 2000).

CONCLUSIONS

Phylogenetic analyses of mtDNA together with morphological synapomorphies and bioacoustical similarities support the monophyly of *Oreobates* and the non-monophyly of *Eleutherodactylus*. *Oreobates* contains 14 species, including former members of the *E. discoidalis* species group, *E. heterodactylus* and species assigned to *Oreobates* by Caramaschi and Canedo (2006). This genus is the sister group of the *E. martinicensis* and *E. conspicillatus* series of the subgenus *Eleutherodactylus* and it is not closely related to members of the *E. dolops* or *E. nigrovittatus* groups, nor it is closely related to members of the *E. binotatus* series or *E. verrucosus* (formerly *Ischnocnema verrucosa*). Members of *Oreobates* occur from Amazonian Colombia to northern Argentina, with an isolated vicariant species in the Precambrian Brazilian Shield (*O. heterodactylus*). The highest diversity of *Oreobates* occurs in humid montane forests of the Andean Cordillera Oriental (1000–2000 m asl).

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Operational key to the species of the genus *Oreobates*

1. Dorsal skin smooth to finely shagreened, enlarged discs on fingers III and IV, vomerine odontophores between choanae……………………………………………………………2

Dorsal skin with warts, granules and/or tubercles…………………………………………………4

2. Discs on fingers III and IV large, ovate; few or no warts on dorsum ……………………………...3

Discs moderately enlarged, truncate, low warts on dorsal skin…………………………..…*O. ibischi*

3. Dorsal colouration with arrow-shaped and X-shaped dorsal marks, Brazilian shield distribution…………………………………………………………………….…*O. heterodactylus*

Dorsal colouration plain or with irregular marks and spots, Andean distribution…..…*O. discoidalis*

4. Dorsal skin coarsely shagreened or warty, keratinized granules absent………………………………5

Dorsal skin coarsely warty or tuberculate, keratinized granules present ………………………….…7

5. Dorsal warts homogeneous in size, finger tips rounded, adult SVL 20.3–33.6…..………………...6

Dorsal warts of different sizes sometimes coalesce in dorsolateral ridges, fingers III and IV moderately enlarged……………………………………………………………..…*O. cruralis*

6. Foot length > 50% SVL, supernumerary tubercles inconspicuous or absent……………..…*O. lehri*

Foot length < 50%SVL, supernumerary tubercles conspicuous, numerous.……………..…*O. madidi*

7. Dorsal skin granular……………………………………………………………………..……8

Dorsal skin tuberculate, composed of enlarged warts, subconical tubercles, and granules…………10

8. Dorsal skin with pungent keratinized granules both on dorsum and extremities. …………………9

Dorsal skin with small, round, keratinized granules, low warts, extremities less granular…..…..…11

9. Pungent granules on forearm present……………………………………………..……*O. simmonsi*

Pungent granules on forearm absent……………………………………………….……*O. zongoensis*

10. Abundant large subconical tubercles on dorsum, heavily mottled venter…………..…*O. quixensis*

Few large subconical tubercles on dorsum, faintly mottled venter…………………..…*O. saxatilis*

11. Finger tips not enlarged.…………………………………………...…………………12

Finger tips enlarged and with indented ungual flap……………………………..…...*O. choristolemma*

12. Supernumerary tubercles conspicuous, prominent, abundant, conical; FL = 50% SVL………..13

Supernumerary tubercles inconspicuous, low, 1–3; FL ≥ 50% SVL……………………*O. granulosus*

13. Dorsal colouration brownish-grey, red or scarlet spots absent, head as wide as long…..*O. sanderi*

Red to scarlet spots in axiles, dorsum and shanks, head wider than long……………*O. sanctaecrucis*

APPENDIX

SPECIMENS EXAMINED

ELEUTHERODACTYLUS BABAX (2 specimens). COLOMBIA: Departamento Nariño: 7 km S de Chucunes, Reserva Natural La Planada, KU 200201–2 (paratypes). *E. DOLOPS* (2 specimens) ECUADOR: Provincia Napo: Loreto, USNM 199990 (paratype); Salto de Agua, 2.5 km NNE of Río Reventador, 1660 m, KU 143505 (holotype); Provincia Pastaza: Río Villano, USNM 199991–2 (paratypes). *E. ELASSODISCUS* (2 specimens). ECUADOR: Provincia Napo: Cuyuja, 7750 ft, USNM 167668 (holotype), 167669 (paratype). *E. OCTAVIOI* (3 specimens). BRAZIL: Rio do Janeiro: Tijuca, Guanabara, BM 1964.2008 (paratype), USNM 152767 (paratype), MCZ 64361 (paratype). *E. VERRUCOSUS* (1 specimen). BRAZIL: Minas Gerais: "Byen [=city] Juiz de Fora i Minas Geraes" ZMUC 51 (R 1180) (holotype). *OREOBATES CHORISTOLEMMA* (5 specimens). BOLIVIA: Departamento Cochabamba: "Old" Chapare road, 1300–1500 m, ZFMK 72569; Departamento La Paz: Provincia Sud Yungas, Boquerón, CBG 765–68. *O. CRURALIS* (181 specimens). BOLIVIA: Departamento Beni: Areruta, CBG 1545–7; Asunción, Biosphere Reserve Pilón Lajas, MNK A 4074; Buena Vista, MZUM 66609–10; Huachi, MZUM 58987, 135343; Laguna Azul, Biosphere Reserve Pilón Lajas, MNK A 3975–76, 3979, 3985–87, 4003; Rurrenabaque, CBG 3667, MNCN 42558–9; San Luis Chico, MNK A 4027, 4030; Serranía del Pilón, Biosphere Reserve Pilón Lajas, MNK A 4182–83, 4209–13; Departamento Cochabamba: Bulo Bulo, TIPNIS, CBG 350, 380, 382–3, 393; Chaquisacha, 1300–1500 m, Parque Nacional Carrasco, CBG 198, 199, 1140; de Paracti a El Palmar, ZFMK 66964, ZFMK 66971–2; El Palmar, 1300 m. Parque Nacional Carrasco, CBG 276, 956, ZFMK 72570; entre Paractito y El Palmar, ZFMK 66964 ZFMK 66971–2, ZFMK 72541–3; Los Guácharos (Chapare, 500 m), CBG 308, MNK A 6617–19, MNCN 43018–9, ZFMK 72532; on Villa Tunari road, USNM 146577; Puerto Villaroel, CBG 1337; Río Grande, 1000 m, Parque Nacional Carrasco, CBG 275; San José de la Angostura, TIPNIS, CBG952–54; Santa Anita, TIPNIS, CBG 739; Santo Domingo 800 m, TIPNIS, CBG 596–7; Serranía de Mosetenes 1580 m, MNCN 43155; Valle de Sajta, MNK A 3633, 1489; Villa Tunari, MNK A 1492; Departamento La Paz: Arroyo Pico Plancha, San José y Apolo, ANMI-Parque Nacional Madidi, MNK A 7180; Aserradero San Francisco, Ixiamas, CBG 1133; Boquerón, Biosphere Reserve Pilón Lajas, CBG 789–93; Camino a Bella Vista, Sud Yungas, MNK A 3792; Camino maderero El Chaval, Arroyo Mikai, Biosphere Reserve Pilón Lajas, MNK A 3759–61; Chalalán, ANMI-Parque Nacional Madidi, MNCN 42560, 43021–2, 43058, 43222, 43223; Flor de Mayo, La Asunta, CBG 2936; Flor de Mayo, río Boopi, CBG 1791; Hornuni, Parque Nacional AMNI-Cotapata, CBG 4208; Irupana, CBG 529; La Paz (locality in error), BM 1947.2.15.70 (holotype); CBG 2484; Puerto Linares, 360 m, USNM 281100–30; road from Caranavi to Palos Blancos, ZFMK 80599; Serranía Tequeje, CBG 5104–7; Serranía del Pilón, Biosphere Reserve Pilón Lajas MNK A 4182–6, 4201, 4208–13, 4218; Torno Azul, Biosphere Reserve Pilón Lajas, CBG 1004; Tumupassa, MZUM 58985–6, 58988; Tunquini, Parque Nacional AMNI-Cotapata, CBG 4224; 5 km W of San Buenaventura USNM 280617; Departamento Pando: Florida, Reserva Manuripi, MNK A 5086, 5122; Lago Bay, Reserva Manuripi, MNK A 6120–21; Departamento Santa Cruz: Buena Vista, USNM 118686; camino a BellaVista desde la carretera a Samaipata, MNCN 42557, 42977, MNK A 7171, ZFMK 71997; La Hoyada, Parque Nacional Amboró, MNK A 5577, ZFMK 72644; Mataracú, Parque Nacional Amboró, MNK A 3950, 3952, 3954, 3968; Santa Cruz de la Sierra, BM 1904.10.29.102–107; S of Cuevas ZFMK 72644; Víbora, CBG 3638. PERU: Departamento Madre de Dios: Colpa de Guacamayos, Zona Reservada Tambopata-Candamo, USNM 332436–37; Puerto Maldonado, 30 km SSW of Tambopata, USNM 284267, 343240, 342989–92; 15 km E of Puerto Maldonado, 200 m, KU 207749, 215461–62, 215479–80; Departamento Cusco: Río los Amigos, MHNC 3170, 3172, 3177. *O. DISCOIDALIS* (113 specimens). ARGENTINA: Province Tucumán: El Indio, km 28 Tafí del Valle-Amaicha del Valle road, 960 m, KU 182815; Horco Molle, "13 km W of Tucumán", Sierra de San Javier, ca. 1200 m, BM 1947.2.15.63–65 (syntypes), KU 154521–29; MCZ 35583; 24 km W of Tucumán, KU 206434–38; without locality, MCZ 117097; Province Jujuy: Abra de las Cañas, Serranía de Calilegua, 1550 m, KU 182813–14; Arroyo Agua Negra, near border Salta-Jujuy, 10 km up to Manolito and Valle Grande, from

Yuto to Ledesma, TNHC 36776, 36778–93; San Lorenzo, BM 98.7.7.19.20. BOLIVIA: Departamento Chuquisaca: Rio Pilipili, CBG 1396–7; Departamento Santa Cruz: Río Parabano, MNK A 5582; Departamento Tarija: Bajada de la Escalera, Reserva Nacional Tariquía, CBG 4978–9; Cambarí. Reserva Nacional de Tariquía, CBG 4889–90; Chiquiacá MNK A 7262–3, MNCN 43133–35; Entre Ríos, MNK A 7257–59, MNCN 43132; La Planchada, CBG 3773, 3780–4; Potrerillas, Reserva Nacional Tariquía, CBG 4891; Serranía Aguarague, a 17 km de Villa Montes en dirección a Entre Ríos, MNK A 7246–56, MNCN 43123, 43125–31, 43136–9; Serrania Aguarague, a 40 km de Yacuiba, CBG 1411–17; subida a Palo Marcado, Reserva Nacional Tariquía, CBG 4970; 12.3 km NW of Entre Ríos on the road to Tarija, 1900 M, MNK A 3877–97, UTA 45645, 45648–50, 45652, 45658–62. *O. GRANULOSUS* (26 specimens). PERU: Department Puno: Agualani, Carabaya, 9000 ft, BM 1905.5.31.14.20 (four specimens); Limbani, Carabaya, 9000 ft, BM 1905.5.31.21 (formerly 1904.10.26.94–99); Ollachea, 11 km (airline NNE of), 1800 m, USNM 299006–12; Santo Domingo, Carabaya, 1400–1500 m, MHNC 5328, 5335; Santo Domingo, Carabaya, 6000 ft (1800 m aprox.), BM 1947.2.15.72 (holotype); Santo Domingo, Carabaya, 6500 ft (2000 m aprox.), BM 1907.5.7.17–18; Juliaca (locality in error), AMNH 6060–2, 6064. *O. HETERODACTYLUS* (7 specimens). BOLIVIA: Departamento Santa Cruz: Bella Boca, MNK A 6482; Cerro del Arco, Serranía de Santiago, MNK A 6356–7, 7175–6, MNCN 43055–6. *O. IBISCHI* (4 specimens). BOLIVIA: Departamento Santa Cruz: km 68.5 on Santa Cruz de la Sierra-Samaipata road, 750 m elevation, CBF 3341 (holotype); km 60 on Santa Cruz de la Sierra-Samaipata road, MNK A 6612, MNCN 42959; Samaipata, ZFMK 60402 (paratype). *O. LEHRI* (31 specimens). PERU: Departamento Cusco: Apurimac River Valley, Camisea Natural Gas Pipeline, Wayrapata Camp, 2445 m asl, (12º 50' 10'' S, 73º 29' 43'' W), USNM 537848 (holotype), USNM 537846–57 (paratopotypes); Buenos Aires, 2400 m, KU 173231; Esperanza, Kosñipata Valley, 2600–2800 m asl, (13° 10' 56'' S, 71° 36' 227'' W), MHNC 3223–31(paratype), 3234–37(paratype), MNCN 43740–1(paratype); Paucartambo, AMNH 153087; Pillahuata, between Puente Kosñipata and road, 2430 m asl, (13º 09' 52'' S, 71º 35' 46'' W), AMNH 11831 (paratype). *O. MADIDI* (6 specimens). BOLIVIA: Departamento La Paz: Arroyo Huacataya, Serranía Eslabón, Área Natural de Manejo Integrado Madidi, MNK A 7856 (holotype), 7197 (paratype), MNCN 42014–15 (paratypes); La Cascada, Biosphere Reserve Pilón Lajas, MNK A 4137–38 (paratypes). *O. QUIXENSIS* (59 specimens). BOLIVIA: Departamento Pando: Luz de América, Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 6525; Murden, 4591; Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 3640–1, 6099; Reserva Nacional de Vida Silvestre Tahuamanu, NKA 4586–90; Río Negro, MNK A 6525–27, 6900; San Antonio, Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 6526–27. COLOMBIA: Departamento Amazonas: Puerto Nariño MCZ 93777–80; Río Amaca–Yacu, MCZ 95734. ECUADOR: Provincia Morona-Santiago: Sucua, MCZ 91361; Provincia Napo: AMO-II drill site, MCZ 111776–7; Archidona, MNCN 1709–21 (paralectotypes); Coca, MCZ 105868, 106017–19, 124825; Hacienda Primavera, MCZ 97881; La Cruz Blanca, South side of Río Napo, MCZ 109213–14, 119093; Limón Cocha MCZ 56307; near Tarapoa, MCZ 101262; Santa Cecilia, MCZ 57793–97, 57799; San José de Moti, MNCN 1708 (lectotype); Provincia Oriente: Canelos MCZ 19734–6. PERU: Departamento Amazonas: MCZ 125896–7. *O. SANCTAECRUCIS* (13 specimens). BOLIVIA: Departamento Cochabamba: Chaquisacha, 1300–1500 m, Parque Nacional Carrasco, CBG 152–3; Diampampa, Parque Nacional Carrasco, CBG 634; Karahuasi, ZFMK 72647; Río Grande, 1000 m, Parque Nacional Carrasco, CBG 291–292; Departamento Santa Cruz: El Bibosi, Parque Nacional Amboró, MNK A 6697; El Chapé, Parque Nacional Amboró, 2060 m elevation, MNK A 1198 (holotype), 7158, MNCN 42010–13. *O. SANDERI* (19 specimens). BOLIVIA: Department La Paz: Arroyo Bilunto, Chunirumi Valley, Bilunto Mountains, Area Natural de Manejo Integrado Madidi, near Santa Cruz de Valle Ameno, CBF 5385 (holotype), MNCN 42016–7 (paratypes), CBF 5383–4 (paratypes); Bajo Hornuni, CBF 4119–22, 4218–19, 4223 (paratypes); Colonia Eduardo Avaroa, ca. 30 km north of Caranavi on the road from Caranavi to Yucumo, ZFMK 80600–1 (paratypes), MNK A 6563 (paratypes); road from Apolo to Sarayo, MNK A 6695–6 (paratypes). PERU: Departamento Puno: Abra de Maruncuna, 10 km SW of San Juán del Oro, 1650 m, KU 206101; Juliaca (locality in error), AMNH 6063. *O. SAXATILIS* (2 specimens). PERU: Departamento San Martín: Pongo de

Shilcayo, about 4 km NNW of Tarapoto, 470 m, KU 212556 (holotype), MHNSM 8431 (paratype). *O. SIMMONSI* (1 specimen). ECUADOR: Provincia Morona de Santiago: Río Piuntza, 1830 m, KU 147068 (holotype). *O. ZONGOENSIS* (1 specimen). BOLIVIA: Departamento La Paz: Valle de Zongo, 1250 m, CBF 2503 (holotype).

APÉNDICE X

SYSTEMATICS OF THE *ELEUTHERODACTYLUS FRAUDATOR* SPECIES GROUP (ANURA: BRACHYCEPHALIDAE)

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ABSTRACT: Members of the *Eleutherodactylus fraudator* species group occur in Bolivia and Peru. This group has not been reviewed since its proposal and description, its putative monophyly has not been tested and its phylogenetic relationships are still unresolved. It was included in the Central American genus *Craugastor* based on a putative morphological synapomorphy but this hypothesis of relationship is doubtful. Its alpha-diversity is unknown and some putative members have still not been studied in detail. To assess the taxonomic status and phylogenetic relationships of this species group, we applied a taxonomic integrative approach based on comparisons of morphological characters (external and internal), bioacoustics (based on advertisement calls) and phylogenetic analyses of partial 16S mitochondrial gene (560 bp). We redescribe *Eleutherodactylus bisignatus* (Werner, 1899) and include it together with *E. mercedesae* in the group. Molecular, bioacoustical and morphological characters support the taxonomic status of all the species. Maximum Parsimony, Bayesian and Neighbor Joining phylogenetic analyses of partial mitochondrial gene, the presence of two shared derived morphological characters (a frontoparietal fontanelle and a new condition of the mandibular ramus of the trigeminal nerve) and two bioacoustical character support the monophyly of this group. Moreover, our data suggest the non-monophyly of the subgenus *Eleutherodactylus* and the monophyly of the genus *Craugastor* when the *C. bufoniformis* species group is excluded. We redescribe the crown clade formed by members of the *E. fraudator* group and named it as the subgenus *Yunganastes*, which contains: *Eleutherodactylus ashkapara* Köhler, 2000, *E. bisignatus* (Werner, 1899), *E. fraudator* Lynch and McDiarmid, 1987, *E. mercedesae* Lynch and McDiarmid, 1987 and *E. pluvicanorus* De la Riva and Lynch, 1997. We describe the advertisement call of *E. bisignatus* for the first time and compare it with available calls of *E. ashkapara* and *E. pluvicanorus*. We suggest the common structure of the advertisement calls and the aggressive calls of the species belonging to *Yunganastes* (single melodic whistles with frequency modulation and no pulses) as synapomorphic for the new subgenus. We exclude members of *Yunganastes* from the Middle American Genus *Craugastor*, to which it was previously tied by the presence of the putative synapomorphic "E" condition of the trigeminal nerve of the mandibular ramus of the abductor muscle. We describe a new condition of the trigeminal nerve and consideredit synapomorphic for *Yunganastes*. Members of this subgenus are endemic of the cloud forests and humid montane forests of the Cordillera Oriental of the Andes in Bolivia and southern Peru.

Key words: Andes, Anura, Bolivia, *Eleutherodactylus fraudator* species group, mtDNA, Peru, phylogenetics, taxonomy.

SPECIES GROUPS are subgeneric arrangements of phenetically similar species intended to facilitate taxonomic work in large genera (Mayr, 1942). Several authors defined and used species groups to manage the genus *Eleutherodactylus*, although these groups are viewed as a previous step until the monophyly can be tested (Lynch and Duellman, 1997). Lynch (1976) performed a large-scale phenetic-arrangement of South American *Eleutherodactylus* in species groups. These groups were composed of species sharing combination of external and internal morphological characteristics. Because the first aim of this procedure was to facilitate the organization and study of these frogs, characters were chosen without testing their phylogenetic significance. Nevertheless, there was still the hope that, at least, some of these groups would coincide with monophyletic clades (Lynch and Duellman, 1997). Thus, in more recent times, there has been a search for synapomorphies (i.e. Lynch, 1993; Lynch, 2001; Guayasamin, 2004) and for phylogenetic redefinition and/or classification of groups (Lynch, 1986; Lynch, 1989; Hedges, 1989). However, species groups are still being described without proposing phylogenetically informative characters (i.e. Lynch and Duellman, 1997; Köhler, 2000*a*). Molecular phylogenetics (i.e. Crawford and Smith, 2005) and comparative morphological studies (i.e. Guayasamin, 2004) are helping to identify clades among the subgeneric units of *Eleutherodactylus*.

Köhler (2000*a*) first proposed and defined the *Eleutherodactylus fraudator* species group to include some phenetically very similar species from central Bolivia: *E. ashkapara* Köhler, 2000, *E. fraudator* Lynch and McDiarmid, 1987 and *E. pluvicanorus* De la Riva and Lynch, 1997. Another species, *E. mercedesae* Lynch and McDiarmid, 1987, was pointed as candidate to join the group but it was not included because of some external dissimilarities. Lynch and McDiarmid (1987) tentatively assigned *E. fraudator* to the *Eleutherodactylus conspicillatus* species group in the original description. Subsequently, Lynch and Duellman (1997) do not assigned *E. fraudator* and *E. mercedesae* to any species group; instead, they placed them in the subgenus *Eleutherodactylus* without further discussion. De la Riva and Lynch (1997) considered *E. fraudator* and *E. pluvicanorus* as the nearest relatives, due to phenetic similarities and presence of a frontoparietal fontanelle. Moreover, they also tentatively assigned this pair of species to the subgenus *Craugastor* based on the presence of the shared "E" condition of the trigeminal nerve of the mandibular ramus of the abductor muscle (see Lynch, 1986). Later, Köhler (2000*a*), by defining the *E. fraudator* group, suspected the species included to differ from the subgenus *Craugastor*. Although the phylogenetic position and relationships of *Craugastor* have recently been analyzed (Crawford

FIG. 1.—Humid Montane Forest from Eastern Cordillera of the Central Andes in Bolivia, habitat of *E. ashkapara*, *E. mercedesae* and *E. pluvicanorus*.

& Smith, 2005), the position of the *E. fraudator* species group remained unclear. Frost et al. (2006) rejected the former hypothesis of relationship between *Craugastor* and the *E. fraudator* species group based on molecular analyses and a new condition of the trigeminal nerve of the abductor muscle detected in *E. pluvicanorus*. Nevertheless, their molecular analysis based on *E. pluvicanorus* is probably erroneous (see below) and they did not included additional taxa of the group. Therefore, some questions remain open: Which is the true alpha diversity of the group? Is the *E. fraudator* species group a mere phenetic cluster or a clade? Which is its relationship with the genus *Craugastor* (Crawford and Smith, 2005; Frost et al., 2006)? Is the "E" condition homoplasic? Do the differences observed by Frost et al. (2006) for the "E" condition of the trigeminal nerve in *E. pluvicanorus* extend to the other taxa of the group?

Due to a recent rediscovery and the accumulation of morphological, bioacustical and molecular data, we are now in good condition to answer these questions. Therefore, the aims of this study are to: (1) test the validity of the species belonging to the *E. fraudator* group by different criteria [i.e. integrative taxonomy, sensu Dayrat (2005), Will et al. (2005) and Padial and De la Riva (2006)]; (2) test the monophyly of the group; (3) clarify its phylogenetic relationships; and, as a consequence of the results, (4) describe and name a new subgenus within *Eleutherodactylus*.

MATHERIAL AND METHODS

FIELDWORK

We conducted fieldwork on several times between 1989 and 2004. The study area includes the Andean slopes of Eastern Cordillera Oriental from southern Peru to northern Bolivia. The habitat sampled for the target species were humid montane rainforest and cloud forest [see Köhler (2000*b*) for further details on the habitats sampled]. Humid montane rainforest (Fig. 1) is characterized as follows: 500–2500 m asl; divided into upper montane rainforests (1500–2500 m), with mean annual temperature 15–24°C, minima below 0°C above 2300 masl, mean annual precipitation 2500 to more than 6000 mm; and lower montane rainforests (500–1500 m), with maximum rainfall between 1500 and 1800 masl, 0–2 arid months; characterized by steep slopes and deep valleys; evergreen forest of medium height (15–30 m). Cloud forest (Fig. 2) is characterized as follows: 2500–3500 masl at the perhumid northeastern versant of the Bolivian Andes; mean annual temperature 10–14ºC; mean annual precipitation 2500–3500 mm; 0–2 arid months; low evergreen forest.

We collected specimens by hand during nights and under wet whether conditions. They were fixed in 10% formalin and preserved in 70% ethanol. Some of the specimens (JKSL collection) from Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) were fixed in absolute ethanol and preserved in 70% ethanol. We clipped fingertips for molecular analyses and deposited tissue samples in the DNA collection of Museo Nacional de Ciencias Naturales, Madrid (MNCN-CSIC). When possible, we took pictures and color notes in the field. We recorded advertisement calls in the field.

TABLE 1.—Numerical characteristics of the advertisement call of some members of the subgenus *Yunganastes*.

Species, Locality and source	**Calls (specimens) analyzed**	**Calls/minute**	**Call length [ms]**	**Frequency band [Hz]**	**Dominant frequency [Hz]**	**°C**	**Vouchers**	**Fonozoo numbers**
E. ashkapara Chapare Köhler (2000*a*)	43(2)	55–74 (67.2±6.6)	24–43 (36.0±5.9)	1150–1830	1454–1493(1476±11)	15.9	CBF3344 ZFMK 70318	5568
E. bisignatus Toriri	35(2)	68–84 (77.5±6.2)	16–25 (20.8±2.7)	1000–5300	1182–1275(1220±37)	20	One from CBF5717–20	5810
E. pluvicanorus Sehuencas, Köhler (2000*b*)	25(1)	65–87 (75.0±9.2)	210–440 (310.5±72.2)	1100–1600	1348–1361(1356±5)	16.4	One from 60186–204	5588
E. pluvicanorus Sehuencas	18(1)	79–91 (84.9±4.7)	209–337 (284.1±30.3)	1000–1600	1242–1264(1251±7)	18.0	MNCN43002	5811
E. pluvicanorus La Yunga	12(1)	15–18 (16.4±0.5)	229–270 (246.8±12.5)	1000–1900	1392–1465(1447±23)	14.5	MNKA6623	5812

MORPHOLOGY

We followed Lynch (1989) and Lynch and Duellman (1997) for morphological and color characteristics used in the diagnosis and description. A single person (JMP) took measurements with a digital caliper to the nearest 0.01mm, but following Hayek et al. (2001), for avoiding pseudo precision, we rounded all measurements to only one decimal. Abbreviations are as follows: snout–vent length, SVL; head length (from posterior margin of lower jaw to tip of snout), HL; head width (measured at level of rictus), HW; eye length (measured horizontally), EL; eye to nostril distance, EN; internarial distance, IND; eye-eye distance, EE; tympanic membrane height, TYH; tympanic membrane length, TYL; width of third finger disc, FIII; arm length (from posterior margin of thenar tubercle to elbow), ARM; tibia length, TL; thigh length, TH (from vent to knee); foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL; width of fourth finger disc, TIV. We do not include values of interorbital distance (IOD) and upper eyelid width (EW). Our experience indicates that these parameters are usually of scarce utility because the preservation condition of specimen highly influences the measurements and makes it difficult to have precise and comparable values. Color characteristics were noted in life and in alcohol. We determined age and sexual condition by dissection or observation of external secondary sexual characters. The condition of the trigeminal nerve (see Lynch, 1986) was determined through dissection of the skin above the tympanic area and through a horizontal cut of the mandibular joint. Museum abbreviations other than cited by Levinton et al. (1985) are: Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Cochabamba, Bolivia (CBG); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA [Amphibian Collection], formerly NKA). In the Appendix, we include a list of specimens examined.

BIOACOUSTICS

Recording equipment included a Sony WM D6C tape recorder, a Sennheiser Me 80 directional microphone and TDK SA60 cassettes. We digitized the sounds at a sampling rate of 44.1 KHz and 32 bit resolution with a Delta 66 digitizing board and Peak 3.2 (OSX) software (Fonoteca Zoológica Digital, MNCN). To obtain numerical information and to generate audiospectrograms and oscillograms we used Cool Edit software. Frequency information was obtained through Fast Fourier Transformations (FFT) (width, 1024 points) at Hanning window function. Air temperature was measured immediately after sound recording. Digitized calls were deposited in the Fonoteca Zoológica Digital (MNCN). Call vouchers and references for calls are shown in Table 1. We analyzed 133 calls belonging to three species and five

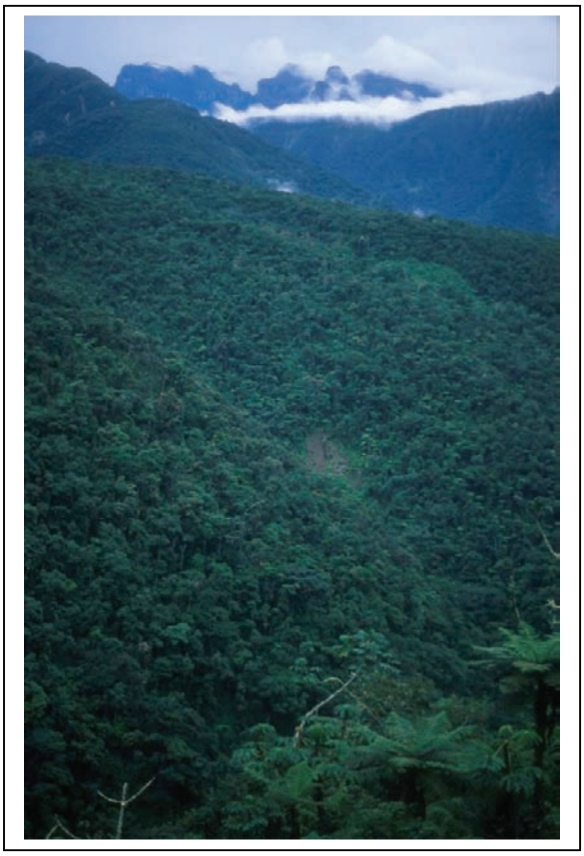

FIG. 2.—Cloud Forest from Eastern Cordillera of the Central Andes in Bolivia, habitat of *E. bisignatus*, *E. fraudator* and *E. pluvicanorus*.

populations (Table 1).

Identification of advertisement and aggressive calls is based on the observed behavior of frogs and our previous experiences (i.e. Márquez et al., 1995; Köhler, 2000*a*, *b*; Bosch and De la Riva, 2004). We analyzed the following qualitative and quantitative parameters: notes per call, call repetition rate, call duration, fundamental frequency, dominant frequency, frequency band, distribution of intensity, and frequency modulation. Terminology in call descriptions generally follows Márquez et al. (1995) as extended by Köhler (2000*b*). Sample sizes do not allow for temperature correction using regression. Mean values for call parameters are shown in Table 1.

MOLECULAR PROCEDURES AND PHYLOGENETIC ANALYSES

For the molecular analyses we sampled a total of 27 individuals belonging to 10 taxa (according to previous classifications) from different localities (Table 2). *Leptodactylus chaquensis* (Anura. Leptodactylidae) was the outgroup. We included sequences from additional species of *Eleutherodactylus* and *Craugastor* (Table 2) downloaded from public databases for genetic sequences (NCBI; http://www.ncbi.nlm.nih.gov/). We used the standard phenol-chloroform extraction protocol (Sambrook et al., 1989) using a with minor changes to isolate genomic DNA. A fragment of approximately 591 bp from the mitochondrial gene 16S was amplified by polymerase chain reaction (PCR) using the primers 16Sar-5' and 16Sbr-3' and previously described PCR conditions (Hillis et al., 1996). PCR products were purified and sequenced in a MegaBACE 1000^{TM} (GR Health Care) instrument following manufacturer protocols. Sequence alignments were performed using the program CLUSTAL X 1.83.1 (Thompson et al., 1997) with a gap opening = 10, and a gap extension = 0.10. A fragment of 30 bp was considered ambiguously aligned and excluded from phylogenetic analyses. We used the program MODELTEST 3.7 (Posada and Crandall, 1998) to select the best substitution model. The model and the parameter estimates were chosen by Akaike's minimum information criterion, or AIC (Akaike, 1974). The model of DNA sequence evolution that required a minimum number of parameters adequate to explain the data was GTR + I + G (General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites). For Bayesian phylogenetic analyses (Rannala and Yang, 1996) we used MrBayes version 3.2.1 (Huelsenbeck and Ronquist, 2001). The majority rule consensus tree was produced from two separate Monte Carlo Markov chains (MCMC; Rannala and Yang, 1997), each run used one cold chain (the head chain) and two heated chains (scout chains). It was run simultaneously for one million generations (Metropolis-coupled MCMC). Trees were sampled every 100 generations. Burn-in was evaluated by examination of the standard deviation of split frequencies (> 0.01). Burn-in occurred in fewer than 25 % of the samples obtained during the first 10,000 generations, so the first 2500 trees were always excluded. Default priors and conditions were used in all cases. Neighbor Joining (NJ) analyses were performed using PAUP* 4.0b10 (Swofford, 1998), with Maximum Likelihood (ML) genetic divergence corresponding to the model GTR + I + G. The relative branch support was evaluated with 1000 Bootstrap replicates. Maximum Parsimony (MP) analyses were done with PAUP* 4.0b10 using heuristic searches under parsimony and tree bisection reconnection (TBR). In order to obtain estimates of clade support, non-parametric bootstrapping was performed with heuristic searches of 1000 replicate datasets.

TABLE 2.—Localities, voucher information, and GenBank accession numbers for sequences and specimens used in this study. Numbers in brackets correspond to sequences used in previous studies: (1) Darst and Cannatella (2004); (2) Faivovich et al. (2005); (3) Frost et al. (2006). Abbreviations: AMNH: American Museum of Natural History (USA); CBF: Colección Boliviana de Fauna (Bolivia); CBG: Centro de Biodiversidad y Genética (Bolivia); CFBH: Celio F. B. Haddad specimen collection; IDlR: Ignacio De la Riva field series; JAC: Jonnathan A. Campbell field series; JMP: José M. Padial field series; KU: Natural History Museum, The University of Kansas (USA); MNCN: Museo Nacional de Ciencias Naturales (Spain); MNK-A: Amphibian Collection, Museo de Historia Natural Noel Kempff Mercado (Bolivia); SIUC: Southern Illinois University at Carbondale (USA); UAZ: Herpetology Collection, University of Arizona, Tucson (USA); USNM: National Museum of Natural History, Smithsonian Institution (USA); ZFMK: Zoologisches Forschunginstitut und Museum Alexander Koenig (Germany).

Species	**Locality**	**Museum no.**	**MNCN DNA**	**16S**
C. alfredi	México: Veracruz: Cruz de los Naranjos, 1100 m.	JAC 21987	-	DQ283318(3)
C. augusti	México: Sonora: Alamos.	UAZ unnumbered	-	DQ283271(3)
C. bufoniformis	Panama: Coclé: Parque Nacional El Copé.	CFBH 5813	-	DQ283165(3)
C. fitzingeri	Costa Rica: Limón: Estación Experimental La Lola.	-	-	AY326001(1)
C. mexicanus	Mexico: Hidalgo: 4.5 km NE Tlanchinol.	-	-	AY326006(1)
C. punctarioulus	Panama: Coclé: Parque Nacional El Copé.	SIUC 7066	-	DQ283168(3)
C. ranoides	Panama: Bocas del Toro: Isla Escudo de Veraguas, West Point.	USNM-FS 195393	-	DQ283105(3)
C. rhodophis	México: Oxaca: El Mirador, Municipio Santa María Chilchotla.	JAC 22721	-	DQ283317(3)
E. (E.) chloronotus	Ecuador: Napo: 3.5 km E Santa Bárbara.	KU 202325	-	AY326007(1)
E. (E.) discoidalis	Bolivia: Tarija: Serranía Aguarague, 17 km from Villa Montes to Entre Ríos.	MNK A 7247	6123	…
E. (E.) duellmani	Ecuador: Carchi: ~5 km W La Gruel; 2340 m.	KU 202404	-	AY326003(1)
E. (E.) fenestratus	Bolivia: Cochabamba: road Los Guácharos - El Palmar, 8 km from Los Guácharos.	MNK A 6633	4109	…
E. (E.) platydactylus	Perú: Quispicanchis Between San Miguel and Marcapata.	IDlR4652	5524	…
E. (E.) rhabdolaemus	Bolivia: Santa Cruz: Serranía de la Siberia, between El Empalme and Carahuasi	MNK A 6628	3940	…

E. (E.) rhabdolaemus	Bolivia: Santa Cruz: La Yunga	MNCN 43036	4120	…
E. (E.) cf. *rhabdolaemus*	Bolivia: Santa Cruz: San Juán Canton, Amboró National Park, 2050 m.	AMNH A 165195	-	AY843586(2, 3)
E. (E.) thymelensis	Ecuador: Carchi; 12 km W Tufino; 3520 m.	KU 202519	-	AY326009(1)
E. (E.) w-nigrum	Ecuador: Carchi; ~5 km W La Gruel; 2340 m.	KU 205076	-	AY326004(1)
E. (Y.) ashkapara	Bolivia: Cochabamba: Old Chapare road.	ZFMK 70318	7312	…
E. (Y.) bisignatus	Bolivia: La Paz: Toriri.	CBF 5718	6332	…
E. (Y.) bisignatus	Bolivia: La Paz: Toriri.	CBF 5719	6331	…
E. (Y.) fraudator	Bolivia: Cochabamba 73.5 km from Cochabamba to Villa Tunari.	MNCN 43107	5984	…
E. (Y.) mercedesae	Bolivia: Cochabamba Old Chapare road.	ZFMK 72571	7318	…
E. (Y.) mercedesae	Bolivia: Cochabamba Old Chapare road.	ZFMK 72572	7319	…
E. (Y.) mercedesae	Bolivia: Cochabamba Old Chapare road.	ZFMK 72573	7320	…
E. (Y.) mercedesae	Bolivia: Cochabamba Old Chapare road.	ZFMK 72597	7321	…
E. (Y.) mercedesae	Bolivia: Cochabamba Old Chapare road.	ZFMK 72598	7322	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	MNCN 43002	3928	…
E. (Y.) pluvicanorus	Bolivia: Santa Cruz: La Yunga de Mairana.	MNK A 6623	4118	…
E. (Y.) pluvicanorus	Bolivia: Santa Cruz: La Yunga de Mairana.	MNCN 43035	4119	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: El Palmar.	CBG 963	5774	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 134	5735	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 133	5750	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 128	5754	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 132	5761	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 129	5771	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 131	5772	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: Sehuencas.	CBG 92	5778	…
E. (Y.) pluvicanorus	Bolivia: Cochabamba: road of Penstock's bridge tubes.	…	6004	…
L. chaquensis	Bolivia: Santa Cruz: Lomas de Arena.	JMP 223)	4042	…

RESULTS

SYSTEMATICS

YUNGANASTES SUBGEN. NOV.

Type species: *Eleutherodactylus pluvicanorus* De la Riva & Lynch, 1997 (Holotype: MNKA 1100).

Diagnosis: moderate to medium-sized frogs (SVL in males 25.9–52.2 mm, in females 33.6–63.3) with head wider or equal than long; snout short; sexually dimorphic in size; cranial crests absent; body robust; limbs moderately long; skin on venter smooth to granular; discoidal fold absent; dorsolateral folds complete or rudimentary; males with faint nuptial pads, vocal slits and large vocal sac; tympanic membrane and annulus visible beneath skin; prominent supratympanic fold; canthus rostralis sharp; disc on Finger III and IV and on toes broad; Finger I slightly longer or equal than II; Toe V equal or slightly shorter than Toe III, not reaching distal subarticular tubercle of Toe IV [condition B sensu Lynch and Duellman (1997)]; webbing absent or rudimentary on toes; no tubercles on heel or tarsus; tarsal tubercle absent in all but one species (*E. mercedesae*); vomerine odontophores conspicuous and oval; mandibular ramus of the trigeminal nerve of the adductor muscle anterior to both the *m. adductor mandibulae anterior*, *m. adductor mandibulae posterior* and *m. adductor mandibulae externus* (E2 condition, Fig. 5); presence of an exposed frontoparietal fontanelle; advertisement call consisting of single melodic whistles with frequency modulation, absence of pulses, and low dominant frequency (1200–1500 Hz).

We include in the subgenus *Yunganastes*: *E. ashkapara*, *E. fraudator*, *E. pluvicanorus* [previously included by Köhler, (2000*a*, *b*) in the *E. fraudator* species group], plus *E. bisignatus* and *E. mercedesae*.

FIG. 3.— A) *Eleutherodactylus ashkapara* from Chapare, Bolivia (CBF 3344, holotype); B) *E. fraudator* from La Siberia, Bolivia (one from the series ZFMK 72660–62); C) *E. mercedesae* from Chapare, Bolivia (one from the series ZFMK 72571–73); D) *E. pluvicanorus* from La Yunga, Bolivia (one from the series ZFMK 66872–75).

Etymology.—From the Quechua word “yunga”, that designates the humid forest of the Andean valleys and the Greek “nastes”, meaning dweller, to indicate the typical habitat occupied by members of this subgenus. The nominal gender is masculine.

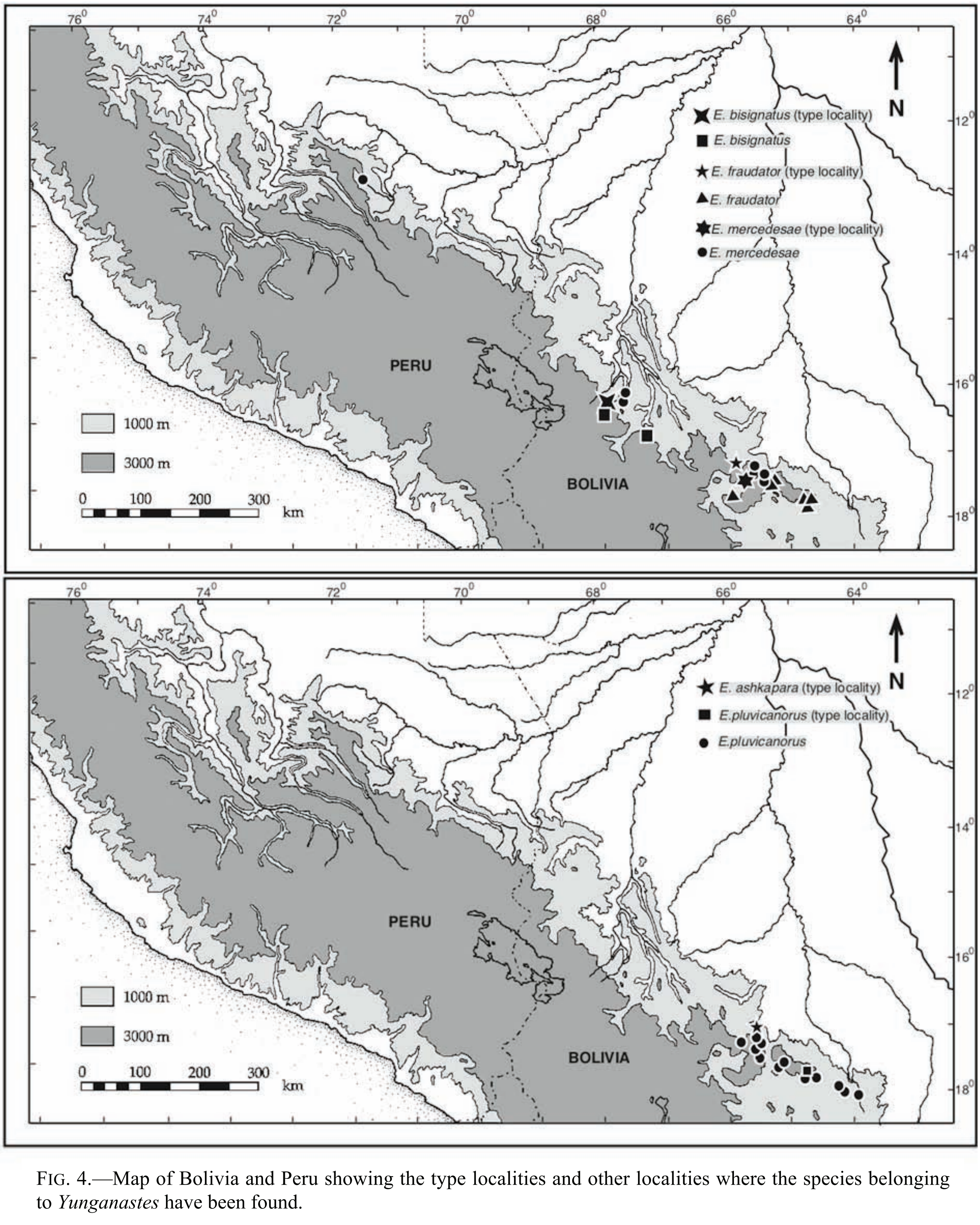

FIG. 4.—Map of Bolivia and Peru showing the type localities and other localities where the species belonging to *Yunganastes* have been found.

SPECIES ACCOUNTS

Eleutherodactylus ashkapara Köhler, 2000

(Fig. 1A)

Diagnosis.—(1) skin on dorsum shagreen; well developed dorsolateral folds; venter smooth, no discoidal fold; (2) tympanic membrane distinct, oval, visible beneath the skin, 50% ED; (3) head wider than long, snout rounded in dorsal view and lateral profile; canthus rostralis sharp; (4) prominent tubercles on upper eyelid; (5) choanae not concealed by palatal shelf of the maxillary arch; vomerine odontophores oval, prominent, well separated, median behind choanae; (6) males with vocal slits posterolateral to tongue; large subgular vocal sac; non spinous nuptial pads; (7) first finger slightly longer than second; (8) tips of outer two fingers truncate, with large pads, tips of inner two fingers rounded; fingers with weak fringes; (9) no ulnar tubercles or folds on heel and tarsus; (10) toes with weak lateral fringes; webbing absent; (11) fifth toe equal in length to third toe, not reaching distal subarticular tubercle on fourth toe [condition B, Lynch and Duellman, (1997)]; (12) toe tips truncate, expanded, slightly smaller than those of outer finger; (13) dorsum brown with darker markings; dark brown canthal and supratympanic stripe, upper lip with irregular brown blotches; belly cream with brown spots and blotches; (14) E2 condition of the mandibular ramus of the trigeminal nerve (Fig. 5); (15) exposed frontoparietal fontanelle.

This species is most similar to *E. pluvicanorus*, from which it can be distinguished by its bigger size, different proportions, and advertisement call (see remarks); from other species of *Yunganastes* it can be distinguished as follows (characters of *E. ashkapara* in parenthesis): *E. bisignatus* has granular belly (smooth), smaller male size (see table 3), and less developed finger discs; *E. fraudator* is also smaller, has several parallel longitudinal dorsal stripes (absent), a broad continuous bold black stripe from snout to above shoulder (discontinuous and thin) and less developed finger discs; *E. mercedesae* differs mainly by slender body and longer feet and tibia in relation to body size, greenish coloration (brownish), presence of basal interdigital membrane on feet, presence of tarsal fold (absent), and faint incomplete dorsolateral folds (complete and conspicuous). Other sympatric species, as *E. patydactyluss*, *E. rhabdolaemus* and *E. danae*, differ mainly by having aerolate belly, first finger shorter than second, all finger discs expanded and smaller size.

Description.—The original description by Köhler (2000*a*) is accurate.

Natural History.—This is an arboreal nocturnal species that calls during the rainy season inside moss of the cloud forest canopy, from 2.5 to 10 m height (Köhler, 2000). See Köhler (2000*a*, *b*) for the description of the advertisement call (see also bioacoustics section above). This species seems to be very scarce, since subsequent fieldwork by JMP and Rodrigo Aguayo (pers. comm.) failed to collect additional specimens.

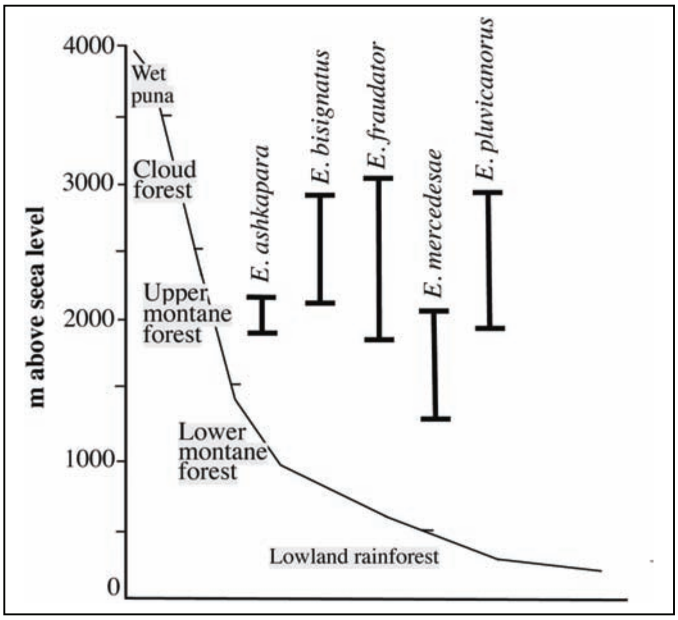

FIG. 5.— Approximate elevation ranges and habitats for each species of the subgenus *Yunganastes* in a cross-section of the Central Andes.

Distribution.—Known only from the type locality in humid montane forests from Central Bolivia (see Appendix and Figs. 4, 5).

Remarks.—Köhler (2000*a*, *b*) stated that *E. ashkapara* is most similar to *E. pluvicanorus* from which it is distinguished mainly by having larger size, smaller upper eyelid width/IOD ratio (maximum value in *E. ashkapara* 70.0%, minimum value in *E. pluvicanorus* 96,8%), and a different advertisement call. Externally it seems that *E. ashkapara* and *E. pluvicanorus* are criptic species, but advertisement call, differences in 16S mtDNA sequence, and larger size allow an unequivocal specific separation (see morphometrics, bioacoustics and phylogenetics sections).

Eleutherodactylus bisignatus (Werner, 1899)

(Fig. 6)

Hylodes gollmeri var. *bisignata* Werner, 1899, Zool. Anz., 22: 483. Holotype: NHMW 16502, according to Häupl and Tiedemann, 1978, Kat. Wiss. Samml. Naturhist. Mus. Wien, 2: 21, and Häupl, Tiedemann, and Grillitsch, 1994, Kat. Wiss. Samml. Naturhist. Mus. Wien, 9: 25. Type locality: "Bolivia (Chaco)".

Eleutherodactylus gollmeri bisignatus — Stejneger, 1904, Annu. Rep. U.S. Natl. Mus. 1902: 582–583.

Eleutherodactylus bisignatus — Parker, 1934, Ann. Mag. Nat. Hist., Ser. 10, 14: 267.

Eleutherodactylus bisignatus — Köhler, 2000, Bonn. zool. Monogr., 48: 63.

Lynch (1980) synonymyzed *E. bisignatus* with *E. fenestratus*, a hypothesis that was subsequently followed by Lynch and Duellman (1997). Heyer and Muñoz (1999) suspected that *Eleutherodactylus bisignatus* (= *Hylodes gollmeri bisignata*) could be a distinct species but did not remove it from synonymy. De la Riva et al. (2000) did not include this species in the Bolivian list, but mentioned the erroneous resurrection by Reichle (1999) of *E. bisignatus* as *H. gollmeri bisignatus*. Finally, Köhler (2000*b*) revalidated *E. bisignatus* and assigned it to the *Eleutherodactylus conspicillatus* species group (sensu Lynch and Duellman, 1997). He redescribed *E. bisignatus* based on the holotype and two specimens from the Zongo valley, a locality in the Yungas of La Paz, Bolivia. However, the re-examination of the same material revealed that specimens from Zongo actually correspond to *E.* cf. *fenestratus* (i.e., they belong to Bolivian populations currently recognized as *E. fenestratus*), a species belonging to the *E. conspicillatus* species group. After re-examination of the holotype, we conclude that *E. bisignatus* is clearly distinct from *E. fenestratus* and that it belongs to the subgenus *Yunganastes*. *E. bisignatus* is a rare species, and for many years it was known only from the holotype. Recent collections now allow a proper redescription off the species based on the holotype and the new material.

Diagnosis.—A robust, medium sized species, distinguished from other species of *Eleutherodactylus* by the following combination of characters: (1) skin of dorsum shagreen with small scattered conical granules; well developed and complete dorsolateral folds; skin on belly smooth in the middle to granular in the posterior and lateral margins; discoidal fold absent; (2) tympanic membrane and annulus distinct, oval, visible beneath the skin, its diameter about two thirds of eye length; (3) snout subacuminate to rounded in dorsal view, rounded in lateral profile; canthus rostralis sharp in frontal profile and slightly concave in dorsal view; (4) upper eyelid bearing small granules; cranial crests absent; (5) vomerine odontophores oval in outline, low, oblique, almost in contact, posteromedial to rounded medial choanae, bearing vomerine teeth; (6) males with vocal slits, subgular vocal sac and non spinous nuptial pads; (7) first finger equal or slightly longer than second; tips of outer two fingers forming truncate discs; tips of inner two fingers rounded, only slightly expanded; (8) fingers with inconspicuous fringes and no webbing; (9) no ulnar tubercles; (10) no tubercles or folds on heel or tarsus; (11) inner metatarsal tubercle large, prominent, oval, three times size of rounded low outer; no supernumerary tubercles, subarticular tubercle prominent, rounded to conical; (12) toe tips rounded to oval, slightly enlarged; toes with inconspicuous fringes and no webbing; third and fifth toes almost equal in length; (13) dorsum grey to brown; canthus rostralis and supatrympanic fold covered entirely or partially by a bold black stripe; two bold black spots under each eye and two others on each side of the scapular region, in lateral contact with dorsolateral folds; belly cream with some brown mottling, throat grey to dark brown in males; posterior surfaces of thighs dark brown with light mottling; (14) E2 condition of the mandibular ramus of the trigeminal nerve (Fig. 7); (15) presence of a frontoparietal fontanelle; (16) marked sexual dimorphism in size [one adult female 47.1 mm SVL and five adult males 28.3–35.0 (mean=31.6)].

Eleutherodactylus bisignatus is most similar to *E. pluvicanorus* and *E. ashkapara*, from which it differs by having slightly granular belly, less enlarged external finger tips, not forming discs, head width equal to head length, and smaller size of adult males (see Table 3). With *E. fraudator* it shares the granular belly (although smoother in this species) and scarcely dilated finger tips, but differs from it by its larger vocal sac (very small, almost indiscernible in *E. fraudator*), by lacking the labial longitudinal stripe and the characteristic broad continuous bold black stripe from snout to posterior margin of tympanic region present in *E. fraudator* (thinner and/or discontinuous in *E. bisignatus*) and by lacking longitudinal and parallel light and dark dorsal stripes (present in *E. fraudator*). *E. bisignatus* differs from another related species, *E. mercedesae*, in that the latter lacks conspicuous dorsolateral folds (when present they are irregular and indistinct), exhibits a tarsal fold, large finger discs, basal membrane on feet, aerolate belly (granular in *E. bisignatus*), larger size, longer tibia and feet in relation to SVL, and intense green coloration. Other species inhabiting the area are *E. platydactylus*, *E. rhabdolaemus* and *E. danae*, all of which have strongly aerolate belly, first finger shorter than second, and enlarged finger and toe discs, additionally all of them but *E. rhabdolaemu*s, lack dorsolateral folds.

TABLE 3.—Morphometrics of members of *Yunganastes*. Means ± standard deviation in parentheses follow ranges (in mm). For abbreviations see material and methods.

	E. pluvicanorus		***E. ashkapara***	***E. fraudator***		***E. bisignatus***		***E. mercedesae***	
	F (n=26)	M (n=30)	M (n=2)	F (n=4)	M (n=31)	F (n=1)	M (N=5)	F (n=2)	M (n=3)
SVL	33.6–46.6(39.5±2.7)	30.7–40.4(34.6±2.4)	44.8–49.8(47.3)	40.1–48.4(43.7)	25.9–33.4(30.0 ±1.8)	47.1	28.3–35.0(31.6)	62.1–63.3(62.7)	41.7–52.2(48.5)
HL	12.1–16.7(14.8±1.2)	11.6–15.6(13.2±1.0)	16.7–18.3(17.5)	13.8–17.3(15.8)	9.4–13.6(11.0±1.0)	17.6	11.3–13.5(12.4)	24.5–24.6(24.6)	16.119.8(18.0)
HW	13.3–18.0(15.6±1.1)	12.7–16.0(14.1±0.9)	17.9–20.0(19.0)	14.3–19.2(17.1)	9.9–13.3(11.6±0.8)	17.5	10.4–13.6(12.6)	24.6–26.1(25.4)	16.518.2(17.6)
EL	4.3–6.2(5.2 ±0.5)	3.8–5.5(4.6±0.5)	4.9–5.2(5.1)	4.6–5.8(5.1)	2.7–4.8(4.1±0.4)	5.7	4.4–4.4(4.4)	5.9–7.0(6.5)	5.1–6.2(5.6)
EN	3.6–5.5(4.2 ±0.4)	2.3–4.5(3.6±0.4)	4.8–5.6(5.2)	4.5–5.6(4.8)	2.8–4.1(3.4±0.3)	5.1	3.4–3.8(3.6)	6.4–7.5(7.0)	4.9–6.5(5.9)
IND	2.9–4.3(3.6 ±0.4)	2.4–3.7(3.3±0.3)	3.7–4.6(4.2)	3.5–4.2(3.9)	2.6–3.5(3.0±0.2)	4.6	2.7–3.3(3.1)	5.5–5.7(5.6)	4.0–4.7(4.4)
EE	5.2–7.5(6.3 ±0.7)	4.8–7.0(5.7±0.6)	6.8–7.5(7.2)	6.3–8.0(7.2)	4.4–6.0(5.2±0.4)	7.6	4.6–5.6(5.3)	10.3–10.8(10.6)	7.4–8.4(7.9)
TYH	1.9–3.3(2.5±0.4)	1.3–2.6(2.0 ±0.3)	2.3–2.7(2.5)	2.5–2.9(2.8)	1.5–3.4(2.0±0.4)	3.9	2.0–2.5(2.3)	3.6–4.0(3.8)	2.3–2.9(2.6)
TYL	1.5–2.7(2.1 ±0.3)	1.3–2.1(1.8±0.2)	2.3–2.6(2.5)	2.2–2.8(2.5)	1.5–2.3(1.8 ±0.2)	3.2	1.7–2.1(1.9)	3.3–3.4(3.4)	1.9–2.7(2.4)
FIII	1.1–2.1(1.7±0.2)	1.1–1.8(1.4±0.2)	1.8–2.0(1.9)	1.3–1.7(1.5)	0.9–1.3(1.1 ±0.1)	1.8	1.0–1.1(1.1)	2.8–3.4(3.1)	2.2–2.9(2.5)
FIV	1.3–2.0(1.6±0.2)	1.1–1.7(1.3±0.2)	1.9–2.1(2.0)	1.4–1.7(1.6)	0.8–1.4(1.1±0.1)	1.8	1.0–1.1(1.1)	2.8–3.6(3.2)	2.1–2.9(2.4)
ARM	7.2–10.0(8.7 ±1.5)	6.3–8.8(7.6 ±0.7)	9.6–11.3(10.5)	7.9–10.8(9.8)	4.6–7.3(6.1±0.5)	10.0	5.5–7.6(6.8)	15.1–15.7(15.4)	9.4–12.6(11.3)
TL	19.6–27.6(22.7±1.8)	17.7–23.2(19.7±1.3)	26.6–28.4(27.5)	22.5–25.2(24.2)	14.3–18.5(16.3±0.8)	25.9	16.5–20.9(19.0)	42.6–43.6(43.1)	28.0–35.5(33.0)
TH	17.7–29.0(21.6±2.6)	16.7–22.3(18.4±1.2)	24.5–25.4(25.0)	21.2–24.3(23.0)	13.1–17.5(15.4±2.1)	24.5	14.9–19.3(17.3)	38.7–40.8(39.8)	24.8–31.7(29.1)
FL	17.6–24.1(20.3±1.7)	14.6–22.3(17.4±1.6)	24.2–25.8(25.0)	22.0–25.3(23.3)	12.8–17.6(15.6±1.0)	24.6	13.4–19.0(16.9)	36.5–38.9(37.7)	23.5–29.4(27.4)
TIV	1.1–2.0(1.6±0.2)	1.0–1.8(1.3 ±0.2)	1.9–1.9(1.9)	1.3–1.5(1.4)	0.9–1.4(1.1±0.1)	1.8	1.0–1.1(1.1)	1.9–2.6(2.3)	1.8–2.2(1.9)

*Description of the holotype.—*An adult female with head wider than body and wider than long (for proportions see Table 4); snout short, subacuminate to round in dorsal view, rounded in lateral profile; EN 90% and 80% of ED in females and males respectively; nostrils protuberant, oriented dorsolaterally; canthus rostralis moderately sharp from frontal view, slightly convex in dorsal profile; loreal region granular, weakly concave, lips slightly flared; upper eyelid bearing small granules; cranial crests absent; supratympanic fold prominent, obscuring upper edge of tympanic annulus; tympanic annulus conspicuous, thin; tympanic membrane evident through skin, large, oval; length of tympanic annulus 56% of eye length; two inconspicuous subconical postrictal tubercles; choanae round, not concealed by palatal shelf of maxillary arch, medially positioned, almost contact with vomerine odontophores; vomerine odontophores posteromedial to choanae, almost contacting each other, low, oval in outline; tongue large, round, not attached posteriorly.

FIG. 6.—Dorsal and Ventral view of an adult male of *Eleutherodactylus (Yunganastes) bisignatus* (CBF 4122) from Plataforma, Department La Paz, Bolivia.

Dorsum shagreen with small scattered conical granules, areas posterior to eyelids and posterior parts of the dorsum with higher density of granules; dorsolateral folds complete; interocular fold weak; flanks smooth with scattered granules dorsally and high density of granules ventrally; no cloacal sheath or enlarged tubercles in cloacal region; skin on throat smooth; skin on belly smooth in central area, granular in lateral and posterior margins (character indistinct due to preservation); skin on groin aerolate; discoidal fold absent; upper surface of thighs and shanks smooth with scattered granules; ulnar tubercles absent; thenar tubercle oval, prominent, equal in size to bifid palmar tubercle; supernumerary palmar tubercles small, round, prominent, one on each finger but two on thumb, smaller than round, nonconical, prominent subarticular tubercles; fingers lacking lateral fringes, first finger equal to longer than second (relative length of Fingers III>IV>I≥II); disc on inner two fingers round, only weakly enlarged; Fingers III and IV with moderately expanded truncate discs bearing non-indented ungual flap and frontal grooves. Tarsal fold absent; heel tubercles absent but bearing small granules; inner surface of tarsus lacking granules; inner metatarsal tubercle large, prominent and slightly oval, outer almost indiscernible; supernumerary plantar tubercles absent; subarticular tubercles subconic, elongate; toes lacking lateral keels; webbing absent; discs on Toes I, II, IV weakly enlarged, round; discs on Toes III and IV more expanded, slightly oval; relative toe length IV>III>V>II>I; tibia length 50% of SVL.

Dorsum dark reddish-brown, flanks lighter with cream ground, narrow dark brown sinuous band from tip of the snout to the anterior margin of the eye along canthus; two dark brown spots under eye; supratympanic fold and tympanic membrane dark brown, annulus cream; two dark brown blotches of different sizes on each side of scapular region, lateral to dorsolateral folds; another spot more ventrally. Forearm grey with a dark brown spot on anterior surface; anterior surfaces of thighs cream with dark brown spots, posterior surface dark brown with fine cream mottling; dorsal surfaces of limbs and shanks grey with weak transverse brown bars; tarsus with clean-cut separation of dark dorsal surfaces and light ventral regions. Throat cream with dense fine grey mottling; lips with weak blotches; belly, ventral surfaces of limbs, and groin cream.

Measurements of the holotype.—SVL 47.1, HL 17.6, HW 17.5, EL 5.7, EN 5.1, IND 4.6, EE 7.6, TYH 3.9, TYL 3.2, FIII 1.8, FIV 1.8, ARM 10.0, TL 25.9, TH 24.5, FL 24.6, TIV 1.8.

Variation.—The description of the variation is based on five adult males and two juveniles from Yungas of La Paz, Bolivia (see Table 3 and 4 for measurements and proportions respectively and Appendix for the exact information on the localities). Males are much smaller than females, have non-spinous brown nuptial pads on thumb, and bear dark subgular vocal sac. Bellies can be densely granular, with ventral granules smaller than lateral granules; the vocal sac can also bear some granules. Density of granules of dorsal skin varies slightly among different dorsal areas within and between individuals. Vomerine odontophores are more prominent in some individuals and have three to four vomerine teeth on each odontophore; odontophores can be very close or distinctly spaced. Subarticular palmar tubercules can be one or two on each finger. Basal toe and finger fringes can be present although weak and basal. Recently collected specimens present bold black coloration in areas that are pigmented with dark brown in the holotype (canthus, supratympanic fold, posterior surfaces of limbs, scapular blotches, and blotches of anterior surface of forearm and knees). Dorsal coloration can be grey to brown or reddish-brown; some individuals are more patterned (i.e. a dark "X" mark on dorsum or thin stripes on scapular region). Some common patterns are: canthal stripe outlined by a thin cream line; upper lip with dark brown blotches; dark brown interocular stripe outlined by a thin cream line oriented anteriorly; belly can be either cream with brown mottling or vice versa. Palms are grey and plantar surfaces are brown. Juveniles are almost identical to adults.

Two specimens (CBF 4122 and 5719) were dissected to assess the condition of the mandibular ramus of the trigeminal nerve. This nerve presented the E2 condition described below (Fig. 5). An exposed frontoparietal fontanelle can be observed through the skin in the slightly desiccated specimen showed in Fig. 4 (CBF 4122) and in CBF 5717–20.

Natural history.—This species inhabits cloud forest (Yungas). Males were observed calling from tree trunks and bushes at 0.3–0.6 m above ground, all night around and during the day when the climatic conditions were foggy and rainy. Specimens were collected at night from 19:30–21:30. For information on the advertisement call, see the bioacoustic section and Fig. 6.

Distribution.—Cloud forest of Department La Paz, Bolivia, from Prov. Inquisivi to Prov. Nor Yungas, Parque Nacional-ANMI Cota Pata National Park (see Appendix and Figs. 4, 5).

Remarks.—One specimen (BM 1901.8.2.43) collected by P. O. Simmons in Actinero, Bolivia, was previously identified as *Hylodes gollmeri* and subsequently as *H. conspicillatus* in the catalogue of the British Museum. This specimen actually is an adult male of *E. bisignatus*. We were not able to trace the locality of Actinero. A specimen from Zamora, Ecuador (BM 1933.6.24.60) catalogued as *E. bisignatus* does not correspond to this species.

Eleutherodactylus fraudator Lynch and McDiarmid, 1987
(Fig. 1B)

Diagnosis.— A medium-sized species distinguished by the following combination of characters: (1) skin of dorsum smooth to shagreen with scarce and scattered warts, that of venter slightly granular; no discoidal fold; low complete dorsolateral folds present; (2) tympanic membrane prominent, visible beneath skin, round, 40% of eye length; annulus prominent, only slightly obscured dorsally by supratympanic fold; (3) snout round in dorsal view and lateral profile; canthus rostralis sharp in cross section, slightly concave in dorsal view; (4) no cranial crests or occipital fold; (5) vomerine odontophores small, round to oval, oblique, bearing 3–4 vomerine teeth; choanae visibles not obscured by maxillary arch of the palatal shelf, round, medial; (6) males with vocal slits and non-spinous nuptial pads on thumb; subgular vocal sac scarcely developed; (7) first finger slightly longer than second; tips of outer two fingers slightly enlarged and truncated, ungual flap not indented; tips of inner fingers rounded, not expanded; (8) lateral fringes between Fingers I and II weakly defined or absent;

(9) no ulnar tubercle; (10) no tubercles on heel and tarsus; (11) inner metatarsal tubercle oval and elevated, outer low and indistinct; no supernumerary plantar tubercles; (12) keels on toes weak or absent; Toe IV enlarged, with slightly oval toe tip, rest of toe tips round, scarcely enlarged; (13) dorsum brown to grey with darker longitudinal stripes from brown to black; bold black canthal-supratympanic and labial stripes; venter cream stippled with brown, heaviest on throat; (14) E2 condition of the mandibular ramus of the trigeminal nerve (Fig. 5); (15) an exposed frontoparietal fontanelle; (16) strong sexual dimorphism in size; mean SVL of males, 30.0; mean SVL of females, 43.7.

Description.—The original description by Lynch and McDiarmid (1987) is accurate, moreover, De la Riva and Lynch (1997) reported additional information about variability of color pattern and morphometrics.

Natural History.—Vocalizations were heard by JMP and Rodrigo Aguayo but they were not able to record them. A single male was calling, perching on a bush in a disturbed cloud forest near the road from Cochabamba to Villa Tunari. The call consisted of a single moderately spaced whistle repeated at constant rate, similar to the described calls of the other species of the group. The weather was dry and no other species were calling. De la Riva and Lynch (1997) described a clutch. This species was rather common when IDlR collected along the roads from Cochabamba to Santa Cruz in 1990. However, in subsequent surveys only very few specimens were observed.

Distribution.—Restricted to cloud forests and humid montane forests of the Departments Santa Cruz and Cochabamba, in Central Bolivia (see Appendix and Figs. 4, 5).

Remarks.—De la Riva and Lynch (1997) examined the position of the trigeminal nerve with respect to the abductor muscle in *E. fraudator* and determined the “E” condition. Contrarily, the “S” condition was reported for the paratype in the original description (Lynch and McDiarmid, 1987). De la Riva and Lynch (1997) considered that the paratype was abnormal; however, a re-examination of the paratype revealed the E2 condition described below (Fig. 5). Moreover, although in the original description the belly was reported as smooth, the holotype and paratype show a granular belly, although difficult to appreciate due to preservation condition. Others specimens of *E. fraudator* examined also show a granular belly, a condition shared with *E. bisignatus*.

Eleutherodactylus mercedesae Lynch and McDiarmid 1987
(Fig. 1C)

Diagnosis.—A large species distinguished from other species of *Eleutherodactylus* by the following combination of characters: (1) skin of dorsum shagreen, that of venter granular; irregular, indistinct dorsolateral folds; (2) tympanic membrane prominent, small, round and visible beneath skin, 1/3–1/5 of eye length; annulus conspicuous, dorsally obscured by prominent supratympanic fold; (3) snout subacuminate in dorsal view, round in lateral profile; canthus rostralis sharp, slightly concave in dorsal view; (4) no cranial crests or occipital folds; (5) choanae not concealed by palatal shelf of the maxillary arc, large, medial; vomerine odontophores oblique, large, nearly triangular, just medial and posterior to choanae, bearing vomerine teeth; (6) males with vocal slits and non-spinous nuptial pads; (7) first finger slightly longer than second or equal in length; fingers long, tips enlarged and truncate in Finger III and IV, round in I an II; ungual flap slightly indented; (8) basal and weak lateral fringes on fingers; (9) no ulnar tubercles; (10) weak (or absent) tarsal fold in contact with inner metatarsal tubercle; no heel or tarsal tubercles; (11) two metatarsal tubercles, inner large, oval, about four times the size of low outer; scarce (1–3) and inconspicuous supernumerary plantar tubercles; (12) toes with narrow lateral fringes and basal webbing; toe tips truncate, with broad pads; ungual flap slightly indented; (13) dorsum brown with a lichenous pattern of cream and gray (green in life); throat streaked and mottled with cream and brown; posterior surfaces of thighs brown with many cream flecks and mottling; (14) E2 condition of the mandibular ramus of the trigeminal nerve (Fig. 5); (15) an exposed frontoparietal fontanelle; (16) adults large, with sexual dimorphism in size, mean SVL of males, 48.5; mean SVL of females, 62.7.

This species can be easily distinguished from other species of the subgenus by its conspicuous coloration and large size. It has proportionately longer feet and tibia. It shares the granular belly with *E. bisignatus* and *E. fraudator*, although the belly is less granular in the two latter species.

Description.—The original description by Lynch and McDiarmid (1987) is accurate.

Natural History.—This species can be found during the day or night on the ground or perching on leaves of bushes in humid montane forests (Köhler, 2000*b*; Padial et al., 2006). The vocalization is unknown.

Distribution.—Cloud forests and humid montane forests from southern Peru to central Bolivia (Padial et al., 2006) (see Appendix and Figs. 4, 5).

Remarks.—Padial et al., (2006) reported the first record for Peru and provided some morphological notes on the types and additional specimens, including the first description of an adult female. De la Riva and Lynch (1997) considered *E. fraudator* and *E. pluvicanorus* as closely related on the basis of a putative synapomorphy (exposed frontoparietal fontanelle), a character also found in *E. mercedesae* by Lynch and McDiarmid (1987). However, De la Riva and Lynch (1997) did not consider *E. mercedesae* related to *E. fraudator* and *E. pluvicanorus*; indeed, they still considered it as "phenetically very dissimilar…a member of the subgenus *Eleutherodactylus*" (p. 156). Lynch and McDiarmid (1987) reported the "S" condition of the trigeminal nerve for *E. mercedesae* while we found the E2 condition in the paratype (Fig. 5). Moreover, a detailed examination of additional specimens of *E. mercedesae* (Padial et al., 2006) reveals that this species is not that dissimilar in habitus from the members of the former *E. fraudator* group proposed by Köhler (2000*a*, *b*). We propose the assignation of this species to the subgenus *Yunganastes*. The only significant differences are the presence of a tarsal fold and basal membrane on feet in some individuals, two characters that are also present in other species groups (e. g. *E. conspicillatus* and *E. discoidalis* species group). Hence, these characters could be tentatively considered as plesiomorphic.

Eleutherodactylus pluvicanorus De la Riva and Lynch 1997
(Fig. 1D)

Diagnosis.—A robust, medium-sized species, distinguished from other species by the following combination of characters: (1) skin of dorsum shagreen, with two low dorsolateral folds, skin of venter smooth; absence of discoidal fold; (2) tympanic membrane conspicuous, visible beneath skin, oval, its longest axis about two-fifths of eye length; (3) snout rounded both in dorsal and lateral views, canthus rostralis sharp; (4) supratympanic fold prominent, cranial crests absent; (5) choanae not concealed by the palatal shelf of the maxillary arch, small, lateral, round; vomerine odontophores large, oval, posteriomedial, almost in contact; (6) males with vocal slits, a large vocal sac, and non-spinous nuptial pads; (7) first finger about equal or slightly longer than second; finger tips expanded on finger III and IV forming discs; ungual flap not indented; tips of Finger I and II rounded; (8) no lateral fringes on fingers; (9) no ulnar tubercles; (10) no tubercles or folds on heel and tarsus; (11) inner metatarsal tubercle oval and elevated, outer smaller, rounded, less conspicuous; supernumerary plantar tubercles absent; (12) toes lacking lateral fringes and webbing; toe tips enlarged, rounded to truncate, all with small discs, Toe III longer than Toe V; (13) dorsum beige to brown with darker markings, dark brown canthal and supratympanic stripe, upper lip with irregular brown blotches, limbs barred, venter cream with brown spots; (14) 'E2 condition of the mandibular ramus of the trigeminal nerve (Fig. 7); (15) an exposed frontoparietal fontanelle; (16) adults large; mean SVL of males, 34.6 mm; mean SVL of females, 39.5 mm.

Externally, *E. pluvicanorus* is most similar to *E. ashkapara*, *E. bisignatus* and *E. fraudator*. *E. pluvicanorus* can be distinguished from *E. ashkapara* by its smaller size, shorter snout in relation to eye length and different advertisement call (see morphometrics and bioacustics). It can be distinguished from *E. bisignatus* by the following features (*E. bisignatus* in parentheses): smooth belly (granular); smaller size; well-developed discs on two external fingers (slightly enlarged, truncate, not forming discs); and head wider than long (equal). From *E. fraudator* it differs by the following features (*E. fraudator* in parentheses): upper lip with irregular dark markings (continuous dark stripe); tympanic stripe narrow (broad); two sharp, symmetric dark blotches on the scapular region, lateral to the dorsolateral folds, dorsum with tenuous chevrons or X-shaped blotches (two symmetric black blotches absent or poorly marked; dorsum with longitudinal stripes more or less continuous); males with a large, slightly bilobate vocal sac (males with small single subgular vocal sac); and venter smooth (granular). Other sympatric *Eleutherodatylus* species are *E. platydactylus*, *E. rhabdolaemus*, and *E. danae*, all of them having granular belly, first finger shorter than second, and enlarged finger tips forming well defined discs with circumferential grooves. Moreover, *E. platydactylus* and *E. danae* lack dorsolateral folds.

*Description.—*The original description by De la Riva and Lynch (1997) is accurate.

*Natural History.—*This species can be found calling during the day or night on the ground or perching on bushes in humid montane forests (Köhler, 2000*b*). The vocalization was described by De la Riva and Lynch (1997) and Köhler (2000*b*) (see bioacoustics and Fig. 8–9).

*Distribution.—*This species is moderately common, occurs only in cloud forest and humid montane from departments Cochabamba and Santa Cruz, in Central Bolivia (see Appendix and Fig. 4 and 5).

*Remarks.—*De la Riva and Lynch (1997) considered *E. fraudator* and *E. pluvicanorus* as close relatives. They described the skull of one adult female of *E. fraudator* and *E. pluvicanorus*, and they stated that both species were very similar in meristic skull characters and share a frontoparietal fontanelle.

MORPHOMETRICS

Measurements and proportions for all the species are shown in tables 3 and 4, respectively. Members of *Yunganastes* are medium-sized frogs with SVL ranging from the smallest adult of *E. fraudator* (SVL=25.9) to the largest adult female of *E. mercedesae* (SVL=63.3). Sexual dimorphism is evident in all species where members of both sexes are known, but it is more conspicuous in *E. bisignatus*, *E. fraudator* and *E. mercedesae*. The order of the relative size (SVL) of males between the species considered is *E. mercedesae*>*E. ashkapara*>*E. pluvicanorus*>*E. bisignatus*>*E. fraudator*; while in females it is *E. mercedesae*>*E. bisignatus*>*E. fraudator*>*E. pluvicanorus*. In *E. pluvicanorus*, some adult males are larger than females. This species is very similar in appearance to *E. ashkapara*, but the later is considerably larger. In *E. fraudator*, females are larger than males; indeed, males of this species are quite small in comparison with the other species of the subgenus. This is also true for *E. bisignatus*, with its emphasized sexual dimorphism. Moreover, the latter species has a considerable large tympanum (TYH) in relation to its small size, contrarily to the small tympanum of the large *E. mercedesae*.

TABLE 4.—Proportions (standard deviation in parentheses) of species of *Yunganastes*. For abbreviations see material and methods.

	E. pluvicanorus		***E. ashkapara***	***E. bisignatus***		***E. fraudator***		***E. mercedesae***	
	F (n=26)	M (n=30)	M (n=2)	F (n=1)	M (n=5)	F (n=4)	M (n=31)	F (n=2)	M (n=3)
TL/SVL	0.6 (0.0)	0.6(0.0)	0.6	0.5	0.6	0.5	0.5(0.0)	0.7	0.7
FL/SVL	0.5(0.0)	0.5(0.0)	0.5	0.5	0.5	0.5	0.5(0.0)	0.6	0.6
HL/SVL	0.4(0.0)	0.4(0.0)	0.4	0.4	0.4	0.4	0.4(0.0)	0.4	0.4
HW/SVL	0.4(0.0)	0.4(0.0)	0.4	0.4	0.4	0.4	0.4(0.0)	0.4	0.4
HW/HL	1.1(0.0)	1.1(0.1)	1.1	1.0	1.0	1.1	1.1(0.0)	1.1	1.0
EN/EL	0.8(0.1)	0.8(0.1)	1.1	0.9	0.8	0.9	0.8(0.1)	1.1	1.1
EE/HW	0.4(0.0)	0.4(0.0)	0.4	0.4	0.4	0.4	0.4(0.0)	0.4	0.4
WD/ARM	0.2 (0.0)	0.2 (0.0)	0.2	0.2	0.2	0.2	0.2(0.0)	0.2	0.2
TYL/TYH	0.8(0.1)	0.9(0.1)	1.0	0.8	0.8	0.9	0.9(0.1)	0.9	0.9

Proportions are very constant within species, but there are some conspicuous differences between them. The most notorious difference between *E. pluvicanorus* and *E. ashkapara* is in the proportion EN/EL (0.8 and 1.1 respectively). *E. mercedesae* has also 1.1 of EN/EL. In other words, the eye is longer than the snout in all species except in *E. ashkapara* and *E. mercedesae*. The head is wider than long in all species but in *E. bisignatus* and males of *E. mercedesae*, in which length and width are equal. *E. mercedesae* is also peculiar by two other proportions: it has the longer TL and FL in relation to SVL. That is, it has comparatively longer hind legs; the opposite can be said for *E. fraudator*. The relation TYL/TYH show some differences between species. While in *E. ashkapara* both measurements seem to be equal, in the rest of species the height of the tympanic membrane is larger than the length, and this pattern is most conspicuous in *E. bisignatus*.

PHYLOGENETICS RELATIONSHIPS

THE TRIGEMINAL NERVE

De la Riva and Lynch (1997) placed *E. fraudator* and *E. pluvicanorus* in the Middle American subgenus *Craugastor* based on the shared "E" condition of the trigeminal nerve (V_3) with respect to the *m. adductor mandibulae externus superficialis* (sensu Starret, 1968 in Lynch, 1986). They considered both species as each other's closest relatives, based on the phenetic similarity and the presence of an exposed frontoparietal fontanelle, a character also considered as a putative synapomorphy for this pair of species.

The "E" condition is one of the three conditions considered by Starret (1968) for the *m. adductor mandibulae* and the pathway of the mandibular ramus of the trigeminal nerve. In the "E" condition, the nerve passes between the *m. adductor mandibulae posterior* and the *m. adductor mandibulae externus superficialis* (Fig. 7) and is therefore not visible externally when the skin anterior to the tympanic region is dissected. Contrarily, in the "S" condition, the nerve passes lateral and externally to the *m. adductor mandibulae externus* and is therefore appreciable as an oblique white thin band of tissue covered by a translucid lay of conjunctive tissue between the tympanic region and the eye. The third possibility ("E+S") is that the two muscles share the origin and insertion and the nerve passes between them (Starret, 1968; Lynch, 1986).

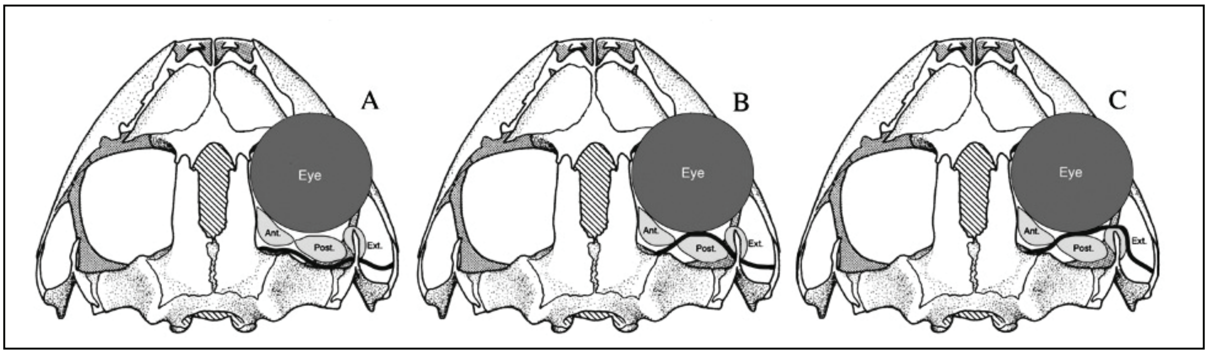

FIG. 7.—Pathway of the mandibular ramus of the trigeminal nerve of the abductor muscle (in bold). A: E2 condition; B: E, condition (sensu Lynch 1986); C: S condition (sensu Lynch, 1986). Abbreviations are as follows: Ant., *m. adductor mandibulae anterior*; Post., *m. adductor mandibulae posterior*; Ext., *m. adductor mandibulae externus*.

Lynch (1986) illustrated both "S" and "E" conditions. In both, the nerve originated in the prootic, just between the *m. adductor mandibulae anterior* and *m. adductor mandibulae posterior*. The nerve then continues along the anterior margin of the *m. adductor mandibulae posterior* and rotates before reaching the *m. adductor mandibulae externus* to pass between the external face of the *m. adductor mandibulae posterior* and the internal face of the *m. adductor mandibulae externus* ("E" condition); or continues and rotates after it surpasses the anterior margin of the *m. adductor mandibulae externus* and continues attached to it ("S" condition) (see Fig. 7).

Lynch (1986) considered the "S" condition as plesiomorphic and "E" as derived. He stated that the "E" condition was rare except in the Middle American *Eleutherodactylus* and common among the members of the subgenus *Craugastor*. At this time, he did not report any strict South American eleutherodactyline having the "E" condition. Although the "E" condition is also present in other groups, including some bufonids, some hyperoliids, most microhylids, some ranids, rhacophorids, *Rhinophrynus* and at least one sooglossid (Starret, 1968; Lynch, 1986). Lynch (1986) preferred to consider this condition as a putative synapomorphy for the Middle American clade *Craugastor* and, hence, implicitly hypothesized an independent origin of the "E" condition in several groups.

Moreover, Lynch (1986) reported an anomalous condition in one specimen of *E. angelicus* and one of *E. maussi* [now *E. biporcatus* (Savage and Myers, 2002)]. In these species, the "E" condition is normal, but in those two particular specimens, the trigeminal nerve does not pas anterior to the *m. adductor mandibulae posterior*. Instead, the nerve originates in the same place described above, continues posterior to the *m. adductor mandibulae posterior* and runs ventrolaterally between the *m. adductor mandibulae posterior* and the *m. adductor mandibulae externus* toward the lower jaw. Hence, the V_3 cannot be seen externally, but also is not passing anteriorly to the *m. adductor mandibulae posterior*.

The same pattern was detected for two specimens of *E. pluvicanorus* (Frost et al., 2006, p. 200). The authors described the route of the trigeminal nerve as follows: "A single muscle (the *m. adductor mandibulae externus*) originates on the zygomatic ramus of the squamosal, and the V_3 does not lie lateral (superficial) to it (so it is not the "S" pattern), but does not extend posteroventrad between that muscle and the deeper *m. adductor mandibulae posterior* ("E" musculature), either. Instead, V_3, lies entirely posterior to both muscles and runs ventrolateral toward the jaw—that is, it does not run around the anterior face of the *m. adductor mandibulae posterior*". Frost et al. (2006) had not the possibility to examine this character state for other members of *Yunganastes* and they mentioned that their observations could also represent individual anomalies. Nevertheless, they consider these differences enough to question the placement of the former *E. fraudator* group within *Craugastor*, a position shared by us.

Our observations further corroborate these observations. We examined the position of the V_3 (both sides) for *E. ashkapara* (ZFMK 70318, paratype), *E. bisignatus* (CBF 4122, CBF 5717), *E. fraudator* (USNM 257846, paratype; CET A 177, 191, 172), *E. mercedesae* (USNM 165753, paratype) and *E. pluvicanorus* (CET A 157, 159, paratypes). For all the specimens of *Yunganastes* examined, the trigeminal nerve passes posteroventrad to the *m. adductor mandibulae posterior* and runs ventrolateral between the *m. adductor mandibulae posterior* and the *m. adductor mandibulae externus* toward the lower jaw (Fig. 7). We propose this character state to be termed "E2 condition". For other Bolivian *Eleutherodactylus*: *E. cruralis*

(MNK A 6618–9), *E. discoidalis* (MNK A 7246, 7262), *E. ibischi* (MNK A 6612), *E. rhabdolaemus* (MNK A 7205, 7207), *E. platydactylus* (MNK A 7226, 7207) and *E. fenestratus* (MNK A 3933, 7290), representing the *E. discoidalis*, *E. unistrigatus* and *E. conspicillatus* species groups, respectively, only the "S" condition was observed. We consider the "S" condition as plesiomorphic and the E2 condition as synapomorphic for *Yunganastes*.

THE FRONTOPARIETAL FONTANELLE

The presence of an exposed frontoparietal fontanelle was another character state described by De la Riva and Lynch (1997) for *E. fraudator* and *E. pluvicanorus* and they proposed it as a putative synapomorphy (see Fig. 7). Although *E. mercedesae* also shows the fontanelle (Lynch and McDiarmid, 1987; De la Riva and Lynch, 1997) this character was not considered a synapomorphy for the three species, because the later was considered to belong to the subgenus *Eleutherodactylus* and the former two species were included in the subgenus *Craugastor*. Köhler (2000*a*, *b*) described the fontanelle for *E. ashkapara* and also mentioned the possible close relationship of *E. mercedesae* with members of the former *E. fraudator* group. The examination of *E. bisignatus* also revealed the presence of an exposed frontoparietal fontanelle, which is also evident under the skin in the specimen CBF 4122. Hence, all known species described to date and assigned to *Yunganastes* exhibit this character.

Guayasamin (2004) reviewed the presence of an exposed frontoparietal fontanelle in *Eleutherodactylus*. He mentioned this character for 19 species, including *E. fraudator*, *E. mercedesae* and *E. pluvicanorus*. No species of *Craugastor* shows an exposed frontoparietal fontanelle (Guayasamin, 2004; Lynch, 2000). If we consider this character to be a synapomorphy of *Yunganastes*, it must have evolved independently in any other *Eleutherodactylus* group (*E. unistrigatus*, *E. discoidalis*, *E. lacteus*, *E. martinicensis* and *E. orcesi* groups). The presence of this character in adults of different groups could be the result of independent heterochronic events, given that juveniles of all *Eleutherodactylus* show an exposed frontroparietal fontanelle (Lynch, 1971). Moreover, there are other conspicuous differences between the groups showing this character, such as the relative length of the parasphenoid, the number of teeth in the dentigerous processes of the vomer (Guayasamin, 2004), and the condition of the V_3. Hence, we consider the presence of the fontanelle as a synapomorphy of *Yunganastes*.

BIOACOUSTICS

Only the calls of *E. ashkapara*, *E. bisignatus* and *E. pluvicanorus* are available for comparisons. The advertisement call of *E. pluvicanorus* was first described by Lynch and De la Riva (1997) and subsequently by Köhler (2000*b*). Köhler (2000*a*) described the advertisement

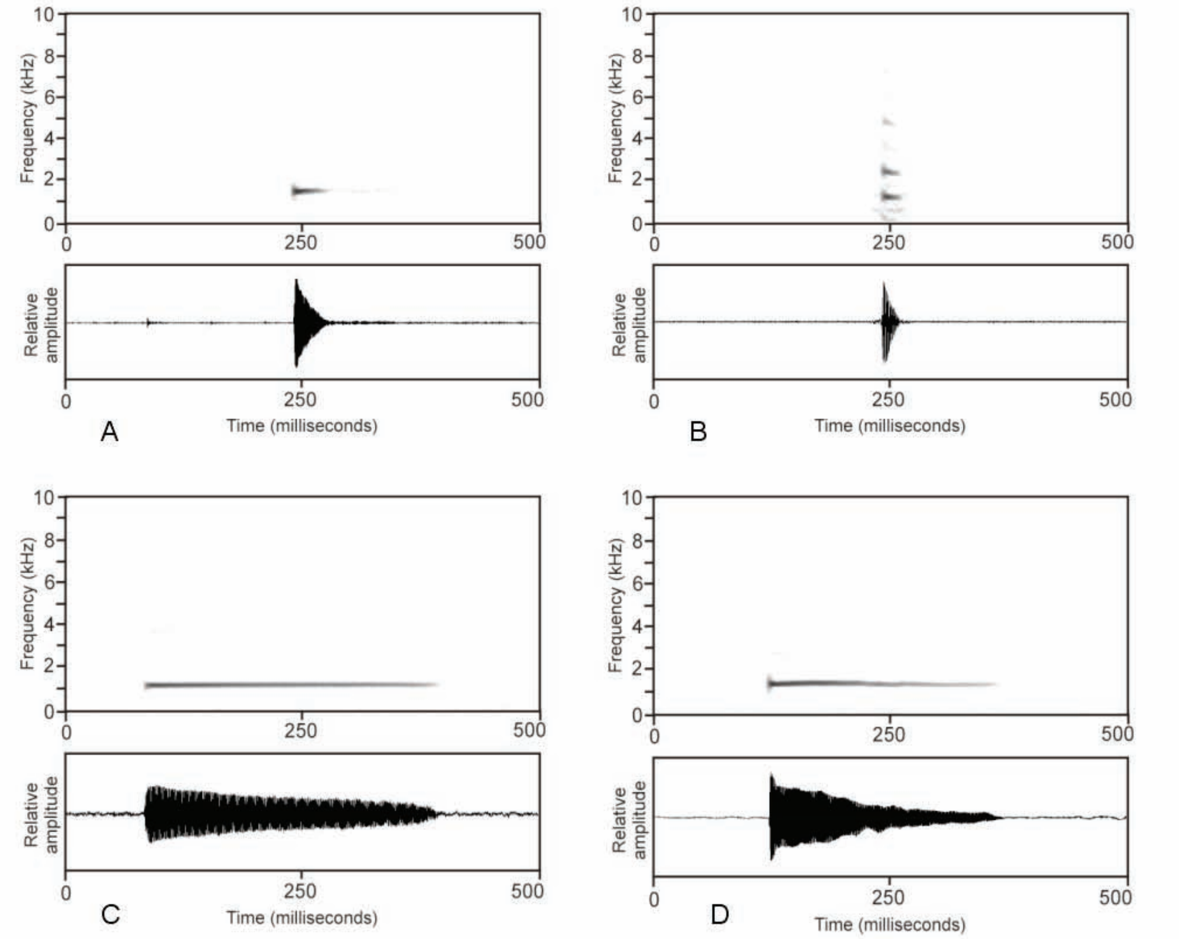

FIG. 8.—Call spectrogram and oscillogram of three species of the subgenus *Yunganastes*: A, *E. ashkapara* from Chapare; B, *E. bisignatus* from Toriri; C, *E. pluvicanorus* from Sehuencas; D, *E. pluvicanorus* from La Yunga.

call of *E. ashkapara*. We describe the advertisement call of *E. bisignatus* for first time and compare it with calls of the other two species. Fig. 8 includes the sound spectrograms and oscillograms of the advertisement call of each species.

The call of *E. bisignatus* consists of a single short note with harmonic structure. It is the shortest call of the three species (Table 1, Fig. 8). It is much shorter (approximately 15 times) than the call of *E. pluvicanorus* and slightly shorter than the call of *E. ashkapara*. Moreover, it has three main harmonics, while harmonic structure is absent in the two other species. The frequency range of its call is clearly greater than in the other two species (Table 1) and, as in *E. ashkapara* and *E. pluvicanorus*, the amplitude decreases to the end of the call. Also, in this three species, the main call can be followed by a series of fast repeated notes that is here termed aggressive call (Fig. 9, see below).

The calls of the three taxa are very similar in structure and dominant frequency (Table 1, Fig. 8). Call/note duration and presence/absence of harmonics are the main characters allowing for an unequivocal distinction (Fig. 8). Despite some variation, calls of different populations of *E. pluvicanorus* are very similar (Fig. 8 and Table 1). The call of *E. ashkapara* is considerably shorter than the calls of *E. pluvicanorus*. The call of *E. bisignatus* is even shorter and exhibits distinct harmonic frequency bands. Apart from advertisement calls, we were able to record a second call type in all three species of *Yunganastes* for which vocalizations are currently known (*E. ashkapara*, *E. bisignatus* and *E. pluvicanorus*). This second call type was only emitted when several conspecific males were calling within close distances. Thus, we suspect this call type to be involved in male-male interactions and we tentatively term it aggressive call. The waveforms of the aggressive calls of the three species are shown in Figure 9. A common character of these calls is a higher note repetition rate when compared to the advertisement call of the same species (in advertisement calls note repetition rate is synonymous with call repetition rate). Compared to the advertisement calls, note repetition rate in aggressive calls is approximately three times faster in *E. ashkapara*, four times faster in *E. bisignatus* and 2.4 times faster in *E. pluvicanorus*. This is considered as a feature, which may support an aggressive function from this type of call (Wagner, 1989). Note duration in aggressive calls is shorter in *E. ashkapara* (25 ms versus 35–43 ms) and *E. pluvicanorus* (90–100 ms versus 210–440 ms), but longer in *E. bisignatus* (35–55 ms versus 16–25 ms) compared to notes in these respective advertisement calls. Another common character in aggressive calls is a longer initial note followed by several (5–12) nearly identical, shorter secondary notes. In all three cases, the duration of the initial note is approximately three times the duration of secondary notes. In addition, amplitude modulation of the initial note of aggressive calls is evident in three species. Compared to secondary notes, the relative amplitude of the initial note is higher in *E. ashkapara*, but lower in *E. bisignatus* and *E. pluvicanorus*. There are not differences in frequency characters between advertisement and aggressive calls within the same species.

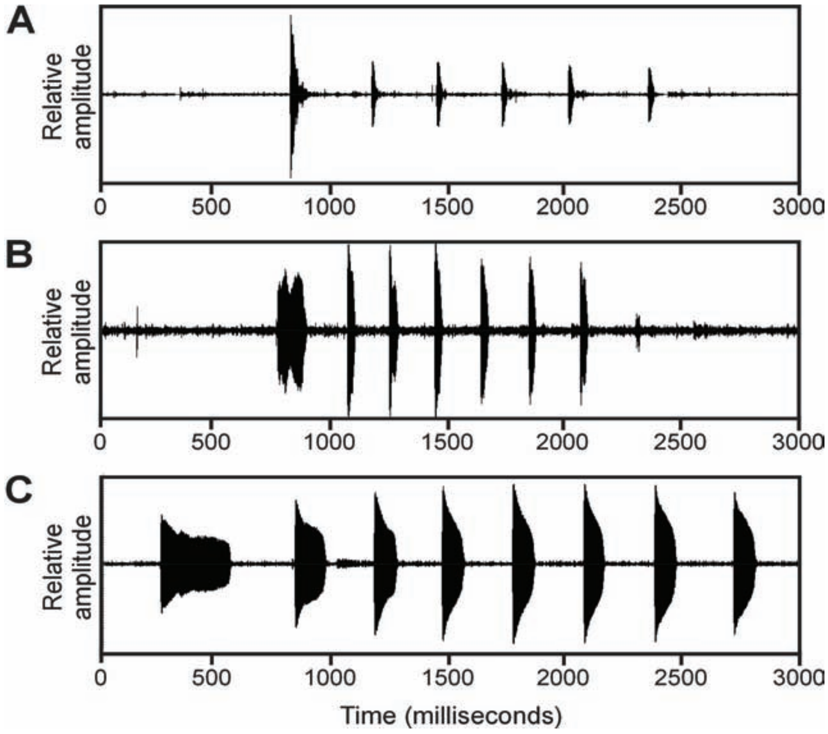

FIG. 9.—Call spectrogram of the aggressive calls of three species of the subgenus *Yunganastes*: A, *E. ashkapara* from Chapare; B, *E. bisignatus* from Toriri; C, *E. pluvicanorus* from Sehuencas.

Although the interspecific differences in aggressive calls of all three species are well pronounced (as are differences in advertisement calls) and also allow for unequivocal species identification, the general structure is quite similar and follows the same pattern (i.e. increase of note repetition rate combined with amplitude modulation of initial note).

Members of other groups of *Eleutherodactylus* occurring in Bolivia have quite different patterns of structure in their (advertisement) calls. For example, the calls of members of the *E. discoidalis* species group are always composed of consecutive pulses (Reichle et al., 2000; Padial et al., 2005). Members of the *E. conspicillatus* species group have also pulsed calls composed of several consecutive notes that are much less melodic compared to those of *Yunganastes* (Márquez et al., 1995; Köhler, 2000*b*). Finally, members of the *E. unistrigatus* species group present a broad diversity in advertisement call characters (Marquez et al., 1995; Köhler, 2000*b*). Therefore, we propose the call structure described above (single melodic whistles with frequency modulation and no pulses) as synapomorphic for *Yunganastes*. Additionally, the existence of aggressive calls with a similar structure further supports close phylogenetic relationships.

ANALYSES OF mtDNA

Inferred phylogenies and nodal support (bayesian posterior probabilities or bpp and bootstrap support or bss) based on MP, NJ and Bayesian analyses are concordant in their support for the monophyly of three main clades: Yunganstes, Eleutherodactylus and Craugastor (Figs. 10–12). Furthermore, the specific status of all species assigned to *Yunganastes* is supported by the monophyly of these taxa (with one exception for one sequence of *E. pluvicanorus* from GenBank, which clusters with *E. rhabdolemus* (bpp = 1.00, NJ bss = 100%, MP bss = 100%).

The clade Yunganastes (bpp = 1.00, NJ bss = 100% and MP bss = 100 %) includes all the species assigned by us to this subgenus. Within the clade, *E. bisignatus* appears as basal to the remaining taxa in the Bayesian (bpp = 1.00) and NJ (bss = 98%) trees but not in the MP tree, where it clusters with *E. fraudator* (bss = 78%). *E. ashkapara* appears always as the sister taxon of *E. pluvicanorus* (bpp = 1.00, NJ bss = 98% and MP bss = 69%). The position in the trees of *E. fraudator* is not concordant between methods. Bayesian analyses placed *E. fraudator* as sister species of a clade containing *E. ashkapara*, *E. pluvicanorus* and *E. mercedesae*, while NJ and MP place *E. fraudator* as a taxon more related to *E. mercedesae*. In summary, there are two clear clades within *Yunganastes*; one includes *E. pluvicanorus* and *E. ashkapara* and the other contains *E. mercedesae*. The relationships between these two clades and *E. bisignatus* and *E. fraudator* are not clearly resolved. Following the Phylocode (Cantino and de Queiroz, 2004), the subgenus *Yunganastes* can be node–based defined as the least inclusive crown clade including *E. bisignatus*, *E. fraudator* and *E. pluvicanorus*.

The clade Eleutherodactylus (bpp = 1.00, NJ bss = 68% and MP bss = 74%) contains species assigned to the subgenus *Eleutherodactylus* (Lynch and Duellman, 1997). Only the position of *E. chloronotus* is ambiguous when the different trees are compared. *E. discoidalis* included in the subgenus *Eleutherodactylus* by Lynch and Duellman (1997), does not cluster with the rest of the species of the clade.

The clade Craugastor (bpp = 1.00, NJ bss = 85% and MP bss = 76%) includes species assigned to the Middle American genus *Craugastor* (Frost et al., 2006; Crawford and Smith, 2005). The only outlier would be *Craugastor bufoniformis* that seems to be more related to *E. discoidalis*. Although the position of these two taxa in the phylogenetic trees is not accurately resolved by our analyses, it suggests that they do not belong to any of the previously described clades.

DISCUSSION

SYSTEMATICS

Morphological, molecular and bioacoustical characters support the specific status of *Eleutherodactylus ashkapara*, *E. bisignatus*, *E. fraudator*, *E. mercedesae* and *E. pluvicanorus*. These five species form a crown clade within *Eleutherodactylus* from the Central Andes and Amazonia. We describe this clade as the subgenus *Yunganastes,* that is well supported by molecular and morphological synapomorphies within *Eleutherodactylus*.

The position of *E. (E.) discoidalis* (a member of the *E. discoidalis* species group) well outside the clade *Eleutherodactylus (Eleutherodactylus*) in our phylogenetic trees rejects the hypothesis of monophyly for the subgenus *Eleutherodactylus* (sensu Lynch and Duellman, 1997). Our molecular data also do not support the monophyly of *Craugastor* (sensu Crawford and Smith, 2005) since *C. bufoniformis* (a member of the *C. bufoniformis* species group) does not cluster with other members of *Craugastor* in our tree. This species was not included in Crawford & Smith's phylogeny, and hence, the relationships of the *C. bufoniformis* species group within *Eleutherodactylus* were not analyzed. Moreover, following the phylogeny of Frost et al. (2006), this species is the most distant taxon within the cluster considered by them as *Craugastor* and could indeed be considered as the sister taxon of *Craugastor*. However, they included the former *E. (Craugastor) bufoniformis* species group in the genus *Craugastor* (Frost, 2006). Nevertheless, following our data, if we retain the members of the *C. bufoniformis* species group within the genus *Craugastor*, the latter would be paraphyletic. Another possibility is that *Craugastor* is composed of two clades, one including *C. bufoniformis* and the species of its group (if monophyletic) and one with the rest of the species. Nevertheless, the scarce taxon sampling by both us and Frost et al. (2006) and the scarce support of external branches in our phylogeny make necessary to gather more data in order to resolve this problem.

Frost et al. (2006) discussed the phylogenetic relationships of the genus *Eleutherodactylus* and concluded that is "rampantly nonmonophyletic" (p. 198). They included in their phylogenetic studies six species of *Craugastor* (sensu Crawford and Smith, 2005) and *E. pluvicanorus*. They explicitly discarded the proposition of Lynch and De la Riva (1997) that members of the *E. fraudator* group belong to *Craugastor* (Frost et al., 2006, p. 200) since in their proposed phylogeny *E. pluvicanorus* did not show any close phylogenetic relationship with *Craugastor*. But when we compared the 16S fragment used by them and Faivovich et al. (2005) for *E. pluvicanorus* (genbank accession number AY843586, AMNH 165195) with the same gene fragment used in our phylogenetic analysis, this sequence clustered with sequences of our specimens of Bolivian *E. rhabdolaemus*, a member of the *E. unistrigatus* species group of the subgenus *Eleutherodactylus.* The Bolivian series of specimens deposited in the AMNH that were used in Frost et al.'s (2006) phylogeny included both *E. pluvicanorus* and *E. rhabdolaemus*. Since small specimens of both species are similar in general appearance, we suspect that a mistake occurred while cataloguing or during any phase of the lab work. Hence, it is very likely that their analysis of the *Craugastor–fraudator* problem based on molecular data is not valid. Nevertheless, this fact does not alter the conclusion of Frost et al. (2006) that members of *Yunganastes* do not belong to *Craugastor* (as confirmed by our molecular data), because they discovered a different pathway in the trigeminal nerve in well identified specimens of *E. pluvicanorus* (AMNH A165194, A165211). Indeed, as explained above, we confirmed that this character is a putative synapomorphy for the subgenus *Yunganastes*. However, although they reject to include the members of *Yunganastes* in *Craugastor*, the name *Craugastor pluvicanorus* appears in their phylogenetic tree. This name is also maintained in Genbank. This was surely the result of an overlooked mistake, since Frost (2006) based on Frost et al. (2006) correctly included *pluvicanorus* in the genus *Eleutherodactylus*.

The recognition of the subgenus *Yunganastes* has relevant implications for future work on the infrageneric arrangement recognized within *Eleutherodactylus* (Lynch and Duellman, 1997). *Eleutherodactylus* has been suspected to be non-monophyletic (Lynch, 2001; Darst and Canatella, 2004; Frost et al., 2006) and will probably split soon into several groups. Moreover, within the large taxonomic unit of the South American species, what is mainly the subgenus *Eleutherodactylus*, several phenetic species groups have been proposed (Lynch, 1986; Lynch and Duellman, 1997) with the hope that these groups would form clades (Lynch and Duellman, 1997). Thus, like the former *E. fraudator* species group, some other species groups could be monophyletic. The question is: should these monophyletic groups be recognized as higher taxonomic categories (genera or subgenera) or should they maintain their status simply as species group? From our point of view, the species group, or species–series (sensu Dubois, 2005) is only a phenetic cluster, a working hypothesis until monophyly can be tested. When monophyly is supported by data, species groups should be recognized as ranked taxonomic categories that reflect evolutionary history. The subgenus is the most immediate category below the genus. Hence, we recommend the elevation to subgenus of the species groups when monophyly is supported by data. The genus is probably too inclusive to be applied to most species groups currently described within *Eleutherodactylus*, and can contain several small monophyletic clusters of species closely related to other such groups.

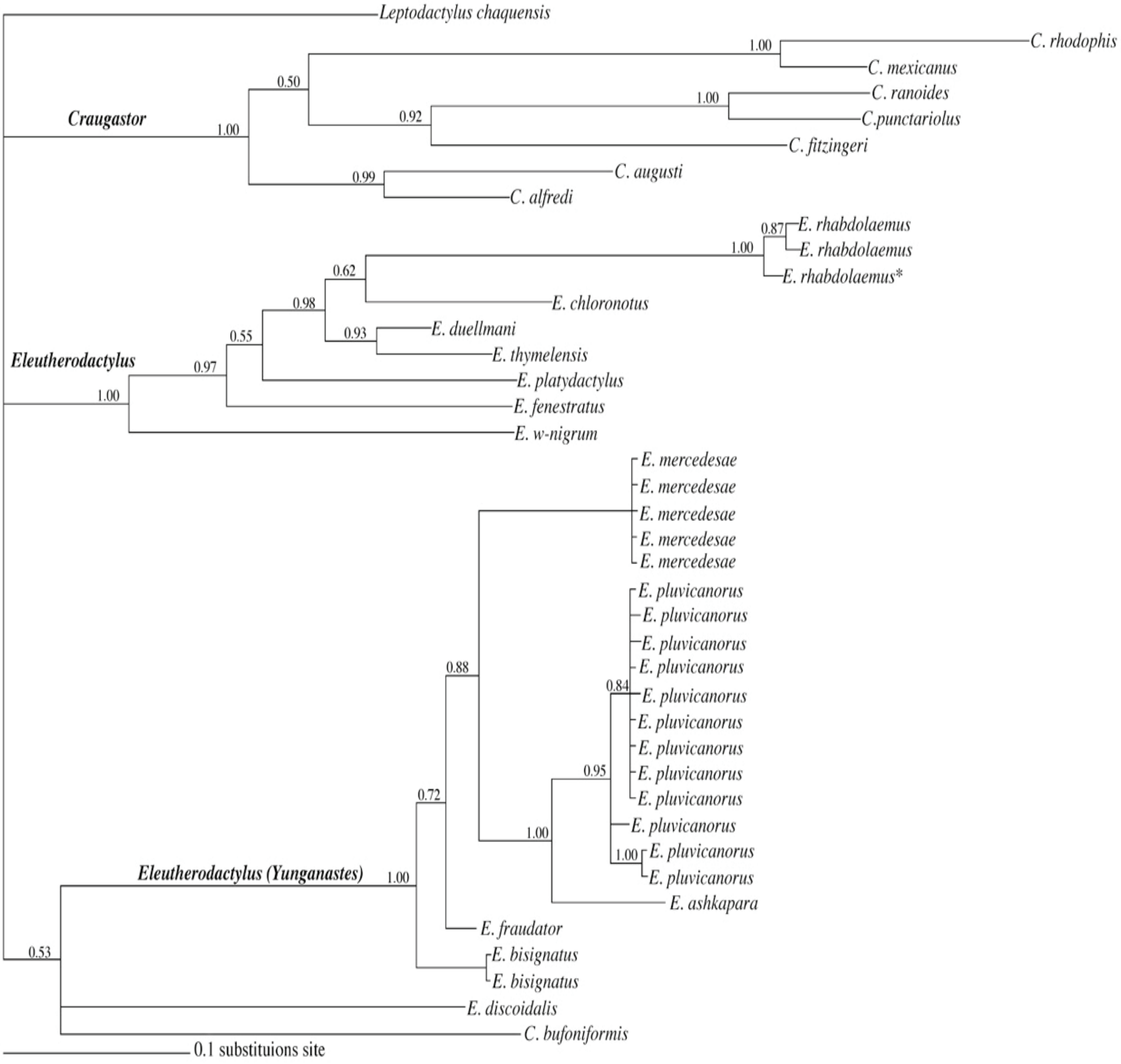

FIG. 10.—Gen tree resulting from Bayesian MCMC phylogenetic analyses of a 560 bp fragment of the 16S mitochondrial gene of members of the genera *Craugastor* and *Eleutherodactylus*, including the subgenera *Eleutherodactylus* and *Yunganastes*. *E. rhabdolaemus** corresponds to a fragment of the 16S sequence used by Faivovich et al. (2005) and Frost et al. (2006) as *E. pluvicanorus*.

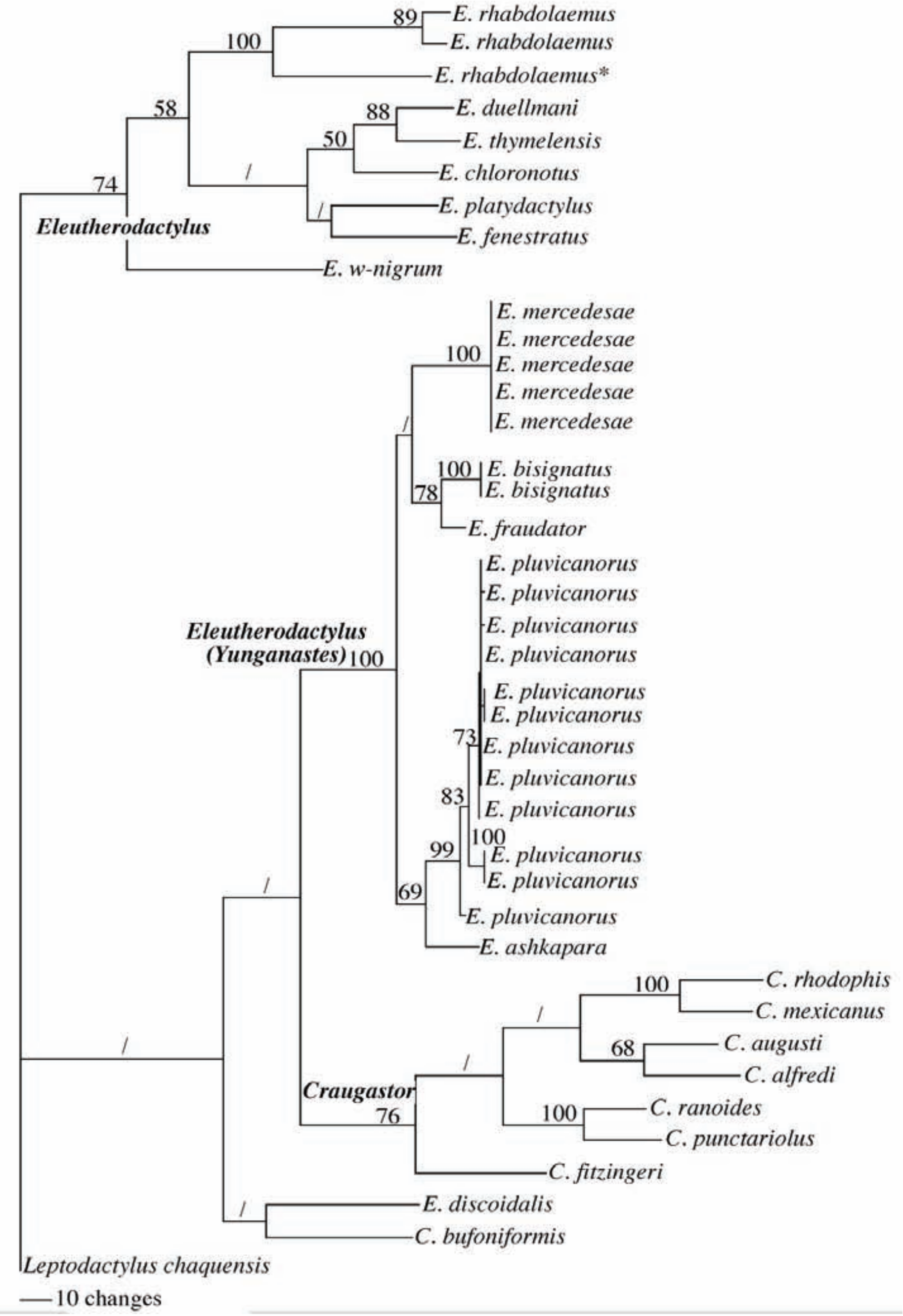

FIG. 11.—Gen tree resulting from Maximum Parsimony phylogenetic analyses of a 560 bp fragment of the 16S mitochondrial gene of members of the genera *Craugastor* and *Eleutherodactylus*, including the subgenera *Eleutherodactylus* and *Yunganastes*. Boostrap nodal support inferior to 50% is indicated by a slash. *E. rhabdolaemus** corresponds to a fragment of the 16S sequence used by Faivovich et al. (2005) and Frost et al. (2006) as *E. pluvicanorus*.

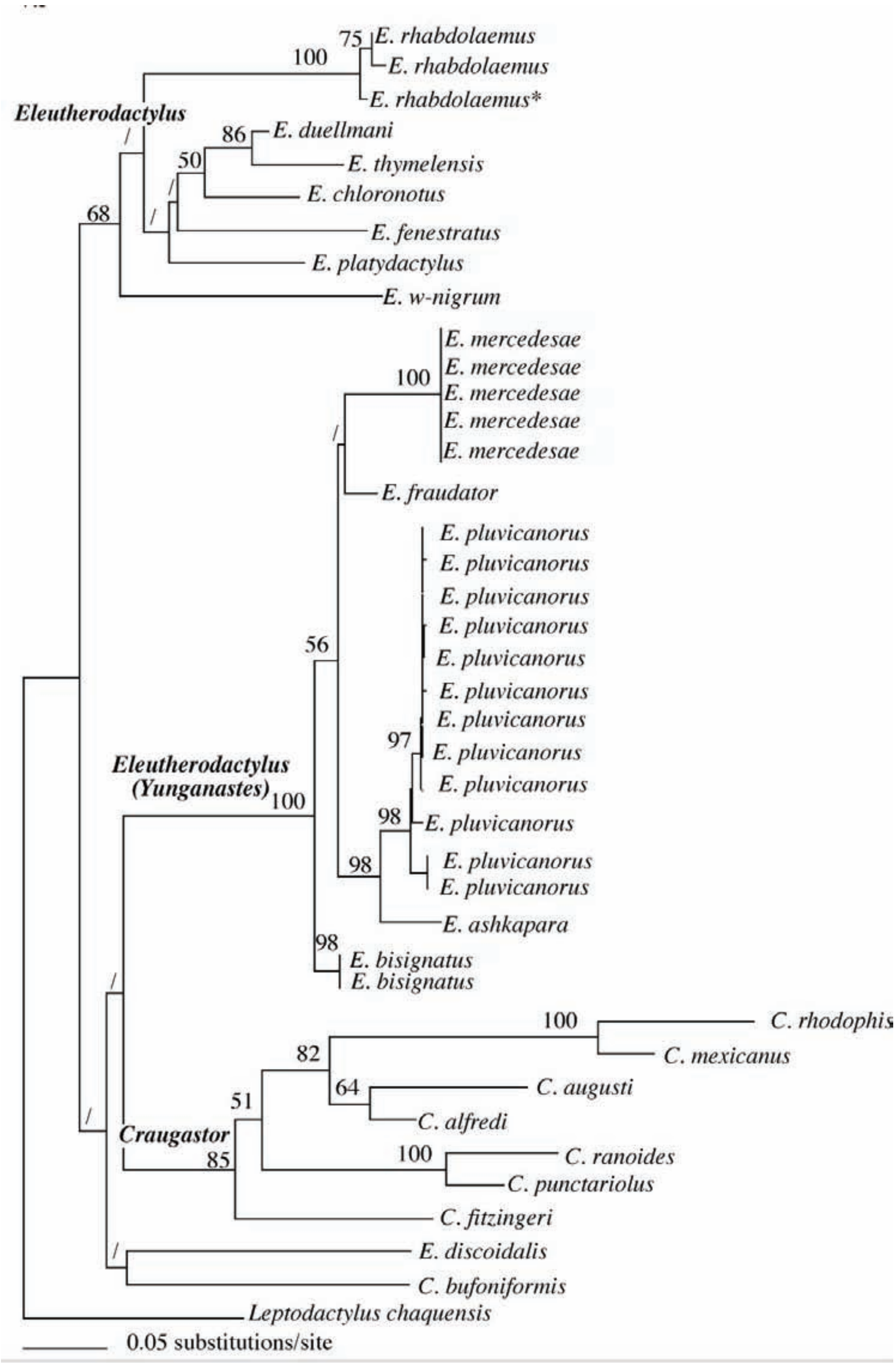

FIG. 12.—Gen tree resulting from Neighbor Joining phylogenetic analyses of a 560 bp fragment of the 16S mitochondrial gene of members of the genera *Craugastor* and *Eleutherodactylus*, including the subgenera *Eleutherodactylus* and *Yunganastes*. Boostrap nodal support inferior to 50% is indicated by a slash. *E. rhabdolaemus** corresponds to a fragment of the 16S sequence used by Faivovich et al. (2005) and Frost et al. (2006) as *E. pluvicanorus*.

THE ORIGIN OF *YUNGANASTES*

The subgenus *Yunganastes* is endemic to the Eastern Cordillera of the Central Andes (sensu Gregory-Wodzicki, 2000), an area rich in endemic anuran species (Köhler, 2000*b*). This high diversity of endemism is probably due to a compound of both intensive vicariant events associated to Andean orogeny and erosion during the final uplift of the Central Andes, when fragmentation of montane habitats began (Gregory-Wodzicki, 2000; Lynch and Duellman, 1997; Doan, 2003), and/or to habitat fragmentation promoted by climatic changes (Haffer, 1969; Weir, 2006; Hughes and Eastwood, 2006).

Table 5.—Relative distribution of the species belonging to the subgenus *Yunganastes*.

	E. ashkapara	*E. bisignatus*	*E. fraudator*	*E. mercedesae*	*E. pluvicanorus*
E. ashkapara	–				
E. bisignatus	Allopatry	–			
E. fraudator	Sympatry, allopatry	Allopatry	–		
E. mercedesae	Parapatry	Parapatry	Parapatry	–	
E. pluvicanorus	Sympatry, allopatry	Allopatry	Sympatry, allopatry	Parapatry	–

Most species of *Yunganastes* are restricted to Central Bolivia, and the only species with a wide range is *E. mercedesae*, that occurs from Southern Peru to Central Bolivia. *E. mercedesae* is mostly parapatric (mainly altitudinal segregation) to the rest of species. *E. bisignatus* occurs in allopatry to all other species except *E. mercedesae*. *E. fraudator*, *E. pluvicanorus* and *E. ashkapara* occur in sympatry and/or syntopy at some localities (see Table 5 for a summary). All but *E. mercedesae* occur mainly in cloud forest of the Andes, while *E. mercedesae* occupies lower altitudes (Figure 5). Hence, combining our information about the distribution, the habitat use, and the phylogenetic relationships of these species, we can hypothesize the speciation processes that could have originated the observed species diversity (Graham et al. 2004).

We hypothesize two different scenarios for the origin of *Yunganastes*. In the first, the ancestor of *Yunganastes* evolved in Central Bolivia with a new condition of the trigeminal nerve. Then, it had split into several species, among which, *E. mercedesae* would be the most derived. This species was able to extend its range to southern Peru through the humid montane forest. The presence of a tarsal fold and basal membrane in this species and its morphological differences within the group must be considered as derived characters of independent origin in relation to other *Eleutherodactylus*.

In the second hypothesis, *E. mercedesae* may have considered the most primitive species of *Yunganastes* (the mentioned exclusive characters being plesiomorphic). This second hypothesis is supported by our morphological and ecological data, but molecular data render ambiguous results (see Fig. 10–12). The presence of a tarsal fold and basal membrane in other *Eleutherodactylus* and its exclusivity in *E. mercedesae* within *Yunganastes* suggest a plesiomorphic condition for these characters. Moreover, *E. mercedesae* occurs at lower altitude than the other species. Hence, we hypothesize that the ancestor of the subgenus *Yunganastes* had a wide distribution range in lower altitudes (probably the humid montane forest). Subsequent isolation by habitat fragmentation may have led some populations to survive and speciate in cloud forests when the humid montane forest was relegated to lower altitudes where *E. mercedesae* evolved. That would explain the altitudinal parapatry-allopatry situation of *E. mercedesaae* with respect to the other species of the group. The wider distribution of *E. mercedesae* would be explained by the higher continuity of its habitat, potentially allowing more gene flow and dispersal.

Eleutherodactylus ashkapara and *E. pluvicanorus* are sister species and occur in syntopy in the southern limit of Andean cloud forests. Both species are similar and close phylogenetically, and molecular, morphological, and bioacoustical characters suggest a recent divergence between both lineages (sensu Wiley, 1981; de Queiroz, 2005). Additionally, there seem to be some niche differences. *E. ashkapara* inhabits the canopy, while *E. pluvicanorus* prefers the forest floor and lower strata of the forest vegetation (Köhler, 2000*a, b*). Following Graham et al. (2004), when two species occur in sympatry and occupy a different environmental space, divergent selection in sympatry can be hypothesized as the origin of one of the species. However, we cannot discard the possibility of a process of speciation in allopatry and subsequent secondary contact to explain this situation. Indeed, the distribution of this pair of species lies just at the southern limits of cloud forest, where the contiguous habitats are dry montane forests or interandean forests, which are not suitable for these species. Currently, in the southern distribution limit of *E. pluvicanorus*, cloud forests are usually found isolated on top of the hills or occur only in the wet versants of the valleys or as gallery forest. Altitudinal migration of montane habitats and the formation of refugia promoted by climatic changes may have resulted in the observed diversity (Haffer, 1969; Hewitt, 2000; Pennington et al., 2000; Hughes and Eastwood, 2006; Weir, 2006; but see Mourguiart and Ledru, 2003). Therefore, it is necessary to gather more data in order to test which speciation model explains better the origin of this pair of species.

RESUMEN: Revisamos el estatus taxonómico de los taxones pertenecientes al grupo de especies de *Eleutherodactylus fraudator*. *Eleutherodactylus bisignatus* (Werner, 1899) es redescrita e incluida en el grupo junto, a *E. mercedesae*. La validez de las especies está corroborada por sus diferencias en caracteres morfológicos, bioacústicos y moleculares. Los análisis filogenéticos de un fragmento del gen mitocondrial 16S utilizando Máxima Parsimonia, métodos Bayesianos y Neighbor Joining, y la presencia de dos caracteres morfológicos derivados y compartidos (la fontanela frontoparietal y una nueva condición de la rama mandibular del nervio trigémino) determinan la monofilia del grupo. A su vez, estos datos sugieren la parafilia del subgénero *Eleutherodactylus* y la monofilia de *Craugastor* si se excluye al grupo de *C. bufoniformis*. El clado formado por los miembros del grupo de especies *E. fraudator* es redescrito y nominado como subgénero *Yunganastes*, que contiene las siguientes especies: *E. ashkapara* Köhler, 2000, *E. bisignatus* (Werner, 1899), *E. fraudator* Lynch y McDiarmid, 1987, *E. mercedesae* Lynch y McDiarmid, 1987 y *E. pluvicanorus* De la Riva y Lynch, 1997. El canto nupcial de *E. bisignatus* es descrito por primera vez y se compara con los cantos disponibles de *E. ashkapara* y *E. pluvicanorus*. Además, se describe por primera vez el canto de agresión de *E. ashkapara*, *E. bisignatus* y *E. pluvicanorus*. Se excluye a los miembros de *Yunganastes* del género centroamericano *Craugastor*, al cual fueron asignados debido a la presencia compartida de una supuesta sinapomorfia, la condición "E" del nervio trigeminal de la rama mandibular del músculo abductor. Se describe una nueva condición del nervio trigémino como sinapomorfía de *Yunganastes*. Los miembros de *Yunganastes* son endémicos del bosque de niebla y bosque montano húmedo del Este de la Cordillera Oriental de los Andes en Perú y Bolivia.

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APPENDIX

Specimens Examined

Eleutherodactylus ashkapara

BOLIVIA: Department Cochabamba: Old road from Paractito via el Palmar to Cochabamba, 2100 m (17º07’58’’S, 65º36’54’’W), CBF 3344 (Holotype), ZFMK 70318 (Paratopotype).

Eleutherodactylus bisignatus.

BOLIVIA: Department La Paz: Chaco, 2200 m (16º20’57’’ S, 67º49’10’’W), NMW 16502 (Holotype); Plataforma, PN-AMNI Cotapata, 2300 m (16º11’44,5’’S, 67º53’14,2’’W), CBF 4113–14, 4122, 4134, 4184; Toriri, 2658 m (16º51’6.36”S, 67º13’17.58”W), Prov. Inquisivi, CBF 5717–20; unknown location: Actinero, BM 1901.8.2.43.

Eleutherodactylus fraudator.

BOLIVIA: Department Cochabamba: Camino antiguo a Aguirre, CBG 53–4; Ceja de Monte antes de Corani hacia Cochabamba, MNCN 43344; Entre Comarapa y Cochabamba, ca. 2600 m, MNCN 43358; La Siberia, 2900 m , MNCN 43329–43338; La Siberia, camino a Locotal y Carahuasi, 2050 m, MNCN 43340–43343, 43345–43347; La Siberia, carretera de Santa Cruz a Cochabamba, MNCN 43348–43356; Monte Punkhu, CBG 059; Río Chua Khocha (17º47’S, 64º42’W), MNKA 635–6; Sehuencas 2000–2500 m (17º29'59.1"S/65º16'30.0"O), CBG 001–5, ZFMK 60244–54; 3.8 km on road from El Empalme to Khara Huasi, tributary of río Hualla Mayu, 2275 m, UTA 45602; 8.6 km N of El Empalme, road to Cochbamba, 2825 m asl, UTA 45603; 73.5 km from Cochabamba to Villa Tunari (17º12'13"S/65º52'19"O), USNM 257847 (Holotype), USNM 257846 (Paratopotype), MNCN 43107; 35 km de Comarapa en dirección a Cochabamba, 2500 m, MNCN 43357; 38 km de Comarapa en dirección a Locotal, 2250 m, MNCN 43339; Department Santa Cruz: Laguna Tingui, 5 km N from Comarapa (17º50’46’’S, 64º32’54’’W), MNKA 1746; Serranía de la Siberia (17º49’S, 64º45’W), CBF 4681–2, CBG 041–5, MNKA 908–919, ZFMK 72660–62.

Eleutherodactylus mercedesae.

BOLIVIA: Department Cochabamba: “Old” Chapare road, 1300–1500 m, ZFMK 72571–73; “Old” Chapare road, 1650 m, ZFMK 72597–99; Pampa Grande, National Park Altamachi, MHNC-AMS 196; 3.3 Km N of Cochabamba to Villa Tunari road on road to San Onofre, at a point 97.5 km from Cochabamba on Cochabamba to Villa Tunary road, 1690 m, (17º10’S/65º46’W), UNSNM 257848 (Holotype); Limbo, 73.5 Km from Cochabamba to Villa Tunari, 1950 m (17º10’S/65º48’W), USNM 165753 (Paratype). Department La Paz: Hornuni, PN-ANMI Cotapata, Prov. Nor Yungas, (16º12’50’’S/67º53’10’’W), CBF 4120; road to Coroico, Serranía Bella Vista, Prov. Nor Yungas (16º14’S/67º43’W), CBF 3701. PERU: Department Cusco: Paucartambo, 68 km by road NE of Puente Unión on río Tachila (Bosque de las Nubes, Km 150 on Paucartambo-Atalaya road), 1700 m, (13º14’13’’S/71º34’00’’W), USNM 346140.

Eleutherodactylus pluvicanorus.

BOLIVIA: Department Cochabamba: Incachaca, 2300 m, ZFMK 66938; La Siberia, camino de Locotal y Carahuasi, 2050 m, MNCN 43285–43288; Pojo (17º45’S, 64º52’W), CBF 1597; “Old Chapare Road” between Paractito and El Palmar, 2250 m, ZFMK 72619–20; Río Chua Kocha (17º47’S, 64º42’W), MNKA 1100 (Holotype), 1101–4 (Paratopotypes), MNKA 627–630; road near Penstock’s bridge (17º12'21.6"S/65º50'14.2"O), MNCN/ADN 6004; Sehuencas 2000–2500 m (17º29'59.1"S/65º16'30.0"O), CBG 91–97, 128–134, MNCN 43002, 43281–43284, 43292, 43293–43296 (paratypes); ZFMK, 60186–91 (Paratypes), 60195–6 (Paratypes), 60203 (Paratypes), 60192–4, 60197–202, 60204; 38 km de Camarapa en dirección a Locotal, 2250 m, MNCN 43289; Department Santa Cruz: Abra de la Cruz, NKA 6712, 6714–17; El Chapé (=La Yunga de Mairana), 15 Km N of Mairana (18º03'34"S/63º54'36"O), MNKA 3486, 3505, 3514, 3528–9, 6623, MNCN 43035, MNCN 43273–43280, 43291, 43297 (Paratype); ZFMK 66872–5; El Corral de China Vilcar, MNKA 6991–2, 6997–7003, 7008, 7010, 7012–5, 7018, 7032, 7041; Entrando desde Mataral por San Juan del Potrero, La Siberia, MNCN 43290; Filo del Rasete, MNKA 6856, 6931, 6939, 6941–6, 6964–7, 6969; Río Amparo, 5 km (aprox.) above the confluence of the Rio Cerro Bravo and Río Amparo (near 17° 50' 8" S 64° 23' 23" W), Amboró National Park, AMNH 165194; Río de las Orquídeas, 2040–2060 m, Amboró National Park, AMNH 165193; Serranía de la Siberia (17º49’S, 64º45’W), CBF 1597, 4677–80, MNKA 5590, ZFMK 72663–4; tree fern forest above base camp (17° 50' 8" S 64° 23' 23" W), closest town San Juan del Potrero, Amboró National Park, AMNH 165211.

ÁPENDICE XI

Integrative taxonomy reveals cryptic Amazonian species of *Eleutherodactylus* (Anura)

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Hypotheses on the taxonomic status of two Bolivian *Eleutherodactylus* with taxonomic problems are assessed by an integrative taxonomic approach that integrates three different lines of evidence: external morphology, prezigotic reproductive barriers (advertisement calls), and reciprocal monophyly (phylogenetic analyses of partial 16S mtDNA). Central Andean Bolivian populations previously assigned to either *E. peruvianus* or *E. dundeei*, and lowland Amazonian populations from southern Peru and northern Bolivia previously considered *E. peruvianus* do not correspond to these species following the result of comparisons of qualitative morphological characters. Indeed, multivariate discriminant analyses of morphometric and bioacoustical characters, and phylogenetic analyses support the hypothesis that they represent different, previously unknown, cryptic lineages. They are herein described as new species taxa. The former is a sibling species of *E. fenestratus* that inhabits the Amazonian and semideciduous forest of the Andean foothills in Central Bolivia. The later is sibling with the Andean *E. danae* and occur parapatryc to it in the Amazonian lowland forests and adjacent foothills of northern Bolivia, southern Peru and adjacent Brazil. The historical habitat dynamics between the Andean foothills and adjacent lowlands seem to have promoted recent lineage divergence as evidenced by sister taxa relationships. Since most species of Neotropical frogs, and specially *Eleutherodactylus*, have been described by using external qualitative morphological characters only, an integrative taxonomic approach, as exemplified herein, may lead to the discovery of many cryptic and sibling lineages that would increase the species numbers of tropical areas.

ADDITIONAL KEY WORDS: Amazon – Andes – bioacoustics – cryptic species – integrative taxonomy – mtDNA – new species.

INTRODUCTION

The Amazonian versant of the Andes and adjacent lowlands house one of the most diverse habitats of the world (Myers *et al.*, 2000), with National Parks as Manu (Peru) or Madidi (Bolivia) as symbols of the preservation of the richest biodiversity hotspots. Among the vertebrates, amphibians show high levels of diversity and endemicity in these areas (Köhler, 2000a). However, Neotropical amphibians are still very porly known despite of the high rate of species discovery (Padial & De la Riva, 2006) and the current conservation concern (Stuart *et al.*, 2004). Nowadays, several Peruvian and Bolivian species are described and named each year both from the Andes (e. g. Padial *et* *al.*, 2006, 2007a) and from the Amazonian lowlands (e. g. Moravec *et al.*, 2006). Nontheless, most of them are discovered by means of standard exploration of remote or poorly explored areas or through the revision of museum specimens. In other words, most newly described species represent quite obvious divergent lineages evidenced by differences in qualitative morphological characters. Nevertheless, the application of bioacoustics (e. g. Padial *et al.*, in press-a) and molecular phylogenetics (e. g. Parra-Olea *et al.*, 2004; Fouquet *et al.* 2007) to tropical taxonomy opens the door to new frontiers of data exploration that may potentially increase the rate of species discovery. Indeed, cryptic and sibling species hidden to the eye of the classical taxonomist may be much more abundant in nature that expected (Bickford *et al.*, 2007). If that is true, the number of, for example, *Eleutherodactylus*, with nearly 500 species having being described by the classical morphological approach based on evident qualitative characters, may increase considerably. Moreover, many *Eleutherodactylus* are candidates for a speciation pattern particularly suitable to originate cryptic and sibling lineages (Lynch & Duellman, 1997). This is exemplified in this study of Andean and adjacent lowland populations of a small assemblage of Bolivian and Peruvian *Eleutherodactylus*, where the use of bioacoustics and DNA taxonomy allowed the discovery and description of two new cryptic species level lineages and solution of some old taxonomic problems.

MATERIAL AND METHODS

TAXONOMIC BACKGROUND AND TAXON SAMPLING

The taxonomic status of Andean populations assigned to *Eleutherodactylus dundeei* by Köhler (2000a) for the humid Andean Amazonian slopes of Departmento de Santa Cruz, Bolivia was considered doubtful by Padial & De la Riva (2005a). They pointed out that Andean populations lacked the basal webbing present in the type series of *E. dundeei*. Padial & De la Riva (2005a) also considered inconclusive the comparisons made by Köhler (2000a) of the advertisement call of these populations and *E. dundeei*. These authors removed this species from the country lists but did not provide enough evidence to assign this population to another species. Previously, Bolivian populations of *Eleutherodactylus* considered as *E. dundeei* by Köhler (2000a) were first assigned to *E. fenestratus* (see De la Riva, 1993) and later to *E. peruvianus* by De la Riva (1994). Köhler (2000a) redescribed *E. peruvianus* and removed this species from the country list since no Bolivian voucher shared the character states of the holotype. De la Riva *et al.* (2000) and Padial & De la Riva (2005a) considered Köhler's (2000a) arguments to be flawed because a large museum series identified as *E. peruvianus* from seven localities in central and southern Peru showed intraspecific variability for these characters. Subsequently, they considered very likely the presence of more than one species hidden under the species name *E. peruvianus*. Padial & De la Riva (2005a) concluded that " Until a taxonomic study is done to confirm or discard the presence of more than one species, the mentioned populations from Bolivia and central and southern Peru should be referred to as *E. peruvianus* or *E.* cf. *peruvianus*…" (as by Padial *et al.*, 2000). Moreover, given the variability of *E. peruvianus* considered by these authors, the diagnosis of another species, *E. danae*, rendered inconsistent, and they attributed this fact to the existence within this series of a putative new species, more similar to *E. danae* than to *E. peruvianus*.

To solve this complex taxonomic panorama, we selected as target "taxa" the Bolivian and Peruvian populations hypothesized to be *E.* cf. *peruvianus* by Padial & De la Riva (2005a), which may include two undescribed species, one morphologically similar to *E. danae* and the other similar to *E. dundeei*. *Eleutherodactylus peruvianus* is a member of the *E. conspicillatus* Series and the *E. conspicillatus* Group of Lynch & Duellman (1997). The distribution-range of this group extends from Central America to central Bolivia, and its representatives occur both in Trans and Cis Andean (Frost, 2006). Only *E. w-nigrum* seems to occur at both sides of the Andes in northern Colombia (Lynch, 1975). The rest of species are either inhabitants of the lowlands or Andean foothills at the western or eastern flanks of the Andes. Since our target taxa are Cis-Andean regions, inhabiting the Upper Amazon basin and adjacent Andean hills of Peru and Bolivia, we reduced the taxon sampling to this area. In other words, we do not compare our target taxa with species restricted to western slopes of the Andes, Guayana Shield or the northern mountain ranges of Ecuador, Colombia and Venezuela. However, we included two species, *E. crepitans* and *E. dundeei*, inhabiting the Cerrado forest formations of the Precambrian Shield in Western Brazil (Heyer & Muñoz, 1999) because the problematic populations have either been considered *E. peruvianus* or *E. dundeei* (see below) and the later is morphologically similar to *E. crepitans* and *E. fenestratus*. The species selected for diagnostic comparisons of qualitative characters with our target taxa are the following members of the *E. conspicillatus* Group: *E. avicuporum*, *E. bipunctatus*, *E. buccinator*, *E. caliginosus*, *E. citriogaster*, *E. condor*, *E.*

cosnipatae, *E. conspicillatus*, *E. crepitans*, *E. cuneirostris*, *E. dundeei*, *E. fenestratus*, *E. lanthanites*, *E. lymani*, *E. malkini*, *E. metabates*, *E. peruvianus*, *E. samaipatae*, *E. skydmainos* [comprising *E. karcharias* (see Padial & De la Riva, 2005b)], *E. vilarsi* and *E. zeutoctylus*. *Eleutherodactylus bisignatus*, a former member of the *E. conspicillatus* Group (Köhler, 2000a), is not included because a recent study (Padial *et al.*, in press-b) places it in a different subgenus. We include *E. danae*, a member of the *E. unistrigatus* Group of Lynch & Duellman (1997), in the diagnosis because, as explained above, Padial & De la Riva (2005a) considered that a putative undescribed species similar to *E. danae* might be hidden under what they called *E.* cf. *peruvianus*. Therefore, to study and diagnose this putative new taxon we compared it with some similar species of the *E. unistrigatus* Group inhabiting the Upper Amazon basin and adjacent hills: *E. altamazonicus*, *E. carvalhoi*, *E. croceoinguinis*, *E. diadematus*, *E. eurydactylus*, *E. martiae*, *E. ockendeni*, *E. platydactylus*, *E. rhabdolaemus*, *E. salaputium*, *E. toftae*, and *E. ventrimarmoratus*. For comparison of advertisement calls and phylogenetics (see below), the taxon sampling was reduced to those species very similar in qualitative morphological characters, that is, those putative species pairs lacking qualitative morphological characters clearly supporting a different specific status.

SPECIES CONCEPT

Following De Queiroz (2005a, b), species are independently evolving metapopulational level lineages, and biological properties historically used to define species are neither necessary nor sufficient for defining the category of species. Under this view, species taxa are hypotheses on the divergence of species level lineages that can be tested under different lines of evidence (De Queiroz, 2005c). Differences in qualitative morphological characters have been the most commonly used line of evidence to describe species taxa. The holotype has two functions: to represent those differences, and as being the name bearing semaphoront that represents the species taxa. Therefore, one way to reanalyse species taxa hypotheses, is the study and comparison of types, paratypes or original descriptions (when accurate enough). Hence, we tried to review all the holotypes or paratypes of species taxa considered in this study (see Appendix). Moreover, we try to compare additional museum specimens for all species to assess intraspecific variation.

COMPARISON OF MORPHOLOGICAL CHARACTERS

We followed Lynch & Duellman (1997) for qualitative morphological characters states used in the diagnoses and descriptions of *Eleutherodactylus*. We followed Grant & Kluge (2004) for the character concept in systematics as transformations series. Therefore, all morphological characters considered herein represent fixed character states in an evolutionary transformation series. We focused on the main characters used (see, for example, Lynch, 1980; Köhler & Jungfer, 1994; Lynch & Duellman, 1997; Duellman & Pramuk, 1999; Padial & De la Riva, 2005b; Duellman & Hedges, 2005) to diagnose species within the *Eleutherodactylus conspicillatus* Group (character states in parentheses): relative length of first and second fingers (Finger I>II, Finger I≥II, Finger I=II, Finger I<II); belly skin texture (smooth, granular, granular posterolaterally); dorsal skin texture (smooth, shagreen, coarsely shagreen, granular, warty); dorsal tubercles (presence/absence of dorsal tubercles or short folds); dorsolateral folds (present, absent); finger fringes (prominent, weak, absent); toe fringes (prominent, weak, absent); basal toe webbing (present, absent); tarsal fold (present, absent); labial bars (well defined, diffuse, absent); color pattern of posterior surfaces of thighs (well defined spots, barely visible or diffuse spots, marmored, plain); color pattern of throat, chest and belly (heavily spotted; weakly spotted; immaculate); and adult male nuptial pad on thumb (double, single, absent).

For morphometrics, a single person (JMP) took measurements with a digital calliper to the nearest 0.01mm, but following Hayek *et al.* (2001), for avoiding pseudo precision, we rounded all measurements to only one decimal. Abbreviations are as follows: snout–vent length, SVL; head length (from posterior margin of lower jaw to tip of snout), HL; head width (measured at level of rictus), HW; eye length (measured horizontally), EL; eye to nostril distance, EN; internarial distance, IND; eye-eye distance, EE; tympanic membrane height, TYH; tympanic membrane length, TYL; width of disc on Finger III, F3; width of disc on Finger IV, F4; arm length (from posterior margin of thenar tubercle to elbow), FA; tibia length, TL; thigh length, TH (from vent to knee); foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL; width of disc on Toe IV, T4. We do not include values of interorbital distance (IOD) and upper eyelid width (EW). Our experience indicates that these parameters are usually of scarce utility because the preservation condition of the specimen highly influences the measurements and makes it difficult to have precise and comparable values for large series (but see Arroyo *et al.*, 2005). Color characteristics were noted in life and in alcohol. We determined age and sexual condition by dissection or observation of external secondary sexual characters. The condition of the trigeminal nerve (see Lynch, 1986) was determined through dissection of the skin above the tympanic area and through a horizontal cut of the mandibular joint. Museum abbreviations other than cited by Leviton *et al.* (1985) are: Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Cochabamba, Bolivia (CBG); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA [Amphibian Collection], formerly NKA); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Peru (MHNSM, formerly MHNJP), Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru (MHNC). Specimens examined are listed in Appendix I.

BIOACOUSTICS

In anurans, taxonomic hypotheses on species taxa usually rely on differences in mating calls as evidences for prezigotic reproductive barriers (Bickford *et al.* 2007). We identified the recorded calls as advertisement calls based on the behavior of observed frogs. Other call types are mostly the result of changes in individual motivation or interactions, while advertisement calls are usually emitted continuously under appropriate conditions with the goal of mate attraction (Duellman & Trueb, 1986). The study area includes the Andean slopes (between 500 and 3000 masl) and adjacent lowlands, from central to northern Bolivia. We collected voucher specimens and recorded advertisement calls along this latitudinal axis, that comprises interandean dry valleys forest, humid forest of the Andeand slopes, humid montane forests, the Yungas (cloud forests) and the Amazonian lowland forests (see Köhler [2000a] for more details about the habitats).

Recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone. The sounds were recorded on TDK SA60 cassettes, and digitized at a sampling rate of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board and Peak 3.2 (OSX) software (Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, CSIC, Madrid). All calls were edited with Audacity 1.2.2 for MacOS X (Free Software Foundation Inc., 1991). Praat 4.2.22 for MacOS X (Boersma & Weenink, 2005) software was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through Fast Fourier Transformations (FFT) (width, 1024 points). Air temperature was measured immediately after sound recording. Digitized calls were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (Madrid). Call vouchers, localities, and track numbers are listed in Appendix II. Sample sizes are indicated in Table 1.

We analysed the following quantitative parameters: call repetition rate, number of pulses per call, call duration (ms), pulse rate within a call, fundamental frequency (Hz), and dominant frequency (Hz). All of these call characteristics are commonly used for call descriptions and taxonomic recognitions (e.g., Márquez *et al.*, 1995; Köhler, 2000a; Bosch & De la Riva, 2004; Padial *et al.*, in press-a). Terminology in call descriptions generally follows Márquez *et al.* (1995) and Köhler (2000a). Sample sizes do not allow for temperature correction using regression. Stepwise discriminant analyses of the mean values of specimen calls were performed with JMP 5.0.1.a (© SAS Institute Inc., 2002) on log10-transformed-variables (see Table 1 for the number of calls analysed for each species).

PHYLOGENETIC ANALYSES OF MOLECULAR CHARACTERS

For the molecular analyses we sampled a total of 39 specimens belonging to six ingroup taxa (according to previous classifications) from different localities (Table 2). Tissue samples or *E. samaipatae* were collected in two localities close to the type locality. The type locality of *E. fenestratus*, Río Mamoré, belongs to the Bolivian-Brazilian Amazon Basin. We gathered tissues from different localities from the Bolivian Amazon basin and adjacent Andean slopes that are considered conspecific with *E. fenestratus* (De la Riva *et al.*, 2000). Tissue samples for *E. danae* were collected both at the type locality and from scattered localities along the Bolivian Andes. Tissue samples of *E.* cf. *peruvianus* where collected along the Andean hills of the Amazon basin in Bolivia and Peru. Unfortunately, there were no available tissues of *E. dundeei*. Other species putatively related to *E. danae* (*E. rhabdolaemus*, *E. toftae* and *E. platydactylus*) from the Bolivian and Peruvian Andean hills were included in the analysis.

Table 1. Numerical characteristics and sample sizes of the advertisement call of six *Eleutherodactylus* species included in this study. Mean ± standard deviation in parentheses follows ranges. See text for further explanations and Appendix II for sound collection, locality, temperatures and voucher information.

	Notes/call	**Call length (ms)**	**Note length (ms)**	**Note rate**	**Pulses**	**Fund. Freq. (Hz)**	**Dom. Freq. (Hz)**	**Notes**	**Calls**	**Nº Spec.**	**Nº Populations**
E. dundeei	4–8 (6±2.8)	263–526 (394±186)	30–42 (37.4±3.8)	15.2	3–6 (5.2±0.9)	1984–2208 (2116.3±74.3)	2147–3961 (3536.6±655.5)	12	2	2	2
E. fenestratus	2.0–4.0 (2.6±0.6)	157–458 (265.2±81.6)	50.0–91.0 (63±11.4)	7.7–12.7 (10.1±1.5)	9–17 (12.9±42.2)	1542–2048 (1746.7±158)	1710–3591 (3086.3±580.7)	55	22	6	4
E. sp. nov. 1	3–8 (5.7±1.0)	173–644 (421±159.8)	20–54 (35.5±6.6)	11.8–17.3 (14.1±1.8)	5–9 (7.5±1)	1732–1971 (1853.5±72.1)	3245–3971 (3662.4±128.9)	119	21	6	2
E. samaipatae	1.0–3.0 (2±0.2)	82.2–1062 (291.7±168.1)	59–141 (89±16.4)	2.7–14.9 (8.5±2.1)	11–23.0 (16.4±2.6)	1535.0–1834.0 (1704.9±64.3)	2922–3853 (3326.7±175.9)	160	98	12	4
E. danae	1	7–13 (11±1.2)	7–13 (11±1.2)	76.9–142.9 (92.3±12.6)	1–2 (1.9±0.2)	1369–2925 (2210±553.4)	1369–2925 (2210±553.4)	87	87	4	2
E. sp. nov. 2	2–3	50–268 (143.9±52.2)	20–58 (32±5.8)	11.2–40 (16.9±6.3)	4.0–11 (6.7±1.2)	2013–2815 (2501.4±197.7)	2013–2815.0 (2501.4±197.7)	137	63	5	3

We selected four species of the genus *Oreobates* as outgroup, since recent phylogenetic hypotheses place this taxon as sister group of *Eleutherodactylus* (Padial *et al.*, in press-c). We used the standard phenol-chloroform extraction protocol (Sambrook *et al.*, 1989) with minor changes to isolate genomic DNA. A fragment of approximately 591 bp from the mitochondrial gene 16S was amplified by polymerase chain reaction (PCR) using the primers 16Sar-5’ and 16Sbr-3’ and previously described PCR conditions (Hillis *et al.*, 1996). PCR products were purified and sequenced in a MegaBACE 1000^{TM} (GR Health Care) instrument following manufacturer protocols. Complete sequence alignment (pairwise and multiple alignment) was performed in CLUSTAL X 1.83.1 (Thompson *et al.*, 1997) under gap penalties of 10.0 for gap opening and 0.5 for gap extension respectively. Two ambiguously aligned regions of around 60 and 20 base pairs were refined under penalties of 10.0 for gap opening and 0.1 for gap extension. This procedure led to an alignment very similar to that resulted from alignment under default parameters and posterior editing by eye, but has the convenience of allowing repeatability. Neighbor Joining (NJ) analyses were performed using PAUP* 4.0b10 (Swofford, 1998), with Maximum Likelihood (ML) genetic divergence corresponding to the model GTR + I + G. The relative branch support was evaluated with 2000 Bootstrap replicates. Maximum Parsimony (MP) analyses were done with PAUP* 4.0b10 using heuristic searches under parsimony and tree bisection reconnection (TBR). In order to obtain estimates of clade support, non-parametric bootstrapping was performed with heuristic searches of 1000 replicate datasets with 10 random addition sequence replicates. Gaps were considered a fifth character state. For Bayesian phylogenetic analyses (Rannala & Yang, 1996) we used MrBayes version 3.2.1 (Huelsenbeck & Ronquist, 2001). The majority rule consensus tree was produced from two separate Monte Carlo Markov chains (MCMC; Yang & Rannala, 1997); each run used one cold chain (the head chain) and two heated chains (scout chains). It was run simultaneously for five million generations (Metropolis-coupled MCMC). Trees were sampled every 100 generations. Burn-in was evaluated by examination of the standard deviation of split frequencies (> 0.01). The first 10.000 trees were excluded.

Table 2. Localities, voucher information, and GenBank accession numbers for sequences and specimens used in this study. Abbreviations: IDlR: Ignacio De la Riva's field series; JMP: José M. Padial's field series; MNCN: Museo Nacional de Ciencias Naturales (Spain); MNK-A: Amphibian Collection, Museo de Historia Natural Noel Kempff Mercado (Bolivia); MHNC: Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru.

Species	**MNCN DNA**	**Vouchers**	**Locality**	**Accession number**
Eleutherodactylus				
danae	547	IDLR 4001	Bolivia: La Paz: Santa Cruz de Valle Ameno	
danae	5798, 5837	MNK-A 7182, MNCN 43062	Bolivia: La Paz: Huairuro, senda San José - Apolo	
danae	6005, 6040	MNCN 43069, MNK-A 7190	Bolivia: La Paz: Arroyo Huacataya. senda San José y Apolo	
danae	6258	MNK-A 7273	Bolivia: La Paz: Serranía Bella Vista	
danae	20677	IDLR 4815	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20682	MNCN 44232	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20683	MNCN 44233	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20684	IDLR 4822	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20685	MNCN 44234	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20686	IDLR 4824	Peru: Cusco: Unión, Valle de Kosñipata	
danae	20687	IDLR 4825	Peru: Cusco: Unión, Valle de Kosñipata	
fenestratus	47.4	IDLR	Bolivia:	
fenestratus	3947	MNK-A 6629	Bolivia: La Paz: Chalalán	
fenestratus	3981	MNK-A 6630	Bolivia: La Paz: Sadiri, Arroyo Yariapo	
fenestratus	4109	MNK-A 6633	Bolivia: Cochabamba: Los Guácharos	
fenestratus	9496	MHNC 3130	Peru: Madre de Dios: Cocha Camungo	
fenestratus	4108, 4109, 4088	MNCN 43031, MNK-A 6633, MNK A 6631,	Bolivia: Cochabamba: Los Guácharos	
sp. nov. 1	3903, 3905	MNCN 42990, MNK-A 6627	Bolivia: Santa Cruz: Km 6 Angostura-Samaipata road	
sp. nov. 1	4001–2, 4016	MNCN 42983, 43013, 42986	Bolivia: Santa Cruz: La Chonta	
platydactylus	3919	JMP054 (MNK-A collection)	Bolivia: Santa Cruz: Siberia	
sp. nov. 2	4084–5	MNCN 43012, MNK-A 6621	Bolivia: Cochabamba: Los Guácharos	
sp. nov. 2	5542	MNCN 43249	Peru: Cusco: A 5 km de San Lorenzo hacia Quince Mil	
sp. nov. 2	20642	IDLR 4779	Peru: Puno: Entre Puerto Leguia y San Gabán	
rhabdolaemus	3940	MNK-A 6628	Bolivia: Santa Cruz: Serranía de la Siberia	

rhabdolaemus	4120	MNCN 43036	Bolivia: Santa Cruz: La Yunga de Mairana
samaipatae	3899–02	MNCN 42987–9, MNK-A 6626	Bolivia: Santa Cruz: Km 6 Angostura-Samaipata road
toftae	4093	MNCN 43025	Bolivia: Cochabamba: Los Guácharos
toftae	5505	MNCN 43246	Peru: Cusco: San Pedro, Valle de Marcapata
Oreobates			
cruralis	6098	MNK-A 7171	Bolivia: Santa Cruz: Camino a Bella Vista
discoidalis	6123	MNK-A 7247	Bolivia: Tarija: Serranía Aguarague
heterodactylus	6061	MNK-A 7175	Bolivia: Santa Cruz: Cerro del Arco, Serranía de Santiago
quixensis	6216	MNCN 43147	Bolivia: Pando: San Sebastián, Tahuamanu

RESULTS

COMPARISON OF QUALITATIVE CHARACTERS

The analysis of qualitative character states for each species (summarized in Table 3) led to the following results. (1) Populations from the Andean slopes of Departmento Santa Cruz assigned by De la Riva (1993) to *E. fenestratus* and subsequently to *E. peruvianus* (De la Riva, 1994), to *E. dundeei* (Köhler, 2000a) and to *E.* cf. *peruvianus* by Padial & De la Riva (2005a), are well distinguished from *E. peruvianus* and from *E. dundeei*, but are morphologically cryptic in relation to *E. fenestratus* and *E. samaipatae*. However, De la Riva (1993) and Köhler (2000a) reported differences in advertisement calls of these later two taxa. Therefore, the taxonomic status of these populations remains uncertain under this line of evidence. We will subsequently refer this taxon as to *Eleutherodactylus* sp. 1 for further analyses (see below). (2) Peruvian specimens reported by Padial & De la Riva (2005a) as *E. cf. peruvianus* (except KU 154863–5) are morphologically distinguishable from *E. peruvianus* and all other species taxa of the *E. conspicillatus* Group, but remain cryptic in qualitative characters with respect to *E. danae*, a species of the *E. unistrigatus* Group. However, data on advertisement call support their distinction (see below). We will subsequently refer this taxon of uncertain status as to *Eleutherodactylus* sp. 2 for further analysis. (3) Specimens identified by Padial *et al.* (2000) as *E.* cf. *peruvianus* from the Andean slopes of Department La Paz are *E. fenestratus*. (4) The diagnosis and redescription provided by Köhler (2000a) for *E. peruvianus* matches with qualitative characters of many Colombian, Ecuadorian and northern Peruvian populations assigned to this species. However, the taxonomic status of *E. peruvianus* remains uncertain because Lynch's (1980) proposed characters to separate it from *E. conspicillatus* seem invalid due to variability. Furthermore, this variability renders the diagnoses of some recently described Peruvian species of this group quite inconsistent (Table 3). However, the resolution of these problems lies outside the scope of this paper and will be treated elsewhere.

MORPHOMETRICS

Stepwise discriminant analyses comparing morphometric characters of adult females of *Eleutherodactylus* sp. 1 (n=5), *E. dundeei* (n= 4), *E. fenestratus* (n=44) and *E. samaipatae* (n=9) resulted in all variables being significant. There were only six missclassifications (9%), one between *E. dundeei*-*E. fenestratus*, four between *E. fenestratus*-*E. samaipatae* and one between *Eleutherodactylus* sp. 1-*E.fenestratus* (). Comparison between adult males of *Eleutherodactylus* sp. 1 (n=10), *E. dundeei* (n= 3), *E. fenestratus* (n=44) and *E. samaipatae* (n=20) resulted in most variables being significant ($p<0.0001$). FL (F=30.6), HW (F=29.6), SVL and EE (F=26.4), TH (F=26.0), TL (F=24.9) and HL (F=24.8) where the most significant. The model including all variables

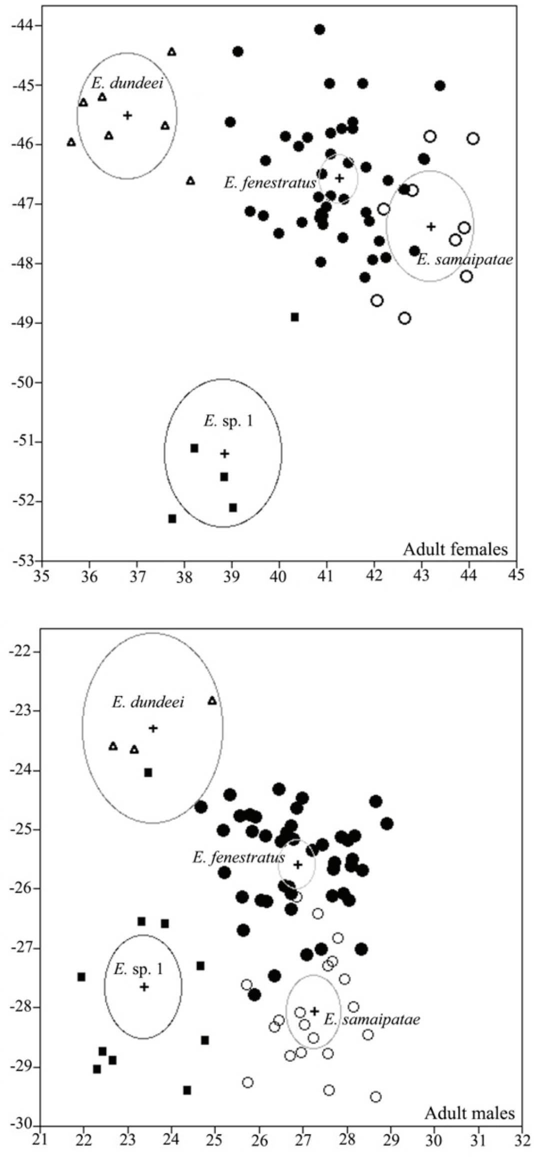

Figure 1. Multivariate discriminant analysis of morphometric characters of adult females (above) and adult males (below) of *Eleutherodactylus* sp. 1, *E. dundeei*, *E. fenestratus* and *E. samaipatae*. Ellipses represent 95 % confidence intervals.

distinguished four roughly defined groups corresponding with the described species taxa plus *Eleutherodactylus* sp. 1. There were eight misclassifications (10.4 %), one between *E. dundeei*-*Eleutherodactylus* sp. 1, six between *E. fenestratus*-*E. samaipatae*, and one between *E. dundeei*-*E. fenestratus* (Fig. 1). In the stepwise variable selection analyses, we selected consecutively all variables significant with a probability limit of 0.01, and the model applied (FL, FA, F3, TYH) led to 14 misclassifications (18%) because the misclassifications between *E. fenestratus*-*E. samaipatae* increased from 6 to 11 and there was an additional misclassification for the pair *E. dundeei*-*Eleutherodactylus* sp. 1. For comparison between adult males of *E. dundeei* and *Eleutherodactylus* sp. 1 alone, we removed some variables (FE, F4, FA, TH, T4) that were not available for two of the type specimens of *E. dundeei*. The only variable with some significance (p<0.05) was SVL (F=6.0); when selected, IND became significant (F=10.5, p<0.01). This model resulted in two missclassifications (13%).

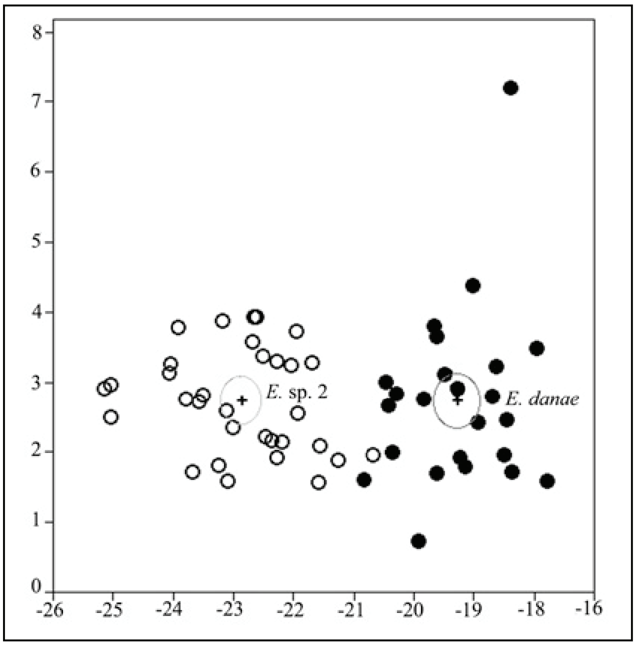

Figure 2. Multivariate discriminant analysis of morphometric characters of adult males of *Eleutherodactylus* sp. 2 and *E. danae*. Ellipses represent 95 % confidence intervals.

Stepwise discriminant analyses for morphometric characters of 24 adult males of *E. danae* and 32 of *Eleutherodactylus* sp. 2, led to EL (F=33.6, P<0.0001) as the only one significant variable. When selected, HW also became significant (F=55.1, p<0.0001). This model led to only two misclassifications, both corresponding to specimens assigned to *Eleutherodactylus* sp. 2 (Fig. 2). These are MNK-A 4743 from Serranía de Chepite (79% probability for *E. danae*) and MNK-A 3705 from Serranía Beu (62% probability for *E. danae*), both in the Andean slopes of Pilón-Lajas Biosphere Reserve. Adult female morphometrics were not compared, since there were only two samples of *E. danae*.

Table 3. Comparison of diagnostic characters between Cis-Andean Amazonian species of the *Eleutherodactylus conspicillatus* Group plus *E. danae* and *E. cosnipatae*: (1) relative length of first and second fingers (Finger I>II, Finger I≥II, Finger I=II, Finger I<II); (2) belly skin texture (smooth, slightly, granular, granular posterolaterally, granular, coarsely granular); (3) dorsal skin texture (smooth, shagreen, coarsely shagreen, granular); (4) dorsal tubercles (presence/absence of dorsal tubercles or short folds); (5) dorsolateral folds (present, absent); (6) finger fringes (prominent, weak, absent); (7) toe fringes (prominent, weak, absent); (8) basal toe webbing (present, absent); (9) tarsal fold (present, absent); (10) labial and subocular vertical bars (present, absent); (11) color pattern of posterior surfaces of thighs (well defined spots, barely visible or diffuse spots, marmored, plain); (12) color pattern of throat, chest and belly (heavily spotted, weakly spotted, immaculate); (13) adult male nuptial pad on thumb (double, single, absent).

	Diagnostic characters states												
	1	2	3	4	5	6	7	8	9	10	11	12	13
E. avicuporum	I>II	granular	shagreen	fin-shaped, interocular	present	weak	absent	present	present	present	barely visible or diffuse spots	weakly spotted	single
E. bipunctatus	I=II	granular posterolaterally	coarsely shagreen	Absent	present	absent	prominent	present	present	present	well defined spots	marmored	-
E. buccinator	I=II	smooth	coarsely shagreen	x-shaped, interocular	present	absent	weak	absent	present	present	barely visible or diffuse spots	weakly spotted, immaculate	single
E. caliginosus	I>II	smooth	shagreen	Absent	present	weak	-	present	-	present	barely visible or diffuse spots	weakly spotted	-
E. citriogaster	I>II	smooth	shagreen	Absent	absent	absent	prominent	absent	present	present	marmored	heavily spotted	single
E. condor	I>II	smooth	shagreen	Absent	present	weak	weak	present	absent	present	well defined spots	heavily spotted	-
E. conspicillatus	I>II	smooth	finelly shagreen	Absent	present	weak	weak	absent	absent	variable	well defined spots	well defined spots	single
E. cosnipatae	II>I	coarsely granular	finelly shagreen	Warts	present	absent	weak	absent	present	present	plain	weakly spotted	-
E. crepitans	I>II	smooth	warty	absent	absent	absent	absent	absent	present	present	plain	inmaculate	single
E. danae	I<II	coarsely granular	finelly shagreen	absent	absent	present	prominent	absent	present	variable	well defined spots	weakly spotted	absent
E. dundeei	I>II	granular	shagreen	flat warts	absent	absent	prominent	present	present	present	plain	weakly spotted	Double
E. fenestratus	I>II	smooth, granular	shagreen	absent	absent	weak	weak	present	present	present	plain	variable	Double

E. lanthanites	I≥II	posterolaterally smooth, granular posterolaterally	shagreen	fin shaped, interocular, calcar	absent	absent	weak	absent	present	present	plain	heavily spotted	absent
E. malkini	I>II	smooth	Finelly shagree	warts	absent	absent	prominent	present	present	present	marmored	inmaculate	single
E. peruvianus	I>II	smooth	finelly shagreen	absent	present	absent	absent	present	present	variable	well defined spots	variable	single
E. samaipatae	I>II	smooth	finelly shagreen	absent	absent	absent	prominent	absent	present	present	plain	inmaculate	double
E. skydmainos	I≤II	granular posterolaterally	finelly shagreen	fin-shaped, interocular	present	weak	weak	present	present	present	plain	barely visible or diffuse spots	single
E. vilarsi	I>II	smooth	coarsely shagreen	absent	absent	absent	absent	present	present	variable	plain	weakly spotted	single
E. sp. 1	I>II	granular posterolaterally	finelly sahgreen	absent	absent	absent	weak	absent	present	present	plain	plain	double
E. sp. 2	I≤II	coarsely granular	finelly shagreen	absent	absent	present	prominent	absent	present	absent	well defined spots	weakly spotted	single

BIOACOUSTICS

The calls of *E. fenestratus*, *E. samaipatae*, *E. dundeei* and *Elutherodactylus* sp. 1 are all very similar in general structure (Fig. 3). These calls are composed of pulsed notes with amplitude modulation and harmonic structure. They differ in the number and rate of notes emitted and in the length and number of pulses of the notes (Table 1). *E.*

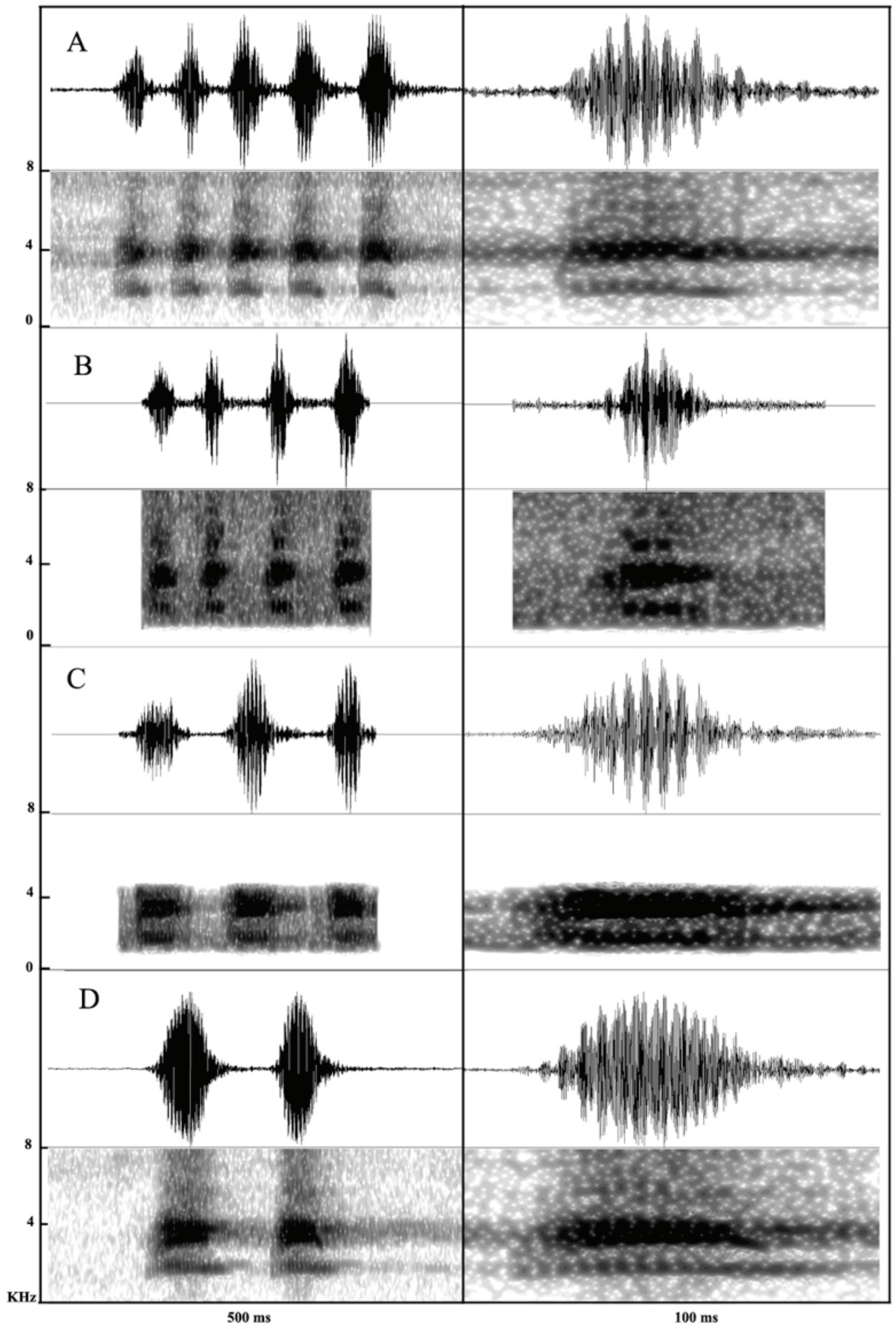

Figure 3. Oscillograms and audiospectrograms of the advertisement call of *Eleutherodactylus* sp. 1 (A), *E. dundeei* (B), *E. fenestratus* (C) and *E. samaipatae* (D).

samaipatae is the species with lower number of notes per call, generally only one. *E. fenestratus* tends to emit 2–3 notes, while the number of notes emitted by *E. dundeei* and *Eleutherodactylus* sp. 1 is usually higher than five. Multivariate discriminant analyses of quantitative parameters of the calls unambiguously separate *E. dundeei*, *E. fenestratus*, *E. samaipatae*, and *Eleutherodactylus* sp. 1 (Fig. 4A and 4B). All variables were significant (p<0.001), in decreasing order of signification: note length, number of pulses, fundamental frequency and dominant frequency. The most divergent call was that of *E. dundeei*, while the calls of *E. samaipatae* and *E. fenestratus* resulted more similar.

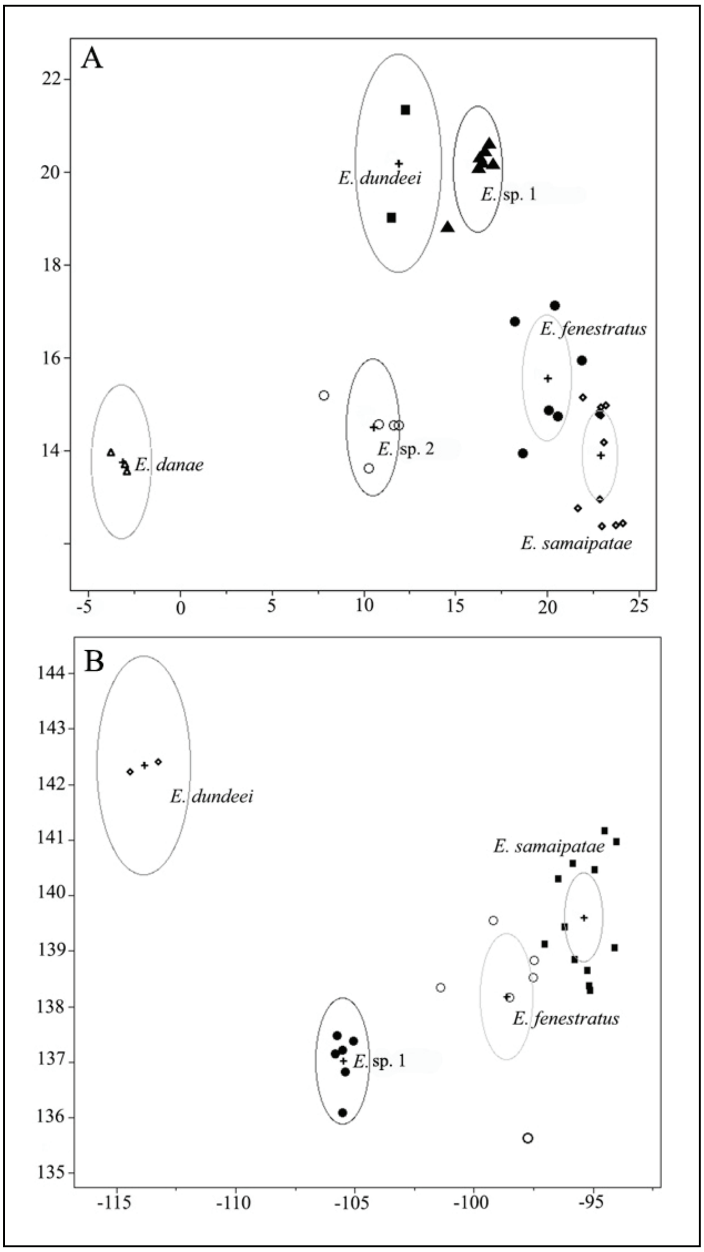

Figure 4. Multivariate discriminant analysis of quantitative characters of advertisement calls. A) Members of the *E. conspicilltus* Group: *Eleutherodactylus* sp. 1, *E. dundeei*, *E. fenestratus* and *E. samaipatae*, plus members of the *E. unistrigatus* Group: *Eleutherodactylus* sp. 2 and *E. danae*. B) Members of the *E. conspicilltus* Group only. Note how the distance between *E. dundeei* and *Eleutherodactylus* sp. 1 increases when *E. danae* and *Eleutherodactylus* sp. 2 are removed from the analysis. Ellipses represent 95 % confidence intervals.

Qualitative (Fig. 5) and quantitative (Table 1, Fig. 4B) call characters also allow the distinction of *E. danae* and *Eleutherodactylus* sp. 2. The call of *E. danae* consists in very short non-pulsed notes while the call of *Eleutherodactylus* sp. 2 is composed by 2–3 highly pulsed amplitude-modulated notes. The first pulsed note of the call of *Eleutherodactylus* sp. 2 is generally shorter, while the second may show some modulation in frequency. Multivariate discriminant analysis of quantitative parameters (note lenght, number of pulses and dominant frequency) resulted in all variables being statistically significant ($p<0.001$) and in no misclassification. The most significant variables are call length and number of pulses.

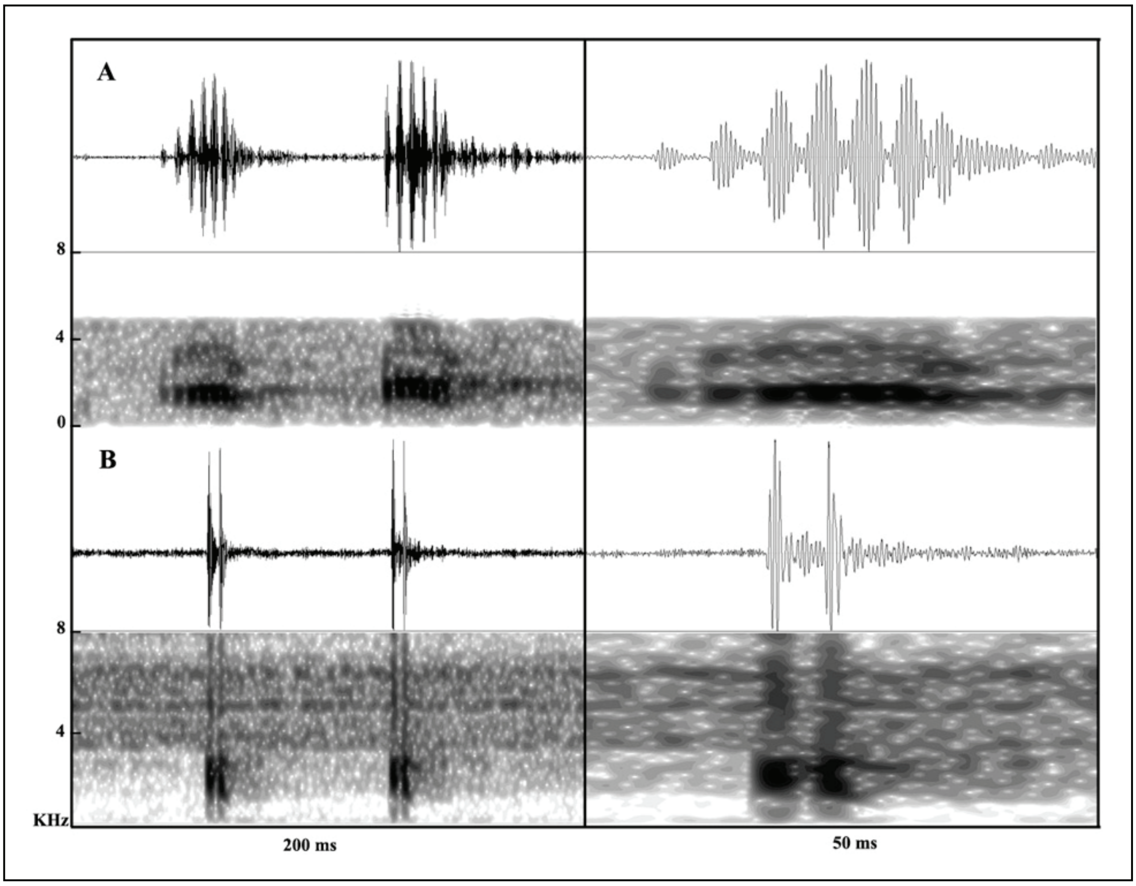

Figure 5. Oscillograms and audiospectrograms of the advertisement call of *Eleutherodactylus* sp. 2 (A) and *E. danae* (B).

ANALYSES OF MTDNA

Maximum Parsimony (MP) and Neighbor Joining (NJ) majority rule-consensus boostrap analyses highly support the monophyly of two main clades (Fig. 6A). The most basal (bss. 100 for NJ and MP) includes two members of the *E. conspicillatus* Group (*E. fenestratus* and *E. samaipatae*) plus *Eleutherodactylus* sp. 1. The monophyly of the three taxa is highly supported (MP: 100, 98, 99 bss. and, NJ: 100, 100 and 93, bss., for *E. samaipatae, E. fenestratus* and *Eleutherodactylus* sp. 1 respectively).

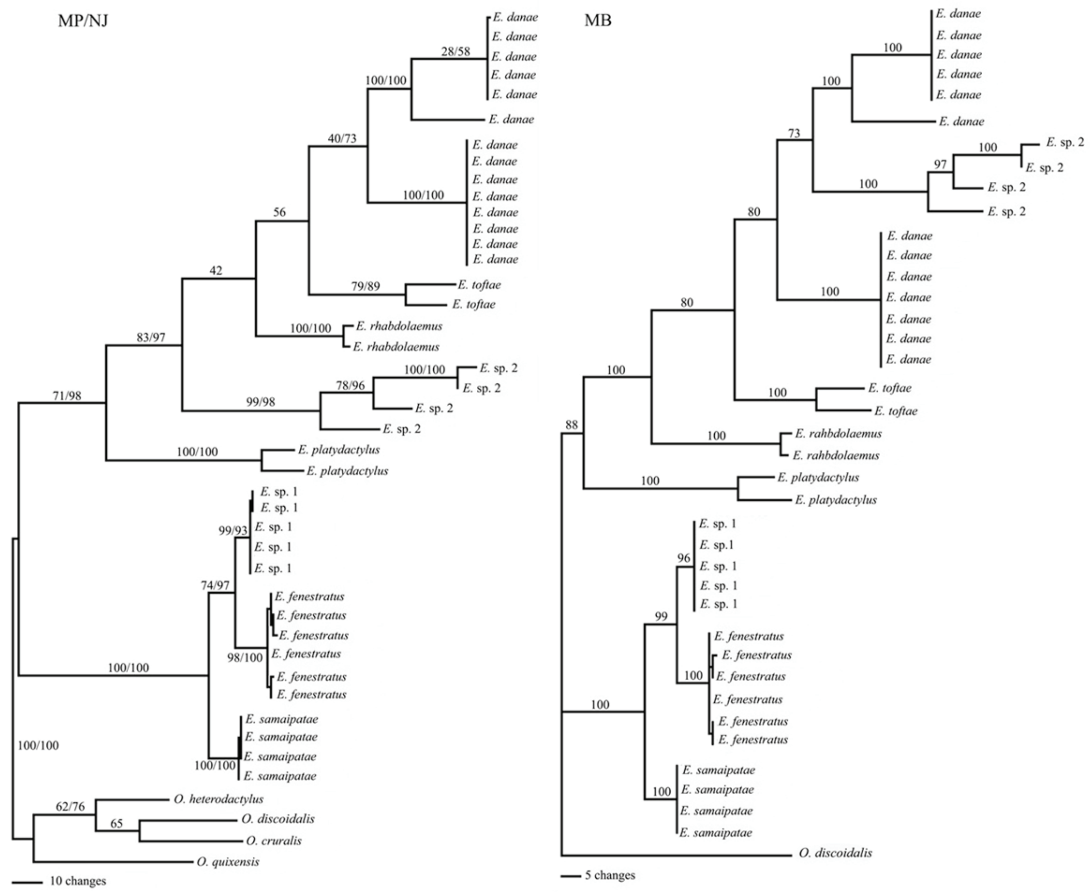

Figure 6. Majority rule concensus trees based on Maximum Parsimony (MP) and Bayesian (MB) phylogenetic analyses of partial 16S (ca. 590 bp) mtDNA of target *Eleutherodactylus* selected for this study. Four members of the genus *Oreobates* conform the outgroup. Single values on the MP tree (left) correspond to MP boostrap values. When the MP topology coincides with Neighbor Joining (NJ) topology (not illustrated) two values are shown, the second representing NJ boostrap values. Values in the MB tree are bayesian posterior probabilities. For further discussion of differences between analyses see text.

Eleutherodactylus sp. 1 and *E. fenestratus* form a well-supported clade (74 and 97 bss. for MP and NJ respectively) nested with *E. samaipatae*.

The other main clade (71 and 98 bss. for MP and NJ respectively) includes members of the *E. unistrigatus* Group. The monophyly of *E. platydactylus*, *E. rhabdolaemus*, *E. toftae* and *Eleutherodactylus* sp. 2 is highly supported (MP: 100, 100, 79, 99; NJ: 100, 100, 89, 98 bss., respectively), while the support for the clade containing *E. danae* is low to moderate (bss. 40 and 73 for MP and NJ respectively). *Eleutherodactylus* sp. 2 and *E. danae* appear as sister taxa in the NJ tree, but with very low support (bss. 26), while in the MP tree *E. toftae* is the sister taxa to *E. danae* (bss. 56) and *Eleutherodactylus* sp. 2 is nested with *E. rhabdolaemus* (bss. 83).

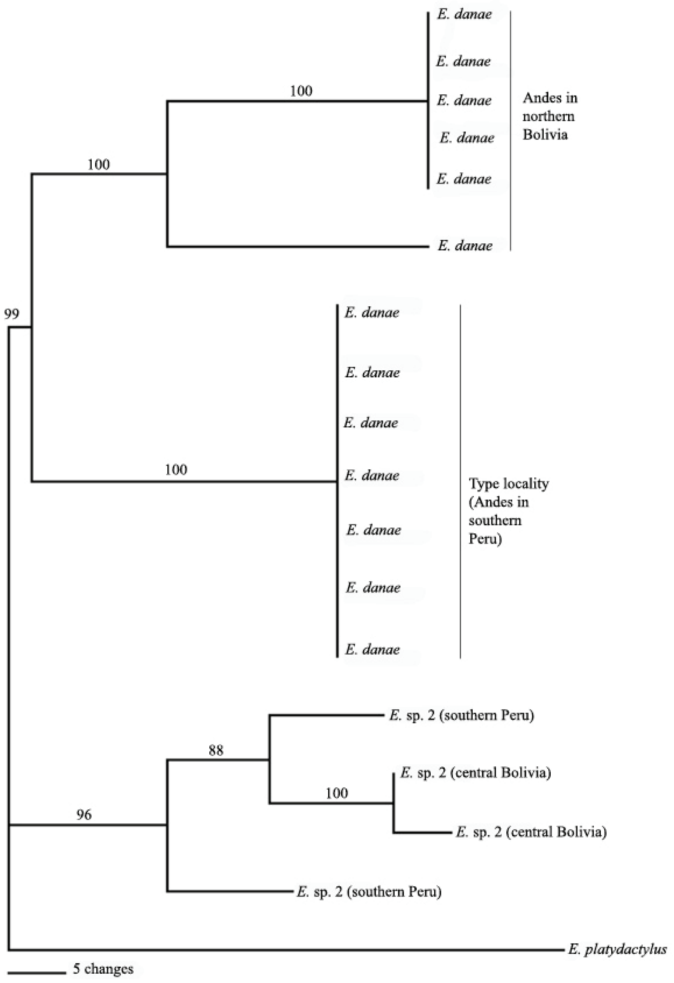

Figure 7. Majority rule concensus tree based on Bayesian (MB) phylogenetic analyses of partial 16S (ca. 590 bp) mtDNA of *Eleutherodactylus* sp. 2 and *E. danae* using *E. platydactylus* as outgroup.

The Bayesian phylogenetic analyses (MB) differ to some extent from MP and NJ (Fig. 6). They coincide in the topology and support for the monophyly of the clade (bpp. 100) containing *E. fenestratus*, *E. samaipatae* and *Eleutherodactylus* sp. 1., and for the monophyly of these three species (bpp. 100, 100, 99 respectively). The clade containing species members of the *E. unistrigatus* Group is moderately to scarcely supported (bpp. 88). However, the monophyly of *E. platydactylus*, *E. rhabdolaemus*, and *E. toftae* is well supported (bpp. 100). The largest discordance between MP and NJ in reference to MB is the placement of *Eleutherodactylus* sp. 2. Contrarily to NJ and MP, this taxon is placed in a clade together with Bolivian *E. danae*, and specimens of *E. danae* from the type locality are the sister group to this clade. The support is high (bpp. 100) for the three different clades containing Bolivian *E. danae*, *E. danae* from the type locality and *Eleutherodactylus* sp. 2. Nonetheless, the support is low for the clades containing Bolivian *E. danae*-*Eleutherodactylus* sp. 2, and these two taxa plus *E. danae* (bpp. 73 and 80 respectively). Given these ambiguous results, we performed a new Bayesian phylogenetic analyses (two million generations) including only *E.* sp. 2 and *E. danae* as ingroup taxa and *E. platydactylus* as otgroup (as derived from Fig. 6). The resulted consensus Bayesian tree (Fig. 7) fully supports the monophyly of *E. danae* (bpp. 99) and of *Eleutherodactylus* sp. 2 (bpp. 96).

Range, mean and standard deviation of uncorrected pairwise distances between *Eleutherodactylus* sp. 1-*E. fenestratus*, *Eleutherodactylus* sp. 1-*E. samaipatae* and *E. fenestratus*-*E. samaipatae* were 2.9–3.3(3.0±0.2), 2.9–4.7 (4.5±0.5) and 5.5–6.2 (5.8±0.2) respectively. Higher genetic distances are shown between the pair *E. danae*-*Eleutherodactylus* sp. 2: 8.9–10.8 (9.7±0.6).

In summary, morphometric, bioacoustics, and phylogenetic analyses of partial mtDNA 16S gene support the independence of *Eleutherodactylus* sp. 1 and *Eleutherodactylus* sp. 2 from those species to which they resemble in qualitative characters. Since three lines of evidence support the specific status of *Eleutherodactylus* sp. 1 and *Eleutherodactylus* sp. 2, they are described respectively as two new species taxa below.

TAXONOMY

ELEUTHERODACTYLUS SP. NOV. 1 new species

(Fig. 8A)

Holotype: MNK-A 6626 (field number JMP 033), an adult male from Km 6 of Angostura-Samaipata road, Departamento Santa Cruz, Bolivia (18º11'S, 63º34'W), collected by José M. Padial on 03 January 2003.

Paratypes: Bolivia, Departamento Santa Cruz: MNCN 42990–1, MNK-A 6627 (adult males, field numbers JMP 031–3), same data as the holotype; MNK-A 7170 (adult male, field number JMP 442), 7172 and 7174 (adult males, field numbers JMP 449 and 451), MNCN 43054 (adult male, field number JMP 448) from Espejillos (17º50'S/63º25'O), collected by José M. Padial and Enrique Ávila on 26 November 2003; MNCN 42983, 42985–6, 43014 (subadult females, field numbers JMP 152, 173, 184, and 153 respectively) from La Chonta, Amboró National Park (17º39'36"S, 63º42'6.6"W) collected by José M. Padial and Rafael de Sá on 21–22 April 2003, and MNCN 43040 (adult male, field number JMP 377) collected at the same locality by José M. Padial & E. Ávila on 05 November 2003; ZFMK 80005–6 (adult males), ZFMK 80007 (adult female) from Macuñucú, Amboró National Park collected by J. Köhler and S. Lötters on 2 December 1998; ZFMK 79991 and 79993 (adult females) and 7992 (juvenile) from Mataracú, Amboró National Park, collected by J. Köhler and S. Lötters on 14 January 1999.

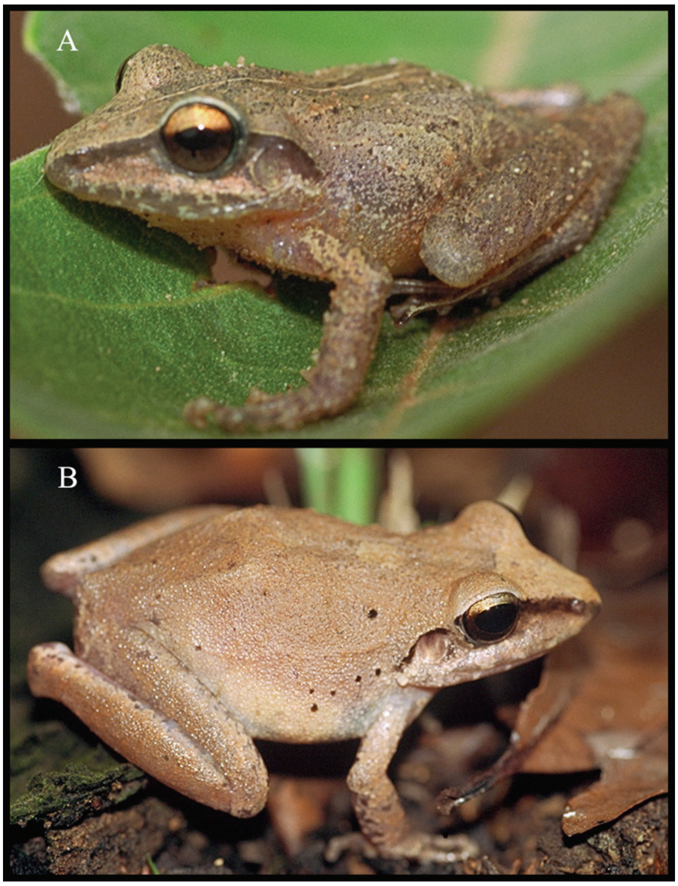

Figure 8. (A) Adult male of *Eleutherodactylus* sp. *nov.* 1 from Km 6 of Angostura-Samaipata road, Departamento Santa Cruz, Bolivia (one from the type series MNK-A 6626–7, MNCN 42990–1) , and (B) adult female of *Eleutherodactylus fenestratus* from Chalalán, Departamento La Paz, Bolivia (one from the series MNCN 43239–44).

Referred specimens: BOLIVIA: Departamento Santa Cruz: Espejillos, MNK-A 6447; Km 29 on Santa Cruz de la Sierra–Samaipata road, MNK-A 1000; Río Saguayo, Amboró National Park, MNK-A 189, 191, 224, 358, 361, 364–5, 374; Río Surutú, Amboró National Park, MNK-A 1197;Santa Cruz de la Sierra,BM 1904.10.29.83–101 (general locality, the origin of these specimens is likely to be in the Andean slopes close to Santa Cruz de la Sierra).

Diagnosis: A member of the *Eleutherodactylus conspicillatus* Group, as defined by Lynch & Duellman (1997), characterized by: (1) skin on dorsum coarsely shagreen, flanks with larger granules; venter finelly granular, smooth only in the middle; posterior surfaces of limbs smooth; discoidal fold conspicuous; dorsolateral folds absent; postrictal glands present; (2) tympanic membrane and annulus round, large, their length about half eye length; supratympanic fold short, very prominent; (3) head slightly longer than wide; snout acuminate in dorsal view, subacuminate in lateral view; canthus rostralis straight in dorsal view, sharp in profile; (4) cranial crests absent; upper eyelid covered by small granules; (5) vomerine odontophores large, situated posteromedial to choanae; (6) males with vocal slits and two nuptial pads on thumb; (7) hands with long and slender fingers, first finger longer than second; subarticular tubercles subconical, prominent; supernumerary round, smaller than subarticular tubercles; terminal discs of inner two fingers round, those of external fingers enlarged, ovate to truncate; circumferential grooves conspicuous, ungual flap not indented; lateral fringes and keels on fingers absent; (8) ulnar tubercles present; (9) tubercles on heel and tarsus absent, tarsal fold prominent; (10) inner metatarsal tubercle ovate, prominent, outer subconical, prominent; single, round supernumerary tubercle on Toe IV; (11) toes long and slender (foot length 50% SVL); lateral fringes or keels weak, basal toe webbing absent; fifth and third toes reaching midpoint of penultimate subarticular tubercle of Toe IV; tips of toes rounded to ovate, enlarged, ungual flap not indented; (12) dorsal coloration grey to brown with variable pattern of dark spots and flecks, ventral coloration white with fine mottling; posterior surface of thighs brown without light spots; (13) mandibular ramus of the trigeminal nerve passing lateral to the *m. adductor mandibulae externus* (S condition sensu Lynch, 1986).

This species is very similar to *E. fenestratus* (Fig. 8B) and *E. samaipatae* in qualitative characters (Table 3). For differences of *E. sp. nov. 1* and other similar members of the group see Tables 3 and 5 and results of morphological, bioacustical and molecular analyses above.

Table 4. Morphometrics of adult specimens of *Eleutherodactylus dundeei*, *E. fenestratus*, *E. sp. nov. 1* and *E. samaipatae*. Means ± standard deviation in parentheses follows ranges (in mm).

	Adult females				Adult males			
	E. sp. nov. 1 (n=5)	*E. dundeei* (n=4)	*E. fenestratus* (n=44)	*E. samaipatae* (n=9)	*E. sp. nov. 1* (n=10)	*E. dundeei* (n=3)	*E. fenestratus* (n=44)	*E. samaipatae* (n=20)
SVL	34.0–39.5 (36.9±2.2)	33.3–43.5 (36.2±3.7)	37.8–57.2 (43.7±4.6)	44.4–51.4 (49.1±2.2)	23.8–29.4 (27.0±1.7)	21.5–27.3 (24.2±2.0)	26.0–34.7 (30.5±2.1)	30.1–40.0 (32.8±2.4)
HL	13.1–14.5 (13.8±0.7)	12.9–18.1 (14.3±1.9)	15.0–22.8 (17.5±2.0)	17.4–20.8 (19.4±1.2)	9.6–12.0 (10.6±0.8)	9.4–11.5 (10.4±0.8)	10.8–14.2 (12.4±0.9)	11.9–15.6 (13.1±0.9)
HW	11.7–13.3 (12.6±0.9)	11.7–14.7 (12.6±1.1)	13.0–21.8 (15.8±2.0)	16.9–19.6 (18.5±0.9)	8.8–10.3 (9.4±0.5)	8.3–9.1 (8.8±0.6)	9.0–13.0 (11.1±0.8)	10.6–14.1 (11.6±0.8)
EL	4.0–4.6 (4.3±0.3)	3.8–5.3 (4.3±0.5)	4.4–7.4 (5.4±0.7)	5.3–6.5 (5.9±0.5)	3.1–4.3 (3.6±0.4)	3.3–3.7 (3.5±0.2)	3.5–4.6 (4.0±0.3)	3.9–5.1 (4.4±0.3)
EN	4.2–5.0 (4.7±0.4)	4.5–6.2 (4.9±0.7)	4.8–7.7 (5.8±0.7)	5.6–6.9 (6.3±0.5)	3.0–4.2 (3.6±0.4)	2.9–3.7 (3.2±0.3)	3.2–4.9 (4.0±0.4)	3.7–5.1 (4.3±0.3)
IND	2.7–3.2 (2.9±0.2)	2.9–3.9 (3.2±0.4)	2.9–4.9 (3.7±0.5)	3.5–4.7 (4.0±0.4)	2.0–2.7 (2.3±0.2)	2–2.8 (2.3±0.3)	2.1–3.1 (2.6±0.2)	2.2–3.3 (2.7±0.3)
EE	6.3–6.4 (6.4±0.1)	6.2–7.7 (6.6±0.6)	6.4–10.4 (7.9±0.8)	8.0–9.4 (8.7±0.4)	4.0–5.4 (4.8±0.4)	4.4–5.1 (4.7±0.3)	5.0–6.6 (5.8±0.4)	5.2–7.1 (6.0±0.4)
TYH	2.3–2.8 (2.5±0.2)	2.6–3.6 (2.9±0.4)	2.6–4.7 (3.1±0.4)	2.8–3.6 (3.3±0.3)	1.8–2.5 (2.2±0.2)	1.8–2.2 (2.0±0.2)	1.7–2.7 (2.2±0.2)	1.8–2.8 (2.3±0.2)
TYL	1.9–2.3 (2.1±0.2)	2.6–3.1 (2.8±0.2)	2.3–4.2 (2.8±0.4)	2.7–3.4 (3.1±0.2)	1.5–2.2 (1.9±0.2)	1.8–2.2 (2.0±0.2)	1.7–2.7 (2.0±0.2)	1.6–2.5 (2.1±0.2)
F3	1.3–1.4 (1.4±0.1)	1.1–15.0 (1.3±0.2)	1.2–2.6 (1.8±0.3)	1.4–2.5 (2.0±0.3)	0.9–1.4 (1.1±0.1)	0.8–1.1 (0.9±0.1)	0.9–1.7 (1.3±0.2)	1.0–1.9 (1.5±0.2)
F4	1.2–1.4 (1.3±0.1)	1.0–1.4 (1.2±0.1)	1.1–2.6 (1.7±0.3)	1.7–2.5 (2.0±0.3)	0.9–1.2 (1.0±0.1)	0.9–1.1 (1.0±0.1)	1.0–1.7 (1.3±0.2)	0.9–2.0 (1.5±0.2)
FA	6.9–7.8 (7.3±0.4)	6.5–8.4 (7.6±0.7)	7.2–11.6 (8.9±1.1)	8.7–10.0 (9.4±0.5)	4.6–5.5 (5.1±0.3)	5.4–6.6 (5.8±0.7)	5.3–7.8 (6.3±0.6)	4.9–7.2 (6.1±0.6)
TL	20.4–22.0 (21.2±0.8)	18.9–22.3 (20.1±1.2)	20.6–32.0 (25.4±2.5)	27.5–31.1 (28.9±1.3)	13.8–19.9 (15.6±1.8)	13.0–16.8 (14.1±1.4)	15.2–20.8 (17.6±1.2)	18.0–21.1 (19.1±0.8)
TH	15.7–17.3 (16.5±0.8)	16.8–20.3 (18.0±1.3)	19.2–28.0 (23.0±2.3)	24.5–28.3 (26.3±1.2)	12.5–15.8 (13.6±1.0)	12.7–14.7 (13.4±1.2)	13.5–18.2 (15.8±1.2)	154–19.9 (17.0±1.0)
FL	18.1–18.9 (18.5±0.4)	16.6–19.1 (17.7±0.9)	17.8–29.0 (22.4±2.6)	24.5–27.8 (26.1±1.4)	11.9–15.2 (13.4±0.9)	11.0–14.9 (12.4±1.4)	13.5–18.8 (15.7±1.2)	15.6–19.5 (16.9±0.9)
T4	1.3–1.6 (1.4±0.2)	0.8–1.5 (1.2±0.2)	1.2–2.7 (1.6±0.3)	1.41–2.1 (1.7±0.2)	1.0–1.4 (1.2±0.1)	0.8–1.0 (0.9±0.1)	0.9–1.7 (1.2±0.2)	0.9–1.7 (1.4±0.2)
HL/HW	1.1–1.1 (1.1±0.0)	1.1–1.2 (1.1±0.1)	1.0–1.2 (1.1±0.0)	1.0–1.1 (1.0±0.0)	1.1–1.3 (1.1±0.1)	1.1–1.3 (1.2±0.0)	1.0–1.2 (1.1±0.0)	1.0–1.2 (1.1±0.0)
TL/SVL	0.5–0.6 (0.6±0.0)	0.5–0.6 (0.6±0.0)	0.5–0.6 (0.6±0.0)	0.5–0.6 (0.6±0.0)	0.5–0.7 (0.6±0.0)	0.5–0.6 (0.6±0.0)	0.5–0.6 (0.6±0.0)	0.5–0.6 (0.6±0.0)
FL/SVL	0.5–0.5 (0.5±0.0)	0.4–0.5 (0.5±0.0)	0.5–0.6 (0.5±0.0)	0.5–0.6 (0.5±0.0)	0.5–0.5 (0.5±0.0)	0.5–0.5 (0.5±0.0)	0.4–0.6 (0.5±0.0)	0.5–0.5 (0.5±0.0)

Description of the holotype: Head longer than wide (head length/head width =1.2); snout acuminate in dorsal view and subacuminate lateral profile; nostrils slightly protuberant, oriented posterolaterally; canthus rostralis straight in dorsal view, sharp in frontal profile; loreal region flat; lips not flared; upper eyelid without tubercles but covered by small granules; no cranial crests. Supratympanic fold prominent, short; tympanic membrane and annulus distinct; tympanic membrane nearly round, its length about half of eye length; postrictal glands conical, conspicuous. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, ovate, separated by distance equal to five times diameter of a choana; vomerine odontophores large, prominent, round in shape, situated posteromedial to choanae, separated by a distance equal than the length of a vomerine odontophore, bearing 4–5 vomerine teeth; vocal sac subgular, vocal slits placed posterolaterally. Skin of dorsal surfaces and posterior parts of hind limbs coarsely shagreen; throat smooth, belly granular, only smooth in the middle; occipital folds absent; dorsolateral folds absent; discoidal fold conspicuous.

Arm with a row of low, round ulnar tubercles; palmar tubercle bifid, flat, equal to elongate, prominent, thenar tubercle; a single supernumerary tubercle on the basis of each finger, low, round, smaller than subarticular; subarticular tubercles prominent, subconical; finger tips small and round on fingers I and II, and large, ovate to truncate on fingers III and IV; fingers lacking lateral fringes; relative length of fingers III>I>II≥IV; a double white glandular, non-spinous nuptial pad on dorsal surface of each thumb.

Toes long and slender (foot length 50% of SVL); heel and tarsus lacking tubercles; tarsal fold prominent, almost in contact with inner metatarsal tubercle and larger than it; inner metatarsal tubercle ovate, prominent, larger than outer; metatarsal tubercle round, subconical; only a single inconspicuous supernumerary tubercle on Toe IV; subarticular tubercles conical, prominent; toes with weak lateral fringes; basal toe webbing absent; toe tips round, moderately developed; ungual flap not indented, circumferential grooves evident; relative length of toes IV>III>V>II>I; toes III and V reaching midpoint of penultimate subarticular tubercle of Toe IV.

Measurements (in mm) of the holotype: SVL 26.6, HL 10.7, HW 9.30, EL 4.0, EN 3.7, IND 2.4, EE 4.81, TYH 2.0, TYL 2.0, FIII 1.1, FIV 1.1, FA 4.9, TL 15.1, TH 12.5, FL 13.2, TIV 1.2.

Colour: In preservative, dorsal surfaces light greyish-brown with diffuse and inconspicuous light brown dorsal chevrons; a pair of bold black occipital spots; flanks light greyish-brown with some fine dark mottling; canthus rostralis dark brown; dorsal and loreal regions of snout grey; a fine bold brown interocular stripe; inconspicuous labial bars dark brown and white; subocular stripes absent; tympanic membrane brown, annulus cream; tympanic fold bold black; hind-limbs and arms with transversal dark stripes; plantar surfaces brown; ventral surfaces white to cream with inconspicuous fine grayish-brown mottling; posterior and anterior surfaces of hind limbs brown without spots. The color pattern in life is very similar, but grayish surfaces tend to be light brown to cream. The ventral surfaces are white and the groin is yellowish-white. The iris is metallic orange with a transverse bold black stripe.

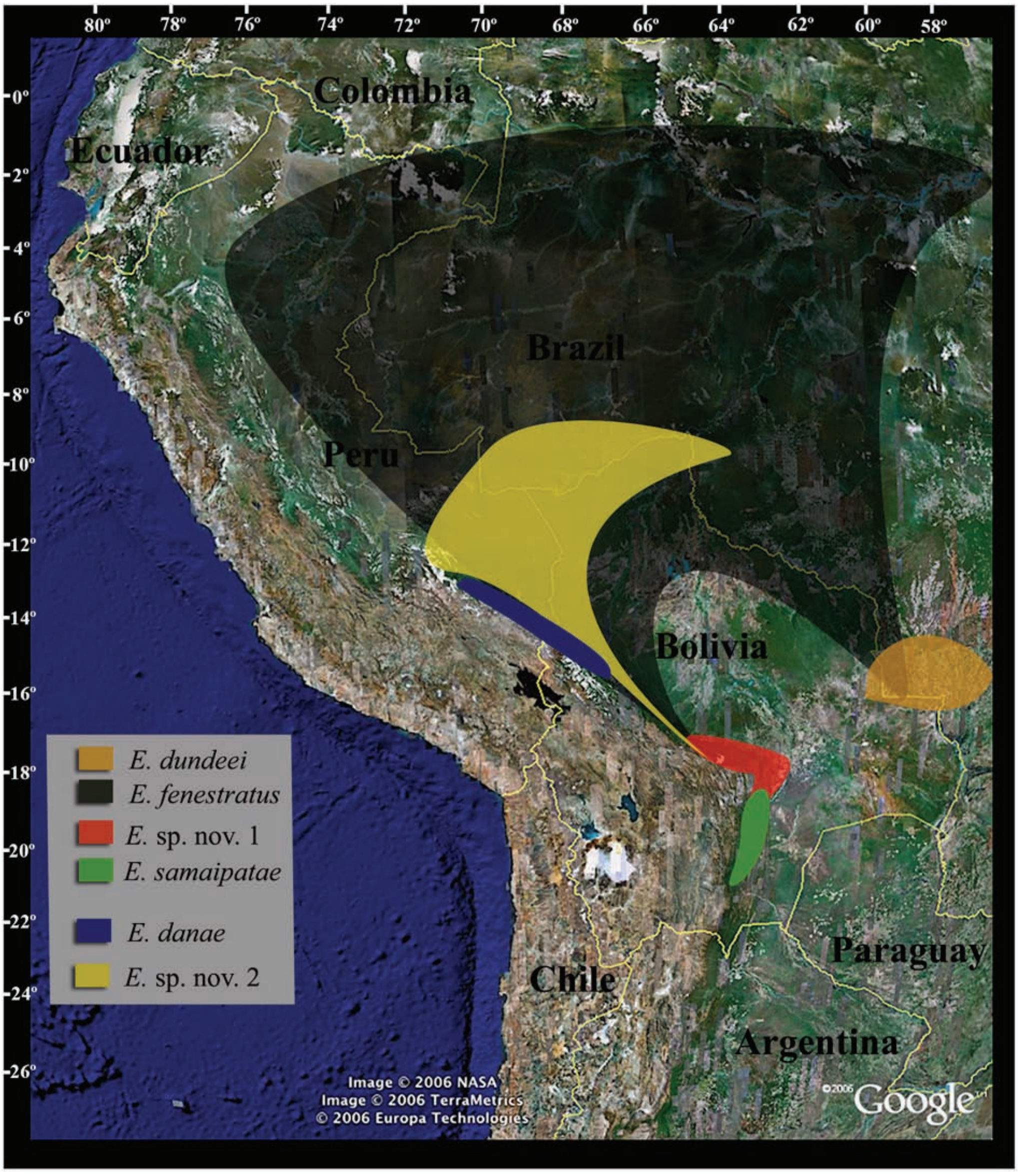

Figure 9. Partial map of South America despicting the approximate distribution of *Eleutherodactylus sp. nov. 1*, *E. sp. nov. 2*, *E. danae*, *E. dundeei*, *E. fenestratus*, and *E. samaipatae*.

Variation: Males and females are similar in all but sexual qualitative external characters. Females are larger than males but are equal in head and limbs proportions (Table 4). Dorsal pattern is quite constant, although some specimens, as MNCN 42986, 43054 or MNK-A 7170, 7172, present a fine mid-dorsal stripe from tip of snout to vent. Some dark dorsal marks, as an interocular stripe, W-shaped occipital mark, X-shaped mid-dorsal mark or sacral chevrons can be present. The tarsal fold can be poorly developed and rounded, as in MNCN 43054. For measurements, see Table 4.

Etymology: The name is a patronym for Jörn Köhler, German herpetologist and friend whose studies have greatly contributed to the understanding of Bolivian amphibian diversity.

Distribution: This is a Bolivian endemic species known from the interandean dry valleys of Departamento de Santa Cruz extending to the northwest along the humid forests of the Andean slopes to the Chapare region of Departmento Cochabamba (see Fig. 9). Köhler (2000a) cited this species as *E. dundeei* for Macuñucú and Mataracú, in the southern edge of Amboró National Park.

Natural History: This species is active by night during the rainy season. Males call from low vegetation of the forest. It has been found in both primary and secondary forest types.

Remarks: Köhler (2000a) described the advertisement call of this species (as *E. dundeei*). His data for the calls are similar to those provided by us, although they differ in that note length reported by Köhler (2000a) was longer. Reichle's (2002) recording for *E. cf. peruvianus* corresponds to *E. sp. nov. 1*. Specimens cited as *E. peruvianus* by De la Riva (1994) also correspond to *E. kohleri* as well as Bolivian specimens cited as *E.* cf. *peruvianus* by Padial & De la Riva (2005a). With the description of *E. sp. nov. 1*, *E. dundeei* does not longer occur in the in Bolivia, accordding to previous data. However, specimens from Noel Kepff Mercado National Park, Departamento Santa Cruz, Bolivia (see Appendix I) represent the first country record. Bolivian specimens coincide both in cualitative and quantitative morphology with the types of *E. dundeei*.

ELEUTHERODACTYLUS SP. NOV. 2 new species

(Fig. 10A)

Holotype: MNK-A 6620 (field number JMP 286), an adult female from Los Guácharos 500 m.a.s.l, Carrasco National Park, Provincia Chapare, Departamento Cochabamba, Bolivia (17º03'51.5"S/65º28'34.7"W) collected by José M. Padial and Dirk Embert on 9 July 2003.

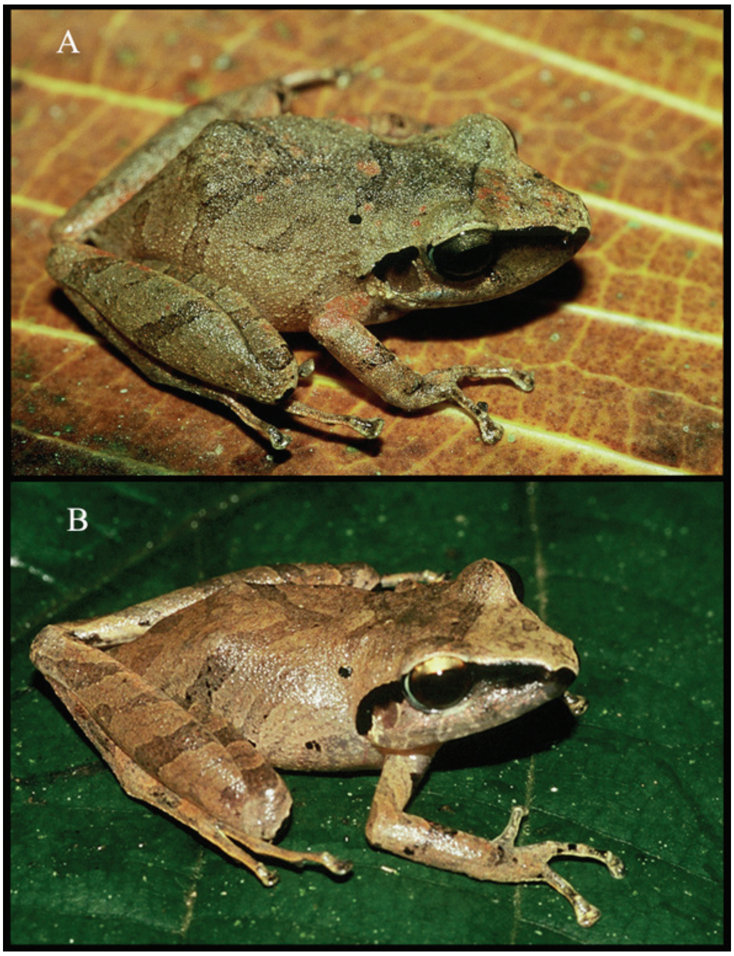

Figure 10. (A) Adult male of *Eleutherodactylus sp. nov. 2* from Chalalán, Departamento La Paz, Bolivia (MNK-A 7178), and (B) adult male of *E. danae* from Huairuro, Departamento La Paz, Bolivia (one from the series MNCN 43054–64, 43067–8).

Paratypes. BOLIVIA: Departamento Cochabamba: ZFMK 72587–9, 72564–5, 72537 from a point between Paractito and El Palmar, Carrasco National Park, collected by J. Köhler and S. Lötters on 16–18 December 1998; ZFMK 66973–6, 66988, from a point between Parajti and El Palmar, Carrasco National Park, collected by J. Köhler and S. Lötters on 3-6 February 1998; MNCN 43012 (adult female, field number JMP 295), 43024 (adult female, field number JMP 303), 43028 (adult male, field number JMP 313), MNK-A 6620 (adult female, field number JMP 286), collected by José M. Padial and D. Embert on 10–14 July 2003, CBG 327 (adult male), 328 (adult female), 329 (adult male), collected by R. Aguayo, all from Los Guácharos (= El Palmar, 500 m), Carrasco National Park;

ZFMK 59574, from Villa Tunari, collected by Pierre Ibisch on 22 August 1991; Departamento La Paz: MNCN 43071–2 (adult males, field numbers 596–7), MNK-7193 (adult males, field number 595), from Arroyo Pico Plancha, Madidi National Park (14º16'19.7"S, 68º05'36.1"W) collected by D. Embert on 16 December 2003; MNK-A 7273 (adult male, field number JMP 952) from Serranía de BellaVista, road between Caranavi and Palos Blancos, collected by José M. Padial and Cinthia Ureña on 07 March 2004 by; MNK-A 7178, from Chalalán, Area Natural de Manejo Integrado Madidi (14º25'28.4"S, 67º55'14.4"O), collected on 13 December 2003 by José M. Padial and D. Embert; Departamento Pando: NMP6V 72578/1–2, from Bioceanica (11°08'S, 69°22'W) (adult males, field number JM 65-66), collected by J. Moravec on 25 January 2005; MNCN 43151, Florida, Reserva Nacional de Vida Silvestre Manuripi (immature female) collected by Marcelo Guerrero; PERU: Departamento Cusco: MNCN 43249 (juvenile), 5 km from San Lorenzo on the road to Quince Mil, collected by I. De la Riva, J. C. Chaparro, S. Castroviejo and J. M. Padial on 22 February 2006. Departamento Huánuco: NMW 28966 (10 specimens, two adult females and 8 juveniles) from Río Llullapichis, Panguana, 220 m, collected by M. Aichinger; Departamento Madre de Dios: KU 154856–57 from Cocha Cashu, Manu National Park, collected by C.A. Toft on 10 and 20 August 1973, KU 205107 collected by T. A. Titus on 16 February 1986, KU 205120 collected by P. A. Burrowes and R. de Sá on 2 February 1986, KU 205132 collected by L. Trueb on 09 January 1986, KU 205133 collected by T. Titus on 1 January 1986, KU 205134 collected 1986 by P. A. Burrowes and R. de Sá on 28 January, KU 205137 collected by R. de Sá on 18 February 1986, KU 205138 collected by P.A. Burrowes on 27 February 1986, KU 205142 collected by P. A. Burrowes on 01 February 1986, KU 207708 collected by A. Channing on 22 Nov 1986, KU 207715, collected by W. E. Duellman on 16 November 1986, KU 207716 collected by B. Quibell on 17 November 1986, KU 207717 collected by B. Quibell on 24 November 1986, KU 215481 collected by V. R. Morales on 15 January 1989, KU 215482 collected by E. R. Wild on 24 January 1989, KU 215483 collected by D. A. Kizirian on 26 January 1989, KU 215484 collected by W. R. Wild on 02 July 1989, KU 215485 collected by D. A. Kizirian on 11 July 1989, KU 215486 collected H. R. Sisniegos on 12 July 1989 by, KU 215487 collected by A. W. Salas on 25 January 1990, KU 215488 collected by L. A. Coloma on 16 February 1990, all from Cuzco Amazónico, 15 km E of Puerto Maldonado; KU 154853–4 colected by C. A. Toft on 03 August 1973, KU 1548535 colected by C.A. Toft on 04 August 1974, all from Manu river, Manu National Park, 365 m; MCZ 136394 (adult female), Puesto Euahuipa, Río Palma Real Grande, Santuario Nacional Pampas del Heath, collected by J. Cadle; USNM 298900 (adult male) and 298901 (subadult female) collected by J. Cadle on 4–5 February 1984; 342623–29 collected by R. McDiarmid on 14–22 September 1988, USNM 3426230–2 collected by R. McDiarmid and V. Morales on 24 January 1989, USNM 342854–55, 345174–76, collected by R. Reynolds and J. Icochea on 2 July 1993, USNM 345177 collected by R. Reynolds and P. Sehgelmeble on 14 February 1992, USNM 345278 collected by R. Reynolds on 21 February 1992, USNM 345279 collected by P. Sehgelmeble on 29 February 1996, USNM 3452780–1 collected by R. Reynolds on 29 February and 2 March 1992, all from Pakitza, Reserve Zone, Manu National Park, ca. 57 km (airline) NW of mouth of Río Manu, on Río Manu (11º52'S, 71º18'W).

Referred specimens: BOLIVIA: Departamento Beni: MNK-A 4178, 4203–7, 4181, Serranía del Pilón, Antena de Entel; Departamento Cochabamba: CBG 437, Altamachi 1000 m; CBG 373–7, Arepucho 1000 m, Carrasco National Park; CBG 200–202, Chaquisacha 1500 m, Carrasco National Park; CBG 1021, Bia Recuate 210 m, Isiboro-Secure National Park; CBG 544, road from Villa Tunari to El Palmar, 1000 m, Carrasco National Park; CBG 333, 524–526, Río Ichilo, brazo muerto; CBG 957–62, Road from Villa Tunari to El Palmar 1300 masl; CBG 746, Santa Anita, Isiboro-Sécure National Park; CBG 604–11, Santo Domingo, Isoboro Secure National Park; CBG 560, Villa Fátima; Departamento La Paz: CBG 378, CBG 845–49, CBG 851–3, Boquerón, 1000 m; CBF 5223–5, Candelaria, Madidi National Park; MNK-A 4128, Lima; MNK-A 4112–3, Quebrada Boquerón 1140 m; MNK-A 4081–2, San Ignacio 1100 m; MNK-A 3692, 3703, 3705, 3710, 3714, 3717, Serranía Beu; MNK-A 4743, Serranía de Chepite; CBF 2485–6, Serranía Pilon Lajas; MNK-A 4119–22, 4126–32, 4139–43, Serranía San Ignacio; Departamento Pando: MNK-A 5178, Arroyo Tulapa, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 6034–5, 6044, 6069–70, Campamento Malecom, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 6083–5, 6095–8, 6090, Campamento Nueva América, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 4401, Campamento Serna-Humaita, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 6896, Curichón, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 4597, El Porvenir road; MNK-A 5085, 5095–110, Florida, Reserva Nacional de Vida Silvestre Manuripi; MNK-A 4596, Mukden; MNK-A 6174, Nueva España, Reserva Nacional de

Vida Silvestre Manuripi; MNK-A 4592–5, 4598–9, Reserva Nacional de Vida Silvestre Tahuamanu; MNK-A 6891, San Antonio, Reserva Nacional de Vida Silvestre Manuripi; USNM 336178, San Juan de Nuevo Mundo, 18 km N of; CBF 2538, 2543–4, San Sebastián; PERU: Departamento Cusco: USNM 537903–34, San Martín-3, ca. 5 km N of the Camisea River; Departamento Huánuco: MHNSM 12444–6, Dantas, Río Pachitea; MNHNSM 603–612, Río Llullapichis, Panguana, 220 m; Departamento Madre de Dios: MHNSM 17347–52, Pakitza, ca. 57 km (airline) NW of mouth of Río Manu, on Río Manu; USNM 222269–73, 247305–21, 247632–3, 343241, 268946–53, Puerto Maldonado, 30 km (airline) SSW of, Tambopata Reserve, Explorer's Inn; USNM 346142, Atalaya, ca. 3 km NW of, on west bank of Río Alto Madre de Dios, Hacienda Amazonia; MHNSM 751–755, 1194, 9302–3, 9259–68; 10070, 15508, 15585, Cocha Cashu, Manu National Park; MHNSM 620–626, 14673, 14676, 14678, Cuzco Amazónico, 15 km E of Puerto Maldonado; USNM 298839–44, Lago Valencia, extreme W bank of, Río Madre de Dios; MCZ 136395–6, Puesto Euahuipa, Río Palma Real Grande, Santuario Nacional Pampas del Heath; MHNSM 14011, USNM 332444–46, Río Tambopata, W bank of, Zona Reservada Tambopata–Candamo, Colpa de Guacamayo; MHNSM 613–16, 628, 1032–7, Río Tambopata; BM 1987.610–2, Tambopata Wildlife Reserve, junction río La Torre and río Tambopata; Departamento Puno: BM 1907.5.7.22, Río Huacamayo, Carabaya, 2000 ft..

Diagnosis: A member of the *Eleutherodactylus unistrigatus* Group, as defined by Lynch & Duellman (1997), characterized by: (1) skin on dorsum homogeneously shagreen; flanks shagreen; venter coarsely granular; posterior surfaces of limbs smooth; discoidal fold not evident; dorsolateral folds absent; postrictal glands present; (2) tympanic membrane and annulus round, large, their length about half eye length; supratympanic fold short, very prominent; (3) head slightly longer than wide; snout round in dorsal and lateral views; canthus rostralis straight in dorsal view, sharp in profile; (4) cranial crests absent; upper eyelid without conspicuous granules; (5) vomerine odontophores large, situated posteromedial to choanae; (6) males with vocal slits and a single white nuptial pad on thumb; (7) fingers short, first finger shorter than second; subarticular tubercles subconical, prominent; supernumerary tubercles round, prominent, smaller than subarticular tubercles; terminal discs of inner two fingers moderately expanded, those external fingers very enlarged, ovate to truncate; circumferential grooves conspicuous, ungual flap not indented; lateral fringes and keels on fingers present; (8) single ulnar tubercles present; (9) tubercles on heel and tarsus absent; tarsal fold prominent, longer than inner metatarsal tubercle; (10) inner metatarsal tubercle ovate, prominent, outer subconical, prominent; a single supernumerary tubercle, round to conical; (11) toes long and slender (foot length 50% SVL); lateral fringes or keels conspicuous, basal toe webbing absent; fifth toe reaching the tip of penultimate subarticular tubercle of Toe IV, third toe reaching the base; tips of toes rounded to ovate, expanded; ungual flap not indented, circumferential groove conspicuous; (12) dorsal coloration variable, mostly tan with dark brown flecks and chevrons; ventral coloration white with fine mottling; posterior surface of thighs brown with conspicuous orange (white in preservative) spots; (13) mandibular ramus of the trigeminal nerve passing lateral to the *m. adductor mandibulae externus* (S condition sensu Lynch, 1986).

The presence of orange spots (white in alcohol) in the posterior surface of thighs has led this species to be frequently mistaken for *E. peruvianus*. However, it differs from *E. peruvianus* by having first finger shorter than second, coarsely granular belly and lacking dorsolateral folds. For differences with other members of the *E. conspicillatus* Group see Table 3. *E. sp. nov. 2* is most similar to *E. danae* from which it cannot be distinguished by qualitative characters (Table 3 and Fig. 10B). Nevertheless, morphometrics, differences in advertisement call and mtDNA does allow a clear separation (see above). This species is ready distinguished from other members of the group by the combination of: canthus rostralis and loreal region bold, dorsum finelly shagreened, and presence of orange spots on posterior part of thighs. Other species of the *E. unistrigatus* group (sharing Finger I<II, granular or aerolate belly and any kind of orange spots) inhabiting the Andean foothills and/or adjacent lowlands are distinguished as follows: *E. altamazonicus*, *E. carvalhoi* and *E. croceoinguinis* all present one or two large red, orange or yellow blotches on the anterior surface of thighs and adjacent flanks, are smaller and have warty skin. *E. diadematus*, *E. eurydactylus* and *E. ventrimarmoratus* present bold black reticulation and spots on belly and limbs and have warty skin. *E. rhabdolaemus*, *E. toftae* and *E. sagittulus* have conspicuous dorsolateral folds. *E. salaputium, E. martiae, E. platydactylus* and *E. ockendeni* all have warty dorsal skin, poorly evident tympanic membrane and lack orange or yellow spots on posterior surfaces of thighs.

Description of the holotype: Head as long as wide (head length/head width =1.0); snout round in dorsal and lateral profile; nostrils slightly protuberant, oriented laterally;

canthus rostralis straight in dorsal view, sharp in frontal profile; loreal region flat; lips not flared; upper eyelid without tubercles or granules; no cranial crests. Supratympanic fold prominent, short; tympanic membrane and tympanic annulus large, distinct; tympanic membrane nearly round, its length about half of eye length; 2–3 postrictal glands, conical, conspicuous. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, ovate; vomerine odontophores large, prominent, drop-shaped, situated posteromedial to choanae but with the anterior margin at the level of choanae, separated by a distance of one half the length of a vomerine odontophore, bearing two rows of around 10 vomerine teeth. Skin of dorsal surfaces and posterior parts of hind limbs homogeneously shagreen; throat smooth, belly and groin coarsely areolate; occipital folds absent; dorsolateral folds absent; discoidal fold not evident.

Table 5. Morphometrics of adult specimens of *Eleutherodactylus sp. nov. 2* and *E. danae*. Means ± standard deviation in parentheses follows ranges (in mm).

	Adult females		Adult males	
	E. sp. nov. 2 (n=23)	*E. danae* (n=2)	*E. sp. nov. 2* (n=32)	*E. danae* (n=24)
SVL	28.2–37.1 (33.0±2.4)	37.5–44.2	23.9–30.7 (26.8±1.7)	23.8–34.3 (27.0±2.6)
HL	11.9–16.0 (13.2±1.0)	14.5–17.1	9.4–12.4 (10.9±0.8)	9.0–13.6 (10.7±1.0)
HW	2.0–15.5 (11.5±3.1)	14.2–16.3	8.3–11.6 (9.9±0.8)	7.9–12.9 (9.8±1.1)
EL	3.6–5.0 (4.1±0.4)	5.0–6.1	3.1–4.6 (3.7±0.3)	3.7–5.6 (4.3±0.5)
EN	3.8–5.2 (4.3±0.3)	5.0–5.4	2.9–4.1 (3.5±0.3)	3.2–4.3 (3.7±0.3)
IND	2.9–4.1 (3.2±0.3)	3.2–3.4	2.1–3.2 (2.7±0. 3)	1.7–3.4 (2.6±0.3)
EE	5.6–7.7 (6.4±0.5)	6.9–8.2	4.6–6.1 (5.3±0.4)	1.7–6.7 (5. 3±0.9)
TYH	1.7–2.0 (2.3±0.3)	2.8–2.9	1.1–2.4 (1.9±0.3)	1.5–2.6 (2.0±0.3)
TYL	1.7–2.8 (2.1±0.3)	2.3–2.5	1.5–2.3 (1.8±0.2)	1.4–2.4 (1.8±0.2)
F3	1.3–2.2 (1.7±0.2)	1.7–2.8	1.1–1.9 (1.5±0.2)	1.0–1.9 (1.4±0.2)
F4	1.3–2.3 (1.7±0.2)	1.7–2.8	0.8–1.9 (1.4±0.2)	1.0–2.1 (1.4±0.2)
FA	6.2–8.6 (7.1±0.6)	7.5–9.9	4.9–7.0 (5.8±0.5)	4.4–7.3 (5.6±0.7)
TL	16.7–24.0 (20.3±1.7)	22.7–27.8	14.4–17.9 (16.4±0.9)	13.2–18.6 (16.1±1.3)
TH	14.5–20.7 (17.4±1.7)	21.1–23.5	12.5–15.8 (14.3±0.9)	11.9–17.5 (14.2±1.3)
FL	13.8–19.2 (16.3±1.5)	19.4–22.9	11.4–15.3 (13.4±1.0)	11.1–16.6 (13.6±1.4)
T4	1.3–2.0 (1.6±0.2)	1.5–2.5	1.2–1.8 (1.4±0.2)	1.0–1.9 (1.3±0.2)
HL/HW	1.0–1.2 (1.1±0.0)	1.0–1.1	1.1–1.1 (1.1±0.0)	1.0–1.1 (1.1±0.0)
TL/SVL	0.5–0.7 (0.6±0.0)	0.6–0.6	0.6–0.6 (0.6±0.0)	0.5–0.7 (0.6±0.0)
FL/SVL	0.4–0.5 (0.5±0.0)	0.5–0.5	0.5–0.5 (0.5±0.0)	0.4–0.6 (0.5±0.0)

Arm with a single low, round ulnar tubercle; palmar tubercle bifid, flat, conspicuous, equal in length to elongate, prominent, thenar tubercle; a single supernumerary tubercle on the basis of each finger, round, prominent, smaller than subarticular tubercles; subarticular tubercles prominent, subconical; finger tips round, moderately expanded on fingers I and II, and large, ovate to truncate on fingers III and IV; Finger III bearing lateral fringes; relative length of fingers: III>IV>II<I.

Toes long and slender (foot length 50% of SVL); heel and tarsus lacking tubercles; tarsal fold prominent, two times longer then inner metatarsal tubercle, not in contact with it; inner metatarsal tubercle ovate, prominent, larger than outer; outer metatarsal tubercle prominent, subconical; one supernumerary tubercle on toes II, III and IV; subarticular tubercles conical, prominent, much larger than supernumerary tubercles; conspicuous lateral fringes on toes I, II and III; balsal toe webbing absent; toe tips round, moderately developed; ungual flap not indented, circumferential grooves evident; relative length of toes IV>III>V>II>I; Toe III reaching the base and Toe V reaching midpoint of penultimate subarticular tubercle of Toe IV.

Measurements (in mm) of the holotype: SVL 32.3, HL 12.6, HW 11.8, EL 3.8, EN 4.0, IND 3.0, EE 6.1, TYH 1.7, TYL 1.7, FIII 1.6, FIV 1.6, FA 6.6, TL 20.51, TH 18.7, FL 17.0, TIV 1.6.

Color: In preservative, dorsal surfaces tan with dark brown chevrons, flanks lighter. Bold black color on canthus rostralis, supratympanic fold, pair of occipital spots, around vent, knees and elbow, that of canthus and supratympanic fold outlined by a thin white stripe; loreal region dark brown to black; interocular dark brown bar; grey diffuse subocular and labial bars; tympanic membrane brown, annulus cream; arms with transverse dark stripes, oblique on hindlimbs; plantar surfaces dark brown; ventral surfaces cream with inconspicuous fine grayish-brown mottling, some enlarged spots on belly; thighs intensely mottled, shanks completely brown ventrally; posterior and anterior surfaces of hind limbs dark brown with well defined white spots. The color pattern in life is very similar, but the dorsum is grayish brown and the spots of posterior surfaces of thighs are orange. The ventral surfaces are white and the groin is yellowish-white. The iris is metallic yellow to orange with a transverse bold black stripe.

Variation: Males and females are similar in all but sexual qualitative external characters. Males commonly bear a single, white, glandular non-spinous nuptial pad on dorsal surface of each thumb, but some males have double nuptial pads. All breeding males present subgular vocal sac and vocal slits. Females are larger than males but are equal in head and limb proportions (Table 5). Gravid females contain large unpigmented eggs on the oviducts. The dorsal pattern is quite constant, although varies in intensity of colours and contrast of stripes. Some specimens may have more reddish, grayish or yellowish-brown colorations. Some dark dorsal marks, as on interocular stripe, a W-shaped occipital mark, an X-shaped middorsal mark or sacral chevrons can be present. The brown mottling in ventral surfaces also varies in intensity. In life, the color of the spots of the posterior surface of thighs vary from yellow to intense orange, they can be anastomosed or well separated, and vary in density, with some specimens showing only one or two spots. Moreover, some specimens also show the pattern of spots in the anterior surface of the thighs. For example, eight (of nine) specimens from Boquerón (in Departamento La Paz, Bolivia) bear this pattern. Contrarily to the holotype, some specimens have enlarged granules on dorsum and eyelids. The shape and development of vomerine odontophores also varies, and the row of vomerine teeth can be single or double. Another character that varies in intensity is fringe development on fingers and toes, although it is always present to some extent. For measurements, see Table 6.

Etymology: The name is a patronym for Steffen Reichle, German herpetologist and friend whose studies have greatly contributed to the understanding of Bolivian amphibian diversity.

Distribution: This species occurs in Departamento Huanuco, in Amazonian Peru, along the Andeand slopes and adjacent lowlands of Peru, Brazil and Bolivia. The southernmost record lies in the Chapare region of Central Bolivia (Fig. 9). It has been recorded in lowland Amazonian forest and humid montane forest of the Andean foot hills up to 1500 m (Chaquisacha, Carrasco National Park, Bolivia). The parapatric altitudinal distribution of the pair *E. danae*-*E. sp. nov. 2* along most of their range makes some identifications uncertain, and doubtful records should be tested by means of morphometric, bioacoustic or molecular analyses. However, *E. danae* has been recorded from higher altitudes and seems to be restricted to southern Peru and northern Bolivia. The southernmost record of *E. danae* corresponds to Valle de Zongo, Department La Paz, Bolivia (16º11'47.5"S, 68º07'35.5"W).

Natural History: This species is active by night during the rainy season. Males call from low vegetation in the forest. It has been found only in primary and secondary forest formations.

Remarks: The advertisement calls described for *E. peruvianus* from Panguana (Peru) by Schlüter (1980), Cocha Cashu (Perú) by Rodríguez (1994) and from Tambopata by Duellman (2005) correspond to *E. sp. nov. 2*. The later was reanalysed herein (USNM tape 265/17; Table 1). The Bolivian record of *E. danae* by Köhler & Jungfer (1994) and the advertisement call of *E. danae* from Chapare 1250 m by Köhler (2000a) correspond to *E. sp. nov. 2*. Specimens from Tambopata reported by Doan & Arizábal (2002) as *E. peruvianus* correspond to *E. sp. nov. 2*. Specimens cited by Padial *et al.* (2004) as *E. danae* for different localities in Bolivia correspond to *E. sp. nov. 2* and are now included herein as referred specimens (see above). Peruvian specimens reported by Padial & De la Riva (2005a) as *E. cf. peruvianus* (except KU 154863–5) also correspond to *E. sp. nov. 2*.

DISCUSION

Two new species taxa, *Eleutherodactylus sp. nov. 1* and *E. sp. nov. 2* are described from the forests of the Amazonian versant of the Andes and adjacent lowlands of Central Bolivia and southern Peru. The former is a member of the *E. conspicillatus* Group that was long confused with *E. fenestratus, E. peruvianus* or *E. dundeei*; the later, belonging to the *E. unistrigatus* Group was also confused with *E. peruvianus*. A reanalysis of qualitative morphological characters allowed unambiguous distinction of *E. sp. nov. 1* from *E. peruvianus* but not from the other two species. However, multivariate discriminant analyses of quantitative morphological and bioacoustic characters allowed to separate *E. sp. nov. 1* from *E. dundeei*. The same analyses plus phylogenetic analyses of partial mtDNA allowed a clear distinction of *E. sp. nov. 1* from *E. fenestratus* and from another similar sympatric species, *E. samaipatae*. Thus, *E. sp. nov. 1* is an endemic sibling species of *E. fenestratus* restricted to the humid and semideciuous forests of the Andean slopes of central Bolivia. It remains to be investigated the relationships between this new species and the Cerrado inhabitant *E. dundeei*, which occurs in the rocky areas of the Precambrian Brazilian Shield and adjacent outcrops of Bolivia. Morphological and bioacoustical analyses indicate that *E. dundeei* could be more closely related to *E. sp. nov. 1* than to *E. fenestratus*. This would also support the biogeographical connection between the Cerrado and the Andean *Eleutherodactylus* through the Precambrian shield, as hypothesized by Köhler (2000a) based on what what was then considered *E. dundeei* in Andean Bolivia, now *E. sp. nov. 1*, and as demonstrated by Padial *et al.* (in press-c) based on *O. heterodactylus*.

Eleutherodactylus sp. nov. 2 is readily distinguished by qualitative characters from *E. peruvianus* and the rest of species of the *E. conspicillatus* Group but it is morphologically cryptic in qualitative characters with *E. danae*, which belongs to the *E. unistrigatus* Group. However, multivariate discriminant analyses of quantitative morphological and bioacoustic characters plus phylogenetic analyses based on mtDNA allowed a clear separation of *E. sp. nov. 2* and *E. danae*. The new species occurs in altitudinal parapatry with *E. danae* along the humid forests of the Andean slopes and adjacent lowlands from central Bolivia to southern Peru.

The origin of both *E. sp. nov. 1* and *E. sp. nov. 2* seems to be related to habitat conditions different from those occupied by their sister taxa. For example, *E. sp. nov. 1* is the sibling species of *E. fenestratus*, but the later has a broad distribution along the humid lowland Amazonian forests and humid forest of the Andean hills, while *E. sp. nov. 1* is mainly restricted to the semideciduous forests (longer dry seasons and cooler temperatures; see Köhler, 2000a) of the Andean hills at the southern edge of the distribution of *E. fenestratus*. Both species share a small area of overlap along the humid Andean slopes of Central Bolivia (Fig. 9). This situation is applicable to *E. samaipatae*, which is the sister taxa to the later pair and is restricted to the semideciduous forest, sharing localities only with *E. sp. nov. 1*. *Eleutherodactylus sp. nov. 2* and *E. danae* also seem to be closely related, even sibling, according to Bayesian and NJ analyses, and they occur in parapatry with altitudinal segregation in different, but similar, habitats (humid montane forest *vs.* humid forest of the Andean hills and lowlands). Therefore, given the parapatry of these taxa in habitats with ranges of precipitations, temperature and humidity, niche conservation driven by differences in seasonal climatic regimes could be hypotesized as the cause of lineage divergence of sister taxa, as evidenced for similar situations in hylid frogs (Wiens *et al.*, 2006). Moreover, recent divergence in isolation in humid refugia (for the pair *E. danae*-*E. sp. nov. 2*) and dry refugia (for *E. sp. nov. 1* and *E. samaipatae*) (Pennington *et al.*, 2000; Killeen *et al.*, 2007) might also have contributed to this speciation process, as in some other Neotropical frogs (Funk *et al.*, 2007).

During the 200 years of Linnean taxonomy, species discovery and description has mainly relied on the study of qualitative morphological characters. The modern synthesis triggered the incorporation of evolutionary concepts into taxonomy, and reproductive isolation, behaviour, ecology and distribution were combined as additional evidences of species limits (Mayr, 1942). For example, reproductive barriers are explicitly or implicitly assumed by many taxonomists when describing species, since the biological species concept has been the most broadly accepted framework. Indeed, it is often overlocked that morphological characters (and many others) are just a proxy to infer reproductive isolation (Vogler & Monaghan, 2006). In more recent times, the utility of molecular and morphological phylogenetic methods was tested to delineate species limits, taking as reference classical species taxa hypotheses (e. g. Sites & Marshall, 2004). The results of some comparative analyses led to the conclusion that many classical species taxa hypotheses were corroborated with new evidence provided by phylogenetics (Wiens & Penkrot, 2002), but also revealed that some putative species level lineages had not been discovered by the classical methods. In other words, cryptic species were most easily discovered by phylogenetic methods, mainly those based on molecular characters (Bickford *et al.*, 2007). Moreover, the high degree of concordance of species boundaries delineated with phylogenetic analyses of some molecular markers with classical species taxa led to the proposal of DNA taxonomy (Tautz *et al.*, 2003). Some problems emerged parallel to this later proposal. For example, species formerly accepted were shown to be not necessarily monophyletic when tested through molecular methods (Funk & Omland, 2003) and some molecular markers (e. g. mitochondrial, vs. nuclear genes) differed in the results and accuracy for each group of organisms (see review of Vogler & Monagham, 2006). Although some biologistz were decidedly inclined toward the use of molecular methods to define species limits (e. g. Blaxter, 2003), some taxonomists pleaded for the use of an integrative approach that included different lines of evidence to support more stable species taxa hypotheses (Dayrat, 2005; Will *et al.*, 2005). This proposal came at the right time mainly for two reasons. First, the steady increase in species discovery during the last decades was interpreted by some biologists as a symptom of taxonomic instability and failure in the identification of true species due to generalized use of molecular phylogenetics (Padial & De la Riva, 2006). Second, the species problem arrived to end (Hey, 2006) due to the recognition that none of the previously proposed criteria to define species were necessary or sufficient to define the species category (De Queiroz, 2005a). In other words, different lines of evidence could be used to delineate and describe equally valid species taxa hypotheses (De Queiroz, 2005b). Therefore, an integrative taxonomy considering all available lines of evidence to test and support species taxa hypotheses would converge in a more reliable and stable taxonomy. Nevertheless, integrative taxonomy is not commonly implemented and most taxonomists still rely on classical descriptive methods. Furthermore, many taxonomists interested in molecular systematics use phylogenetics only to unravel relationships of species and higher taxa, but not for classification (Franz, 2005) or to describe species. Indeed, this trend is causing molecular phylogenetics to absorb investment formerly destined to species discovery and description (Wheeler, 2004).

This study is an example of how an integrative taxonomic approach combining morphology, bioacoustics and molecular phylogenetics can be applied to discover and describe species of Neotropical anurans. We exemplify how tree lines of evidence can be integrated as a powerful tool to solve long-standing taxonomic problems and to discover cryptic lineages within a highly diverse and taxonomically complex genus. Since tree lines of evidence converged in the recognition of the new species taxa described, we consider each of them as stable taxonomic hypothesis. A corollary of this study is that similar results are expectable when applying this approach to a broader scale revision of *Eleutherodactylus*. Indeed, the use of bioacoustics to infer species limits through the discovery of prezigotic reproductive barriers has already allowed the discovery of cryptic *Eleutherodactylus* (e. g. Köhler & Lötters, 1999; Köhler, 2000b; Reichle *et al.*, 2001; Padial *et al.*, 2005). This shows us that the numbers of cryptic species could be much higher than previously expected. However, in the Neotropics, bioacoustics has not been sufficiently incorporated into taxonomy. For example, within *Eleutherodactylus*, representing the largest Neotropical vertebrate genus, with nearly 500 species (Frost, 2006), most species have been described by the classical morphological approach. Therefore, both cryptic species and a great number of synonyms could be hidden under many current species names. A desirably, broad scale study of *Eleutherodactylus* systematics, integrating morphological, bioacoustics, and molecular characters could lead to an important increase in species discovery and to the resolution of many taxonomic problems.

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APPENDIX I

SPECIMENS OF *ELEUTHERODACTYLUS* EXAMINED

ELEUTHERODACTYLUS ACHATINUS (1 specimen): ECUADOR: Esmeraldas: Cachabé, BM 1947.2.15.69 (holotype, formerly 98.4.28.106). *E. ALTAMAZONICUS* (11 specimens): Upper Amazon Basin, MCZ 2038 (holotype); BOLIVIA: La Paz: Candelaria, CBF 5311; Pando: Curichón, Reserva Nacional de Vida Silvestre Amazónica Manuripi-Heath, MNK-A 3878; Luz de America, MNK-A, 6879; Reserva Nacional de Vida Silvestre Amazónica Manuripi-Heath, MNK-A 3637; Federico Román**,** MNKA 6880; ECUADOR: Napo: 6.5 km ESE Puerto Misahualli, MCZ 118822–6. *E. AVICUPORUM* (1 specimen): PERU: Amazonas: 10 km NNE Balsapata, 1700 masl., KU 288628 (paratype). *E. BIPUNCTATUS* (1 specimen): PERU: Pasco: 0.7 km South and 4.5 km East (airline) of Oxapampa, approx. 10 34′ S, 75 24′ W, 2120 m elevation, KU 291638 (holotype). *E. BUCCINATOR* (8 specimens): PERU: Madre de Dios: Cocha Cashu Biological Station, Manu National Park, KU 220919 (paratype); MHNSM 3842 (holotype), 3844–7, 3865 (paratypes), USNM 299779. *E. CALIGINOSUS* (1 specimen): PERU: Huanuco: Cordillera Azul, Pass of Carretera Central, 1650 masl, ZFMK 27634 (holotype of *E. nebulosus*). *E. CARVALHOI* (82 specimens): PERU: Loreto: Bellavista, Yubineto, NMW 24896 (2 specimens); Requena, USNM 332963–68; USNM 537771–844; *E. CITRIOGASTER* (1 specimen): PERU: San Martín: Cataratas Ahuashiyacu, 14 km NE Tarapoto, 370 m, KU 212277 (holotype). *E. COSNIPATAE* (10 specimen): PERU: Cusco: San Pedro, AMNH 157011–2; Santa Isabel, 4 km SW of, Río Cosñipata, 1700 m, KU 162298–304 (holotype and paratypes); Santa Isabel, 2 km SW of, Río Cosñipata, 1500 m, KU 138876 (paratype). *E. CONSPICILLATUS* (131 specimens): COLOMBIA: Putumayo: Puesto de Bombeo Gómez, 1000 m, KU 140299–300; San Antonio, Río Suárez, 400 m, KU 140301; 10 km S of Mocoa, AMNH 84830–1; ECUADOR: Andes of Ecuador, BM 1947.2.16.20 (holotype, formerly 58.7.25.24); Morona Santiago: Taisha, MCZ 100267; Napo: Río Yasuní (150 km upstream from), KU 175106; Río Yasuní (200 km upstream from), KU 175107–10; road to Tivacuno km 9, PUCE, Yasuní, KU 289437; Santa Cecilia, 340 m, KU 104552–63, 106959–60, 106966, 108983–7, 110784–88, 123408–26; 146071–8, 148785–94, 148820–23, 148796–818; 14825–6; 3.1 km S río Tiputini, KU 297110; 1350 m from topographic trail PUCE, KU 289436; Pastaza: Chantoa, KU 119524; Mera, KU 119525; Pastaza River, MCZ 19637 (5 specimens); Sarayacu, KU 119526; 1 km W of Puyo, MCZ 89963-8, 92131, 92133; 10 KM ESE of Veracruz, MCZ 89958–59; Sucumbios: Lago Agrio, KU 126154–5, 297111; Limoncocha, KU 183524; PERU: Loreto: Explorama Lodge, junction Río Yanamomo and Río Amazonas, KU 220444; Quebrada Grande, ca. Junction Sucusari and Napo rivers, KU 220353, 220569, 222348. *E.* CREPITANS (2 specimens): BRAZIL: Mato Grosso: São Vicente, MZUSP 85628 (holotype), 73671 (allotype). *E.* CROCEOINGUINIS (29 specimens): ECUADOR: Napo: Santa Cecilia, KU 110789 (holotype); Pastaza: 1 km SW of Puyo, MCZ 134398; 10 km ESE of Veracruz, MCZ 9124–51. *E.* DANAE (221 specimens): BOLIVIA: La Paz: Arroyo Huacataya, Madidi National Park, MNCN 43069–72, MNK-A 7190, 7199; Arroyo Huairuro, Madidi National Park, MNCN 43059–64, 43067–8, MNK-A 7181–3, 7186; Valle de Zongo, MNCN 4279, 43144; PERU: Cusco: Km 137 on Paucartambo–Atalaya road, 2200 m, USNM 346118; Km 150 on Paucartambo–Atalaya road, 1700 m, USNM 346123–34; Km 154 on Paucartambo–Atalaya road, 1500 m, USNM 346137; Paucartambo, 72–75 km N of, USNM 298247–55, 298257–97; Paucartambo, 68 km by rd. NE of Puente Unión on río Tachila, USNM 346346–51; Puente Quitacalzón, 1180 m, MHNSM 17203–7, USNM 345925–7, 346119–22, 346356–9; Quebrada Morro Leguia, km 137 on Paucartambo–Atalaya rd., 2200 m, USNM 346344–5; Río Cosñipata, 2.5 km (by road) SW of Santa Isabel, 1620 m, KU 138878; Río Cosñipata, 4 km (by road) SW of Santa Isabel, 1700 masl, KU 162307 (holotype) KU 162308–57 (paratypes), MCZ 93305–10 (paratypes); Río Cosñipata, 6 km (by road) SW of Santa Isabel, 1270 masl, KU 162358–64 (paratypes), MHNSM 13957 (paratype); Río San Pedro, 72 km by rd. NE of Paucartambo, 1500 m, USNM 342769, 345928–54, 346352–55; Santa Isabel, km 157 on Paucartambo–Pilcopata road, 1300 m, MHNSM 17193–202, 17208–10, 17211–3, USNM 346135–6. *E.* DUNDEEI (18 specimens): BOLIVIA: Santa Cruz: Boca del Paucerna, Parque Nacional Noel Kempff Mercado, MNK-A 3124; Flor de Oro, Parque Nacional Noel Kempff Mercado, MNK-A 2811–2, 28189, 2821, 2828, 5596; BRAZIL: Mato Grosso: Burity, 30 miles NE of Cuiabá, BM 1928.1.12.9; Casa de Pedra, Chapada dos Guimarães, USNM 507897–90 (paratypes); Chapada, close to Cuiabá, BM 92.4.20.16, 1903.3.26.30–31; Río Coxipozinho, Veu da Noiva, Chapada dos Guimarães, MZUSP 79834 (holotype), 79835–7 (paratypes). *E.* EURYDACTYLUS (1 specimen): PERU: Huánuco: Río Llullapichis, Panguana, ca. 220, KU 218292 (paratype). *E.* FENESTRATUS (323 specimens): BOLIVIA: Beni: Arroyo Agua

Clara, MNK-A 3820; Asunción, MNK-A 4058, 4066, 4070–1; Laguna Azul, MNK-A 4007; Misiones Mosetenes, BM 98.6.9.21; Rurrenabaque, MNK-A 3784–9, 3790–1; San Luis Chico, MNK-A 4013–5, 4032; Serranía del Pilón, Antena de Entel, MNK-A 4150–5, 4175–6, 4199–200; Río Yucumo, MNCN 43149–50, 43838, 43841; Cochabamba: Altamachi, CBG 486–7; between Parajti and El Palmar, ZFMK 66965–6; between El Palmar and Paractito, ZFMK 72545–6, 72536; road to San Onofre from Cochabamba-Villa Tunari road, MNCN 43109, 43119; Chaquisacha, Carrasco National Park, 1500 masl CBG 219; Charuplaya, 1300 m BM 1902.5.29.106–109; Los Guácharos (=El Palmar, 500 m), CBG 301–7, 349–358, MNCN 43031, 43142; ríos Altamachi–Malpaso, CBG 439, 492–8; Santa Anita, Isiboro-Sécure National Park, CBG 744–5, 747–9; Santa Domingo, Isiboro-Sécure National Park, CBG 613–15, 617; Valle de Sajta, MNK-A 3561; Villa Fátima, CBG 556–559; Villa Tunari, MNK-A 1493; La Paz: Arroyo Amahuachi, Camino Maderero Bellavista, MNK-A 3801–3; Arroyo Mikai, Camino Maderero el Chaval, MNK-A 3757, 3763–8, 3762; Boquerón, ANMI Pilón-Lajas, 1000 masl, CBG 773; Chalalán, A.N.M.I. Madidi, MNCN 42980, 43057, 43239–40, 43242–44; Chulumani, 1700 m, BM 1940.4.5.59; Huachi, MCZ 10094–5; Juapi, A.N.M.I Pilón-Lajas, CBG 681–7; La Cascada, MNCN 43037; Sadiri, Arroyo Yariapo, Madidi National Park, MNCN 42982, 42984; Serranía Beu, A.N.M.I Pilón-Lajas, CBG 901–6; San Ernesto, Mapiri 500 m, BM 1901.8.2.53; San Ignacio, MNK-A 4083–7, 4151; Serranía de Bella Vista, MNCN 43146; Serranía de Chepite, 3730, 3735, 3736, 3746–8; Valle de Zongo, ZFMK 72524–5; Pando: Arroyo Capinduro, Santa Rosa del Abuná, MNK-A 6881; Arroyo Tulapa, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 5177; Barraca Hiroshima, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 5193–6; Barraca San Carlos, Madre de Dios, MNK-A 4562; Campamento Malecom, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 6052, 6059; Campamento Serna–Humaita, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 4404; Chivé, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 5011–2, 6124–7, 6883, 6888–9, MNCN 43153–4; Cobija, ZFMK 66795–99; Curichón, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 6894; Florida, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 5084, 5091–4, 5115; Gran Progeso, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 5070; Hiroschima, MNCN 43152; Laguna Bay, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 4945, 6114–7; Mukden, 4620–4628; Nueva España, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 3664, 3668–9, 3673–4; Puerto Loreto, río Madre de Dios, MNK-A 6882, 6884–7;

Reserva Tahuamanu, MNKA–A 4629; Río Negro, Federico Román, MNK-A 6909; San Antonio, Reserva Nacional de Vida Silvestre Manuripi, MNK-A 6890, 6892–3, 6895; Santa Cruz: Buenavista, 500 m AMNH 34004, 34008, 34010; Lago Caimán, Parque Nacional Noel Kempff Mercado, MNK-A 2810, 2815–7, 2820. 2822–7, 2829; Mataracú, MNK-A 3928, 3930–49, 3951, 3953, 3955–67, 3969–70; Quebrada Tesoro, Parque Nacional Noel Kempff Mercado, MNK-A 169; BRAZIL: Amazonas: Borba, NMW 19940:2 (paralectotype); ca. 2 km N of km 70 on Manaos–Itacoatiara road, AMNH 139278; Manaos, AMNH 71437; Matto Grosso: Tumbador, between Mantino and Rosario rivers, 450 m, BM 1928.1.12.10–12; Rondonia: río Mamoré, NMW 1940:1 (lectotype); PERU: Cusco: Quincemil, 40 km SE of, 800 m, KU 175097–9, 196462–3; 6,2 km of Puente Fortaleza to Quince Mil, MNCN 43250; Madre de Dios: Cuzco Amazónico, 15 km E of Pto. Maldonado KU 194906–8, 209174–5, 205109–16, 205118–19, 205121–31, 215463–73; Juliaca, río Heath, Santuario Nacional Pampas del Heath, MCZ 136344; Tambopata Wildlife Reserve, junction río La Torre and río Tambopata, BM 1987.609; Puno: between Santa Rosa and San Juán del Oro, MNCN 43245; Juliaca (in error), AMNH 6119–20; La Unión, río Huacamayo, Carabaya, BM 1907.5.7.14–16, 1911.12.20.40–1; Marcapata Valley BM 1902.5.29.196; Ucayali: Balta, río Curanja, 200 m, KU 175095; Coengua River, upper Uyacali river AMNH 42067. *E. LANTHANITES* (5 specimens): ECUADOR: Province Napo: Santa Cecilia, 340 masl, KU 146144 (holotype); BM 1971–1796–99 (paratypes). *E. LYMANI* (1 specimen) PERU: Cajamarca: Bellavista, USNM 118189 (paratype). *E. MALKINI* (18 specimens): PERU: Loreto: Estirón, Río Ampiyacu, AMNH 94228 (holotype), AMNH 94229 (paratopotype); Yagua Indian Village, headwaters of Río Loretoyacu AMNH 94230–34 (paratypes); Olalla, Río Pastaza, AMNH 94219 (paratype). ECUADOR: Eastern Ecuador, AMNH 94213–7 (paratypes); Morona Santiago: Cusuine, Río Cusuine, 320 masl, 60 km airline SE of Macas AMNH 93686–87, 93690 (paratypes); Pastaza: Olalla, Intuto, río Tigre, AMNH 94250 (paratype); Santa Rosa, Río Tigre, AMNH 94218 (paratype). *E. MARTIAE* (8 specimens): ECUADOR: Napo: Hacienda Primavera, MCZ 95580, 91832; Limoncocha, MCZ 98043; Santa Cecilia, MCZ 58004; Pastaza: 1 km W of Puyo, KU 90315–17; 10 km ESE of Veracruz, MCZ 90342; *E. METABATES* (1 specimen) PERU: Amazonas: ca. 20 km SW Chiriaco, KU 196504 (holotype). *E. OCKENDENI* (3 specimens): PERU: Puno: La Unión, Carabaya, 2000 ft., BM 1947.2.16.88–90 (types). *E. PERUVIANUS* (248 specimens): BRAZIL: Amazonas: Igarapé Belém, near río Solimoes, ca. 70 km E from Leticia, AMNH 96903–97042;

ECUADOR: Morona Santiago: Ashuara Village on Río Macuma, 300 m, ca. 10 km above río Morona (ca. 83 km ESE Macas), AMNH 94656–78; Cusuine, AMNH 93583–655; Río Piuntza, 1830 m, KU 147034–38; Napo: Río Azuela, 1700 m, AMNH 22202–4, KU 143502–3; Río Salado, 1 km from río Coca, 1410 m, KU 165859–65; southern slope of Cordillera del Dué, above río Coca, 1150 m, KU 123446, 123502; 3.2 km NNE, Oritiyacu, 1910 m, KU 165860; 16 km NNE of Santa Rosa, 1700 m, KU 143498–501; Pastaza: Río Pastaza, from Canelos to Marañón, MCZ 19635, 89314, 19639; PERU: Cusco: Cordillera Vilcabamba 1680 m, AMNH 79812–13; Huánuco: Cordillera Azul, La Divisoria, Río Azul, AMNH 86496–7; Loreto: Teniente López, 200 m, KU 222030; Yagua Indian Village, headwaters of Río Loretoyacu, AMNH 96303–30; 1.5 km N Teniente López, 310–340 m, KU 222024–9; San Martín: Río Cainasache, 33 km NE Tarapoto on road to Yurimaguas, KU 209475–77; Roque, NHMG 490 (holotype); 15.4 km SW Zapateros, 950 m, KU 217313; 20 km NE Tarapoto on road to Yurimaguas, KU 209473–4; 48 km NE Tarapoto, KU 217312. *E. PLATYDACTYLUS* (28 specimens): BOLIVIA: Cochabamba: Los Guácharos (=El Palmar, 500), MNCN 43029–30, 43033–4; La Paz: Serranía de Bellavista, MNCN 43147; Santa Cruz: La Siberia, MNCN 42992–43001, 43082–88; PERU: Cusco: Enre San Miguel y Marcapata, MNCN 43248; Puno: Santa Domingo de Carabaya, 6000 ft., BM 1947.2.15.91–96 (types, five specimens). *E. RHABDOLAEMUS* (26 specimens): BOLIVIA: Santa Cruz: Paredones del Amboró, Amboró National Park, MNCN 43074–80; PERU: Cusco: Buenos Aires 2400 m, KU 173236 (holotype of *E. pharangobates*), 173237–53 (paratypes); West side of Cordillera Vilcabamba, 2125 m, AMNH 82511 (paratype). *E. SAGITTULUS* (2 specimens): PERU: Pasco: Río San Alberto, 0.9 km N, KU 291635–6. *E. SALAPUTIUM* (1 specimen): PERU: Cusco: 4 km SW Santa Isabel, Río Cosñipata, 1700 m, KU 162292 (holotype). *E. SAMAIPATAE* (25 specimen): BOLIVIA: Santa Cruz: El Fuerte, Samaipata, ZFMK 59600 (holotype); Espejillos, MNK-A 6444, 6448; Estancia Cuevas, 101 km from Santa Cruz de la Sierra to Samaipata, MNK-A 672, ZFMK 66882–3; Km 6 Angostura-Samaipata road, MNCN 42987–9; Río Parabano, MNK-A 6689–91; Río Seco, 40 km W of, ZFMK 67073; Río Seco, 45 km W of, ZFMK 67071–2; Road to Bella Vista from Santa Cruz-Samaipata road, MNCN 43048–51, ZFMK 71998–9; Samaipata, ZFMK 83086; Serranía del Toce, Los Vidrios, MNK-A 3497; 7 km from Samaipata to Santa Cruz de la Sierra, MNK-A 1744–5. *E. SKYDMAINOS* (38 specimens): BOLIVIA: Pando: Río Negro, MNKA 6911–12; BRAZIL: Acre: Humaitá, at km 29 on río Blanco to Pto. Acre road, AMNH 139279–81; PERU:

Amazonas: Alva, btw. Chachapoyas and Bagua Grande, 1000 masl, MCZ 89074–80 (type series of *E. karcharias*); Huánuco: Río Llullapichis, Panguana, 220 m, KU 218292 (paratype); Madre de Dios: Cocha Cashu Biological Station, Manu National Park, AMNH 134165–6 (paratypes), 139010–6 (paratypes), MCZ 88304 (holotype), 88305–9 (paratypes), MHNSM 9090–1 (paratypes); Colpa de Guacamayos, río Tambopata, 700 masl, USNM 324334–6 (paratypes); La Colpa, río Tambopata, MHNSM 9081 (paratype); vicinity of Cocha Cashu, approx. 70 km (airline) NW of mouth of río Manu, on río Manu, USNM 298914–5 (paratypes); Pasco: Bosque Castilla, Oxapampa, MHNSM 11176–7; Puno: ridges above mouths of Candamo and Guacamayo Rivers, MHNSM 9080 (paratype). *E. TOFTAE* (32 specimens): BOLIVIA: Cochabamba: Los Guácharos (=El Palmar, 500 m), MNCN 43025; PERU: Huánuco: Río Llullapichis, 4.5 km upstream from Río Pachitea, Finca Panguana, 200 m, KU 171863 (holotype), KU 154806–19 (paratypes), KU 171852–62 (paratypes), KU 171864–66 (paratypes); Cusco: San Miguel, Marcapata valley, MNCN 43246–7. *E. VENTRIMARMORATUS* (12 specimens): BOLIVIA: Pando: San Sebastián, Tahuamanu Biological Station, CBF 2537, CBF 2539; ECUADOR: Pastaza: Mera, 1140 m, KU 119805–6, 119809; 1 km E of Mera, Río Alpayacu, 1100 m, KU 119807–8; 8 km NW of Mera, Abitagua, 1300 m, KU 119811; PERU: Junín: Chanchamayo, 2600 ft., BM 1947.2.15.73–6 (types, four specimens). *E. VILARSI* (4 specimens): BRAZIL: Amazonas: Taracuá, Río Urapés, GNM 491 (2 syntypes); VENEZUELA: Amazonas: Teniche, Monte Marahuaca, MCZ 30397 (holotype of *E. conspicillatus ileamazonicus*); Upper Cunncuma River, MCZ 28568 (holotype of *E. brachypodius*).

APPENDIX II

SOUND COLLECTION REFERENCE NUMBERS, LOCALITY DATA AND SPECIMEN VOUCHERS FOR ANALYSED CALL RECORDINGS

ELEUTHERODACTYLUS DANAE: Fonozoo Number…, Bolivia, Departamento La Paz, Valle de Zongo, aprox. 24 ºC, voucher MNCN 42979 (one specimen analyzed); Fonozoo Number…, Bolivia, Departamento La Paz, Serranía Eslabón, Huairuro, 22 ºC, one from the series MNCN 43054–64, 43067–8 (three specimens recorded). *E. DUNDEEI:* USNM Tape 320 cut 2–3, Brazil, Matto Grosso, Chapadas dos Guimaraes, Estancia Monarca 21. 6 ºC, voucher USNM 507899 (two specimens recorded). *E. FENESTRATUS*: USNM Tape 206, Cut 2, Peru, Madre de Dios, Tambopata, 24 ºC, USNM 342993 (one specimen recorded); USNM Tape 266, cut 19, Peru, Madre de Dios, Tambopata, 27 ºC, unvouchered (one specimen recorded); Fonozoo Number 1096, Peru, Madre de Dios, Cusco Amazónico, temperature not given, unvouchered (one specimen recorded); Fonozoo Number…, Bolivia, Departamento La Paz, Arroyo Pico Plancha, 24 ºC, unvouchered (one specimen recorded); Fonozoo Number…, Bolivia, Departamento La Paz, río Maije, aprox. 24 ºC, unvouchered (one specimen recorded); Fonozoo Number…, Bolivia, Departamento Beni, Rurrenabaque, 27 ºC, unvouchered (one specimen recorded). *E. SP. NOV. 1*: Fonozoo Number…, Bolivia, Departamento Santa Cruz, Espejillos, 26º C, vouchers MNCN 43054 and MNK-A 7170, 7172, 7174 (three specimens recorded); Fonozoo Number…, Bolivia, Departamento Santa Cruz, Km 6 Angostura-Samaipata road, 27 ºC, vouchers from the series MNCN 42990–1, MNK-A 6626–7 (three specimens recorded). *E. SP. NOV. 2*: USNM Tape 365, cut 17–8, Peru, Madre de Dios, Tambopata, 24.2 ºC, USNM 343241 (three specimens recorded); Fonozoo Number…, Bolivia, Departamento La Paz, Arroyo Pico Plancha, 24 ºC, one from the series MNCN 43071–2 (one specimen recorded); Fonozoo Number…, Bolivia, Departamento La Paz, Chalalán, aprox. 26 ºC, Voucher, MNK-A 7178 (one specimen recorded). *E. SAMAIPATAE*: Fonozoo Number…, Bolivia, Departamento Santa Cruz, Km 6 Angostura-Samaipata road, 27 ºC, vouchers from the series MNCN 42987–9 (eight specimens recorded); Fonozoo Number…, Bolivia, Departamento Santa Cruz, Road to Bella Vista from Santa Cruz-Samaipata Road, 19 ºC, on from the series MNCN 43048–51 (three specimens recorded); Fonozoo Number…, Bolivia, Departamento Santa Cruz, Espejillos, 26º C, unvouchered (one specimen recorded); Fonozoo Number 1065, Bolivia, Departamento Santa Cruz, Masicurí, temperature not given, unvouchered (one specimen recorded).