Zoological Journal of the Linnean Society, 2009, **155**, 97–122. With 9 figures

Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae)

JOSÉ M. PADIAL and IGNACIO DE LA RIVA*

Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales, CSIC. C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain

Received 6 June 2007; accepted for publication 9 October 2007

Hypotheses on the taxonomic status of two Bolivian *Pristimantis* with taxonomic problems are assessed by an integrative taxonomic approach that integrates three independent lines of evidence: external morphology, prezygotic reproductive barriers (advertisement calls) and reciprocal monophyly (phylogenetic analyses of partial 16S mtDNA sequences). Central Andean Bolivian populations previously assigned to either *P. peruvianus* or *P. dundeei*, and lowland Amazonian populations from southern Peru and northern Bolivia previously considered *P. peruvianus* do not correspond to these species. Indeed, multivariate analyses of qualitative and quantitative morphological and bioacoustic characters, and phylogenetic analyses support the hypothesis that they represent different, previously unknown, cryptic lineages. They are herein described as new species. The former is a sibling species of *P. fenestratus* that inhabits the Amazonian and semideciduous forests of the Andean foothills in central Bolivia. The latter is sibling to the Andean species *P. danae* and is parapatric to it in the Amazonian lowland forests and adjacent foothills of northern Bolivia, southern Peru and adjacent Brazil. Most species of Neotropical frogs, and especially *Pristimantis*, have been described by using external qualitative morphological characters only. An extended integrative taxonomic approach, as exemplified herein, may lead to the discovery of many other cryptic and sibling lineages that would increase the species numbers of tropical areas.

ADDITIONAL KEYWORDS: Andes – bioacoustics – 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 such as Manu (Peru) or Madidi (Bolivia) as symbols of the preservation of the richest biodiversity hotspots. Among vertebrates, amphibians show high levels of diversity and endemicity in these areas (Köhler, 2000a). Several Peruvian and Bolivian species are today described and named every year both from the Andes (e.g. Padial, Chaparro & La Riva, 2006, 2007b; Duellman & Lehr, 2007; Lehr & Duellman, 2007) and from the Amazonian lowlands (e.g. Moravec, Aparicio & Köhler, 2006; Lehr, Torres & Suárez, 2007). However, these areas are still very poorly known in spite of the high rate of species discovery (Padial & De la Riva, 2006) and current conservation concern (Stuart *et al.*, 2004). Most species are discovered by means of standard exploration of remote or scarcely 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. The application of bioacoustics (e.g. Heyer, García-López & Cardoso, 1996; Angulo, Cocroft & Reichle, 2003; Padial *et al.*, 2008b) and molecular phylogenetics (e.g. Parra-Olea, García-París & Wake, 2004; Fouquet *et al.*, 2007; Lehtinen *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 than expected (Bickford *et al.*, 2007) both across taxa and across

*Corresponding author. E-mail: iriva@mncn.csic.es

geographical regions (Pfenninger & Schwenk, 2007). If this is true also for amphibians, the number of, for example, *Pristimantis*, with around 400 species described by the classical morphological approach based on evident qualitative characters, may increase considerably. Many *Pristimantis* are candidates for a speciation pattern particularly suitable to originate cryptic and sibling lineages (Lynch & Duellman, 1997).

Integrative taxonomy

Taxonomy provides the way to distinguish and communicate about living and fossil species. For taxonomists, morphology has been the commonest criterion to delineate and identify those species and, even today, most species descriptions are morphological. The incorporation of non-morphological suits of characters into taxonomy has been criticized, sometimes by taxonomists and sometimes by other users of species. For example, molecular phylogenetics contributed to a boost of new species that some applied biologists consider 'taxonomic inflation' rather than a real increase in species numbers (Isaac, Mallet & Mace, 2004). On the other side, molecular biologists who proposed new ways to identify species based on the comparison of short gene fragments (DNA barcoding or DNA taxonomy) received much criticism from a great part of the taxonomic community (for a recent review see Vogler & Monaghan, 2006). During this debate, a proposal arguing for the combined use of different suits of characters for species descriptions arose from the taxonomic community. This has been termed integrative taxonomy (Dayrat, 2005).

Several phylogenetic methods using combined evidence have been proposed to delineate species boundaries (Sites & Marshall, 2004). However, differences in the results obtained by different methods or different suites of characters (Wiens & Penkrot, 2002) hamper the incorporation of such methods into practice. This is one of the main sources of criticism against taxonomic practices, because uncertainty may lead to arbitrary decisions for species descriptions. As recognized by integrative taxonomists (Dayrat, 2005), the solution might lie in considering species as hypotheses. The conceptualization of species taxa as hypotheses is grounded in a general concept of species that consider a species to be a lineage of populations (or metapopulations) diverging separately from all other such lineages (De Queiroz, 2005a, b, c). The species, thus conceived, becomes a category of biological organization instead of a rank, and the only necessary and sufficient property of a species is that it represents a separately evolving fragment of a metapopulation lineage. Properties considered necessary by former species concepts (monophyly, diagnosability, potential interbreeding, etc.) are now considered contingent properties that represent thresholds crossed by diverging lineages after speciation, and are therefore indicators of the divergence of those lineages. Under this concept, the species is the only biological category above organism, speciation is the process of lineage splitting, and characters are not expected to differ in any predictable extent. Under integrative taxonomy, when naming new species, taxonomists should present different lines of evidence to support the hypothesis that a population is evolving independently. Thus, integrative taxonomy aims to break the circle of considering some characters better than others. Any kind of character is equally good. And any kind of character may be useful to propose species taxa hypotheses. By bringing together additional sorts of evidence, empirical analyses would allow us to reinforce, reject or reconcile hypotheses, making taxonomy a more reliable and scientific activity. Species taxa supported by several independent and coincident kinds of characters could be considered stable hypotheses. Integrative taxonomy thus becomes a new conceptual framework in which species are hypotheses, and in which independent suites of characters are used to construct stable species taxa hypotheses.

The practical application of this theoretical basis is exemplified in this study, where the integration of several independent lines of evidence (morphology, advertisement calls and phylogenetic analyses of partial 16S rDNA sequences) allows the description of two new cryptic species of *Pristimantis* and the solution of some old taxonomic problems.

Taxonomic background

This study centres on several species of Bolivian and Peruvian frogs of the genus *Pristimantis*. These species were formerly assigned to the genus *Eleutherodactylus*, which was subdivided into several species groups, species series and subgenera by Lynch & Duellman (1997). Frost *et al.* (2006) partitioned *Eleutherodactylus* into several genera that were formerly considered subgenera (*Eleutherodactylus*, *Craugastor*, *Syrrophus* and *Euhyas*). However, their analyses did not support the monophyly of *Eleutherodactylus*. A more recent phylogenetic analysis with broader taxon sampling has proposed new taxonomic rearrangements (Hedges, Duellman & Heinicke, 2008). The genus *Eleutherodactylus* was now restricted to a clade comprising Central American and Antillean species, *Craugastor* was restricted to a middle American clade, and *Pristimantis* was resurrected for the clade including South American species formerly included in the subgenus *Eleutherodactylus*. We follow the latter authors for the supraspecific taxonomy and therefore all species mentioned in our

study (formerly *Eleutherodactylus*) are considered *Pristimantis*.

Some Bolivian populations of *Pristimantis* remain with uncertain taxonomic status. On the one hand, this uncertainty was caused by the previous assignment of central Bolivia Andean populations of *Pristimantis* to four similar species: *P. fenestratus* (De la Riva, 1993), *P. peruvianus* (De la Riva, 1994), *P. samaipatae* (Köhler & Jungfer, 1995) and *P. dundeei* (Köhler, 2000a). On the other hand, several lowland and Andean foothills populations from southern Peru to central Bolivia were considered either *P. danae* or *P. peruvianus*. These problems have been recently discussed by Padial & De la Riva (2005a) and are resumed as follows.

Heyer & Muñoz (1999) described *Pristimantis dundeei* from the Cerrado savannahs of western Brazil. Köhler (2000a) cited this species 800 km south-westward in the Andean Amazonian slopes of Departmento de Santa Cruz, Bolivia. Padial & De la Riva (2005a) considered the comparisons of the advertisement calls reported by Köhler (2000a) to be inconclusive, and also stated that Andean populations lacked the basal webbing present in the type series of *P. dundeei*. They removed this species from the Bolivian species list but did not provide enough evidence to assign this Andean population to any other species. On the other side, Köhler (2000a) redescribed *P. peruvianus*, rejected that these Andean populations were *P. peruvianus* and removed this species from the Bolivian country list, as 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 *P. peruvianus* from seven localities in central and southern Peru showed intraspecific variability for these characters. Nevertheless, another species, *P. danae*, fell within this variability, and they attributed this fact to the existence of a putative cryptic species, more similar to *P. danae* than to *P. peruvianus*. Padial & De la Riva (2005a: p. 377) 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* . . .'

In summary, these problematic populations represent the target taxa for this study. The goal is to test the independence of these taxa from species to which they have been previously assigned: (1) *Pristimantis* sp. 1 (central Bolivia Andean populations previously assigned to *P. dundeei*, *P. fenestratus*, *P. peruvianus* and *P. samaipatae*); and (2) *Pristimantis* sp. 2 (lowland populations from southern Peru to central Bolivia assigned to *P. danae* and *P. peruvianus*).

MATERIAL AND METHODS

Taxon sampling

The analysis is structured in two steps. The first is the comparison of qualitative characters in species belonging to the same species group and biogeographical area as the target taxa. Species well distinguished by qualitative characters are not included in morphometric, bioacoustic or phylogenetic analyses. The second step lies in comparing the target taxa with those species morphologically similar in qualitative characters by means of three independent lines of evidence: morphometrics, quantitative and qualitative bioacoustics, and molecular phylogenetics.

Pristimantis dundeei, *P. fenestratus*, *P. peruvianus* and *P. samaipatae* are members of the *P. conspicillatus* Series and the *P. 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 South America (Frost, 2006). Only *P. w-nigrum* seems to occur on both sides of the Andes in Colombia and Ecuador (Lynch, 1975; Lynch & Duellman, 1997). The remaining species are either inhabitants of the lowlands or Andean foothills at the western or eastern flanks of the Andes. We reduced our sampling to cis-Andean regions where our target taxa occur (Upper Amazon basin and adjacent Andean hills of Peru and Bolivia). We exclude from our analyses those species inhabiting the western slopes of the Andes, Guayana Shield or the northern mountain ranges of Ecuador, Colombia and Venezuela. The species selected for diagnostic comparisons of qualitative characters with our target taxa are the following members of the *P. conspicillatus* Group: *P. avicuporum*, *P. bipunctatus*, *P. buccinator*, *P. caliginosus*, *P. citriogaster*, *P. condor*, *P. cosnipatae*, *P. conspicillatus*, *P. crepitans*, *P. cuneirostris*, *P. dundeei*, *P. fenestratus*, *P. lanthanites*, *P. lymani*, *P. malkini*, *P. metabates*, *P. peruvianus*, *P. samaipatae*, *P. skydmainos* [comprising *P. karcharias* (see Padial & De la Riva, 2005b)], *P. vilarsi* and *P. zeutoctylus*. *Pristimantis bisignatus*, a former member of the *P. conspicillatus* Group (Köhler, 2000a), is not included because molecular, bioacoustic and morphological evidence places it in a different group (Padial *et al.*, 2007a). We include *P. danae*, a member of the *P. unistrigatus* Group of Lynch & Duellman (1997), because Padial & De la Riva (2005a) considered that a putative undescribed species similar to *P. danae* might be hidden under what they called *P.* cf. *peruvianus*. Therefore, to study and diagnose this putative new taxon we compared it with some similar species of the *P. unistrigatus* Group inhabiting the Upper Amazon basin and adjacent hills: *P. altamazonicus*, *P. carvalhoi*, *P. croceoinguinis*, *P. diadematus*, *P. eurydactylus*, *P. martiae*, *P. ockendeni*,

P. platydactylus, *P. rhabdolaemus*, *P. salaputium*, *P. toftae* and *P. ventrimarmoratus*.

Morphological characters

Qualitative morphology has been the most commonly used line of evidence to describe and name species taxa. In this sense, the holotype has two functions: to point out specific differences, and to be the name-bearing semaphoront that represents a species taxon. Therefore, an integral part of any testing of species taxa hypotheses is the study and comparison of types, paratypes or original descriptions (when accurate enough). We reviewed holotypes or paratypes of species taxa considered in this study (see Appendix), and also compared additional museum specimens for all species to assess intraspecific variation. We followed Lynch & Duellman (1997) for qualitative morphological character states used in the diagnoses and descriptions of *Pristimantis*. We followed Grant & Kluge (2004) for the character concept in systematics as transformations series. Therefore, all morphological characters considered herein represent character states in an evolutionary transformation series. We focused on the main characters used (see, for example, Lynch, 1980; Köhler & Jungfer, 1995; Lynch & Duellman, 1997; Duellman & Pramuk, 1999; Duellman & Hedges, 2005; Padial & De la Riva, 2005b) to diagnose species within the *Pristimantis conspicillatus* Group (character states in parentheses): relative length of first and second fingers (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); colour pattern of posterior surfaces of thighs (well-defined spots, barely visible or diffuse spots, marmored, plain); colour pattern of throat, chest and belly (heavily spotted, weakly spotted, immaculate); and adult male nuptial pad on thumb (double, single, absent). Qualitative morphological characters are independent of morphometric characters (see below).

For morphometrics, a single person (J.M.P.) took measurements with a digital calliper to the nearest 0.01 mm, but following Hayek, Heyer & Gascon (2001), to avoid 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 of Finger III, F3; width of disc of 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 of 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 greatly influences the measurements and makes it difficult to have precise and comparable values for large series (but see Arroyo *et al.*, 2005). Colour characteristics were noted in life and in alcohol. We determined age and sexual condition by dissection or by 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 S1.

Bioacoustics

In anurans, taxonomic hypotheses on species taxa often rely on differences in mating calls as evidence for prezygotic reproductive barriers (Vences & Wake, 2007). 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). The study area includes the Andean slopes between 500 and 3000 m asl and adjacent lowlands, from central to northern Bolivia. We collected voucher specimens and recorded advertisement calls along this latitudinal axis. This comprises inter-Andean dry valley forest, humid forest of the Andean slopes, humid montane forests, the Yungas (cloud forests) and the Amazonian lowland forests [see Köhler (2000a) for more details about these 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 for MacOS X (BIAS, 2002) 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 & Weenick, 2006) software was used 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 S2. 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, La Riva & Bosch, 1995; Köhler, 2000a; Bosch & De la Riva, 2004; Padial *et al.*, 2008b). Terminology in call descriptions generally follows Márquez *et al.* (1995) and Köhler (2000a). Sample sizes do not allow for temperature correction using regression.

Statistical analyses of quantitative data

Principal component analyses (PCAs) of bioacoustic and morphometric characters are aimed to identify groups corresponding to species cryptic in qualitative morphological characters. PCA is not a clustering technique nor is it designed to discriminate groups, but can provide a representation of data useful to identify groups that may be related to previous assumptions about taxa without a priori subdivisions of the samples into discrete units (Wiley, 1981). By contrast, stepwise discriminant function analysis (DFA) is used to distinguish predefined groups by minimizing variation within groups and maximizing variation between groups. PCA is used to detect groups representing putative cryptic species, and DFA is subsequently applied to identify the set of characters that better diagnose those groups. Both PCA and DFA were performed with JMP 5.0.1.a (SAS Institute Inc.) on log10-transformed variables. PCAs were performed on correlations. DFAs were performed stepwise with an alpha limit of $P < 0.01$ for the inclusion of additional variables. Bioacoustic com-

Table 1. Numerical characteristics and sample sizes of the advertisement call of six *Pristimantis* species included in this study

	Notes/call	Call length (ms)	Note length (ms)	Note rate	Pulses	Fundamental frequency (Hz)	Dominant frequency (Hz)	Notes	Calls	No. of species	No. of populations
P. fenestratus	2.0–4.0	157–458	50.0–91.0	7.7–12.7	9–17	1542–2048	1710–3591	55	22	6	4
	(2.6 ± 0.6)	(265.2 ± 81.6)	(63 ± 11.4)	(10.1 ± 1.5)	(12.9 ± 42.2)	(1746.7 ± 158)	(3086.3 ± 580.7)				
P. koehleri	3–8	173–644	20–54	11.8–17.3	5–9	1732–1971	3245–3971	119	21	6	2
	(5.7 ± 1.0)	(421 ± 159.8)	(35.5 ± 6.6)	(14.1 ± 1.8)	(7.5 ± 1)	(1853.5 ± 72.1)	(3662.4 ± 128.9)				
P. samaipatae	1.0–3.0	82.2–1062	59–141	2.7–14.9	11–23.0	1535.0–1834.0	2922–3853	160	98	12	4
	(2 ± 0.2)	(291.7 ± 168.1)	(89 ± 16.4)	(8.5 ± 2.1)	(16.4 ± 2.6)	(1704.9 ± 64.3)	(3326.7 ± 175.9)				
P. danae	1	7–13	7–13	76.9–142.9	1–2	1369–2925	1369–2925	87	87	4	2
		(11 ± 1.2)	(11 ± 1.2)	(92.3 ± 12.6)	(1.9 ± 0.2)	(2210 ± 553.4)	(2210 ± 553.4)				
P. reichlei	2–3	50–268	20–58	11.2–40	4.0–11	2013–2815	2013–2815.0	137	63	5	3
		(143.9 ± 52.2)	(32 ± 5.8)	(16.9 ± 6.3)	(6.7 ± 1.2)	(2501.4 ± 197.7)	(2501.4 ± 197.7)				

Mean ± standard deviation in parentheses follows ranges. See text for further explanations and Appendix S2 for Fonozoo collection numbers, locality, temperature and voucher information.

parisons were performed on mean values of specimen calls. See Table 1 for bioacoustic variables and Table 4 for morphometrics. The scarce number of samples for *Pristimantis danae* did not allow morphometric comparisons with females of *Pristimantis* sp. 2.

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 of *P. samaipatae* were collected in two localities close to the type locality. The vague type locality of *P. fenestratus* 'Río Mamoré' belongs to the Bolivian–Brazilian Amazon Basin. We gathered tissues from different localities in the Bolivian Amazon basin and adjacent Andean slopes that are considered conspecific with *P. fenestratus* (De la Riva *et al.*, 2000). Tissue samples for *P. danae* were collected both at the type locality and from scattered localities along the Bolivian Andes. Tissue samples of *P.* cf. *peruvianus* were collected along the Andean hills of the Amazon basin in Bolivia and Peru. Other species putatively related to *P. danae* (*P. rhabdolaemus*, *P. toftae* and *P. platydactylus*) from the Bolivian and Peruvian Andean hills were included in the analysis.

According to Hedges *et al.*'s (2008) review, *Oreobates* is basal to *Pristimantis*. The choice of *Oreobates* as outgroup seems appropriate. We selected four species assigned to *Oreobates* by Padial *et al.* (2008a). We used the standard phenol/chloroform extraction protocol (Sambrook, Fritsch & Maniatis, 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 (GR Health Care) instrument following the manufacturer's 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. Two ambiguously aligned regions of around 60 and 20 bp 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 resulting from alignment under default parameters and posterior editing by eye, but has the convenience of allowing repeatability. Sequences are available from GenBank (Table 2). We used the program MODELTEST 3.7 (Posada & 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). Neighbour-joining (NJ) analyses were performed using PAUP* 4.0b10 (Swofford, 1998), with maximum-likelihood (ML) genetic divergence corresponding to the model. 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 ten 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.

RESULTS

Pristimantis sp. 1.

Comparative analyses of qualitative morphological characters allow distinguishing *Pristimantis* sp. 1 from most members of the *Pristimantis conspicillatus* Group (Table 3). It remains cryptic to *P. fenestratus* and barely distinguishable from *P. samaipatae*. In PCAs of female and male measurements (Fig. 1) the first component explains 78.2 and 60.3% of variability, respectively. For both data sets, the first component seems to represent a cline in body size from *P. samaipatae* (the largest species) to *Pristimantis* sp. 1. This analysis distinguishes almost completely *Pristimantis* sp. 1 from *P. fenestratus* (overlap in larger sizes) and completely from *P. samaipatae*. *Pristimantis fenestratus–P. samaipatae* are not distinguished. In DFA, the most significant diagnostic variables for adult females were TH ($F = 17.9$, $P < 0.001$), FL ($F = 9.9$, $P < 0.001$) and FA ($F = 5.36$, $P < 0.01$). This model resulted in eight misclassifications (13.8%, $N = 58$), six for the pair *P. samaipatae–P. fenestratus*, and two for the pair *Pristimantis* sp. 1–*P. fenestratus*.

Table 2. Localities, voucher information, and GenBank accession numbers for sequences and specimens used in this study

Species	DNA collection MNCN	Vouchers	Locality	Accession number
Pristimantis				
danae	547	IDLR 4001	Bolivia: La Paz: Santa Cruz de Valle Ameno.	EU192260
danae	5798, 5837	MNK-A 7182, MNCN 43062	Bolivia: La Paz: Huairuro, senda San José – Apolo	EU192261-2
danae	6005, 6040	MNCN 43069, MNK-A 7190	Bolivia: La Paz: Arroyo Huacataya. senda San José y Apolo	EU192263-4
danae	6258	MNK-A 7273	Bolivia: La Paz: Serranía Bella Vista	EU192265
danae	20677	IDLR 4815	Peru: Cusco: Unión, Valle de Kosñipata	EU192266
danae	20682	MNCN 44232	Peru: Cusco: Unión, Valle de Kosñipata	EU192267
danae	20683	MNCN 44233	Peru: Cusco: Unión, Valle de Kosñipata	EU192268
danae	20684	IDLR 4822	Peru: Cusco: Unión, Valle de Kosñipata	EU192269
danae	20685	MNCN 44234	Peru: Cusco: Unión, Valle de Kosñipata	EU192270
danae	20686	IDLR 4824	Peru: Cusco: Unión, Valle de Kosñipata	EU192271
danae	20687	IDLR 4825	Peru: Cusco: Unión, Valle de Kosñipata	EU192272
fenestratus	3947	MNK-A 6629	Bolivia: La Paz: Chalalán	EU192273
fenestratus	3981	MNK-A 6630	Bolivia: La Paz: Sadiri, Arroyo Yariapo	EU192274
fenestratus	9496	MHNC 3130	Peru: Madre de Dios: Cocha Camungo	EU192277
fenestratus	4108, 4109, 4088	MNCN 43031, MNK-A 6633, MNK-A 6631,	Bolivia: Cochabamba: Los Guácharos	EU192276, EU1922561, EU192275
koehleri	3903, 3905	MNCN 42990, MNK-A 6627	Bolivia: Santa Cruz: Km 6 Angostura–Samaipata road	EU192278-9
koehleri	4001–2, 4016	MNCN 42983, 43013, 42986	Bolivia: Santa Cruz: La Chonta	EU192280-2
platydactylus	3919	MNK-A6594	Bolivia: Santa Cruz: Siberia	EU192283
platydactylus	3929	MNCN43003	Bolivia: Cochabamba: Sehuencas	EU192284
reichlei	4084–5	MNCN 43012, MNK-A 6621	Bolivia: Cochabamba: Los Guácharos	EU192286-7
reichlei	5542	MNCN 43249	Peru: Cusco: 5 km from San Lorenzo hacia Quince Mil	EU192288
reichlei	20642	IDLR 4779	Peru: Puno: Entre Puerto Leguia y San Gabán	EU192285
rhabdolaemus	3940	MNK-A 6628	Bolivia: Santa Cruz: Serranía de la Siberia	EU192258
rhabdolaemus	4120	MNCN 43036	Bolivia: Santa Cruz: La Yunga de Mairana	EU192257
samaipatae	3899–02	MNCN 42987–9, MNK-A 6626	Bolivia: Santa Cruz: Km 6 Angostura–Samaipata road	EU192289-92
toftae	4093	MNCN 43025	Bolivia: Cochabamba: Los Guácharos	EU192293
toftae	5505	MNCN 43246	Peru: Cusco: San Pedro, Valle de Marcapata	EU192294
Oreobates				
cruralis	6098	MNK-A 7171	Bolivia: Santa Cruz: Camino a Bella Vista	EU192295
discoidalis	6123	MNK-A 7247	Bolivia: Tarija: Serranía Aguarague	EU192296
heterodactylus	6061	MNK-A 7175	Bolivia: Santa Cruz: Cerro del Arco, Serranía de Santiago	
quixensis	6216	MNCN 43147	Bolivia: Pando: San Sebastián, Tahuamanu	EU192297

Abbreviations: IDlR, Ignacio De la Riva'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.

Table 3. Comparison of diagnostic characters between cis-Andean Amazonian species of the *Pristimantis conspicillatus* Group plus *P. danae* and *P. cosnipatae*: (1) relative length of first and second fingers (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) colour pattern of posterior surfaces of thighs (well-defined spots, barely visible or diffuse spots, marmored, plain); (12) colour pattern of throat, chest and belly (heavily spotted, weakly spotted, immaculate); (13) adult male nuptial pad on thumb (double, single, absent)

	Diagnostic character states												
	1	2	3	4	5	6	7	8	9	10	11	12	13
P. avicuporum	I > II	Granular	Shagreen	Fin-shaped, interocular fold	Present	Weak	Absent	Present	Present	Present	Barely visible or diffuse spots	Weakly spotted	Single
P. bipunctatus	I = II	Granular posterolaterally	Coarsely shagreen	Absent	Present	Absent	Prominent	Present	Present	Present	Well-defined spots	Marmored	–
P. buccinator	I = II	Smooth	Coarsely shagreen	X-shaped, interocular fold	Present	Absent	Weak	Absent	Present	Present	Barely visible or diffuse Spots	Weakly spotted, immaculate	Single
P. caliginosus	I > II	Smooth	Shagreen	Absent	Present	Weak	–	Present	–	Present	Barely visible or diffuse spots	Weakly spotted	–
P. citriogaster	I > II	Smooth	Shagreen	Absent	Absent	Absent	Prominent	Absent	Present	Present	Marmored	Heavily spotted	Single
P. condor	I > II	Smooth	Shagreen	Absent	Present	Weak	Weak	Present	Absent	Present	Well-defined spots	Heavily spotted	–
P. conspicillatus	I > II	Smooth	Finelly shagreen	Absent	Present	Weak	Weak	Absent	Absent	Variable	Well-defined spots	Well-defined spots	Single
P. cosnipatae	II > I	Coarsely granular	Finelly shagreen	Warts	Present	Absent	Weak	Absent	Present	Present	Plain	Weakly spotted	–
P. crepitans	I > II	Smooth	Warty	Absent	Absent	Absent	Absent	Absent	Present	Present	Plain	Inmaculate	Single
P. danae	I < II	Coarsely granular	Finelly shagreen	Absent	Absent	Present	Prominent	Absent	Present	Variable	Well-defined spots	Weakly spotted	Absent
P. dundeei	I > II	Granular	Shagreen	Flat warts	Absent	Absent	Prominent	Present	Present	Present	Plain	Weakly spotted	Double
P. fenestratus	I > II	Smooth, granular posterolaterally	Shagreen	Absent	Absent	Weak	Weak	Present	Present	Present	Plain	Variable	Double
P. lanthanites	I ≥ II	Smooth, granular posterolaterally	Shagreen	Fin shaped, interocular, calcar	Absent	Absent	Weak	Absent	Present	Present	Plain	Heavily spotted	Absent
P. malkini	I > II	Smooth	Finelly shagreen	Warts	Absent	Absent	Prominent	Present	Present	Present	Marmored	Inmaculate	Single
P. peruvianus	I > II	Smooth	Finelly shagreen	Absent	Present	Absent	Absent	Present	Present	Variable	Well-defined spots	Variable	Single
P. samaipatae	I > II	Smooth	Finelly shagreen	Absent	Absent	Absent	Prominent	Absent	Present	Present	Plain	Inmaculate	Double
P. skydmainos	I ≤ II	Granular posterolaterally	Finelly shagreen	Fin-shaped, interocular	Present	Weak	Weak	Present	Present	Present	Plain	Barely visible or diffuse spots	Single
P. vilarsi	I > II	Smooth	Coarsely shagreen	Absent	Absent	Absent	Absent	Present	Present	Variable	Plain	Weakly spotted	Single
Pristimantis sp. 1	I > II	Granular posterolaterally	Finelly shagreen	Absent	Absent	Absent	Weak	Absent	Present	Present	Plain	Plain	Double
Pristimantis sp. 2	I ≤ II	Coarsely granular	Finelly shagreen	Absent	Absent	Present	Prominent	Absent	Present	Absent	Well-defined spots	Weakly spotted	Single

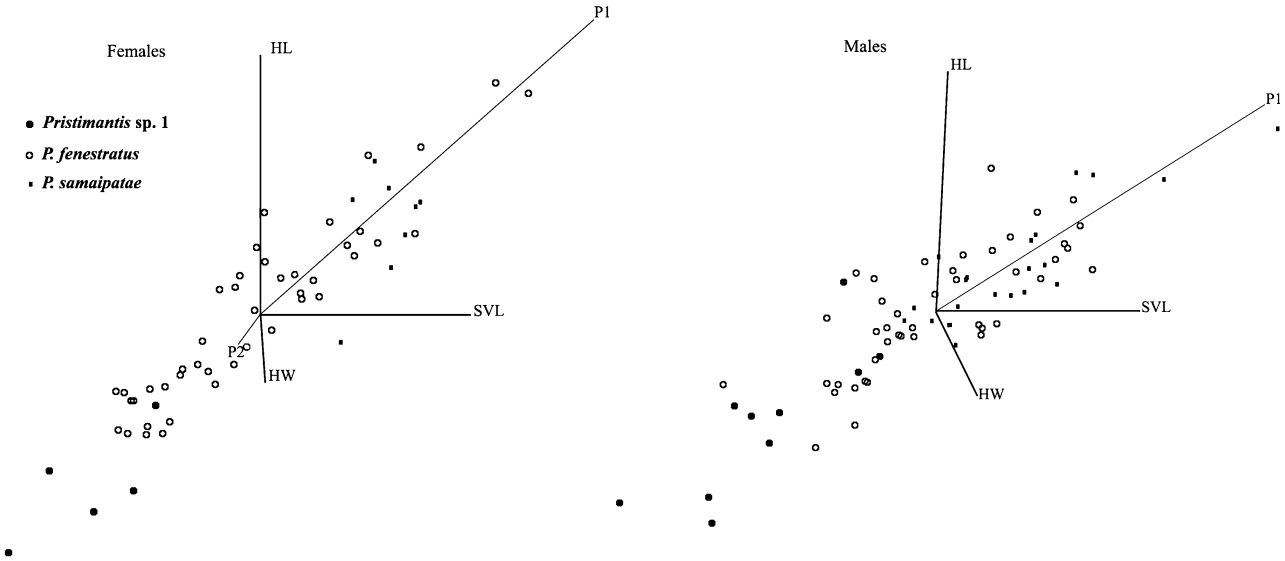

Figure 1. Principal component analysis (PCA) of morphometric characters of adult females and adult males of *Pristimantis* sp. 1, *P. fenestratus* and *P. samaipatae*. Abbreviations are: P, principal component; SVL, snout–vent length; HL, head length; HW, head width.

For adult males, FL ($F = 13.3$), FA ($F = 13.2$) and F3 ($F = 4.3$) were the most significant variables, with 15 misclassifications (20%, $N = 74$), 11 for *P. fenestratus*–*P. samaipatae*, three for *Pristimantis* sp. 1–*P. fenestratus* and one for *Pristimantis* sp. 1–*P. samaipatae*.

The call of *Pristimantis* sp. 1 is very similar in general structure to the call of *P. fenestratus* and *P. samaipatae* (Fig. 2). 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). *Pristimantis samaipatae* is the species with the lowest number of notes per call, generally only one. *Pristimantis fenestratus* tends to emit 2–3 notes, while the number of notes emitted by *Pristimantis* sp. 1 is usually higher than five. PCA separates the three species (Fig. 3). The three first components explain most variation (63.1, 15.8, 13.0, respectively) related to the length of the call, the numbers of notes and the number of pulses. The second component mostly explains variation in dominant frequency. In DFA, the most significant diagnostic variable was number of pulses ($F = 93.2$, $P < 0.0001$). This model resulted in four misclassifications (17%, $N = 24$), three for *P. fenestratus*–*P. samaipatae* and one for *Pristimantis* sp. 1–*P. fenestratus*.

MP and NJ majority rule-consensus boostrap analyses and Bayesian phylogenetic analyses (MB) support the reciprocal monophyly of *Pristimantis* sp. 1, *P. fenestratus* and *P. samaipatae* (Fig. 4). These three taxa form a well-supported clade in which *Pristimantis* sp. 1 is sister to *P. fenestratus*. Additionally, uncorrected pairwise distances between *Pristimantis* sp. 1–*P. fenestratus*, *Pristimantis* sp. 1–*P. samaipatae* and *P. fenestratus*–*P. samaipatae* range from 2.9 to 3.3 (3.0 ± 0.2), 2.9 to 4.7 (4.5 ± 0.5) and 5.5 to 6.2 (5.8 ± 0.2), respectively (mean and standard deviation in parentheses). The lowest distances (*Pristimantis* sp. 1 and *P. fenestratus*) fall within values for interspecific genetic distances in relation to other neotropical amphibians, where the mean value has been established at around 3% (Fouquet *et al.*, 2007). Among the genus *Pristimantis*, this value is similar to interspecific distances found between some members of the subgenus *Yunganastes* (Padial *et al.*, 2007a), while it is lower than those shown by species belonging to the genus *Oreobates* (Padial *et al.*, 2008a).

In summary, the independence of *Pristimantis* sp. 1, *P. fenestratus* and *P. samaipatae* is not supported by qualitative morphological characters; it is supported, however, for *Pristimantis* sp. 1 by morphometric characters, and for the three taxa by bioacoustic and molecular characters. As different independent lines of evidence support the independence of *Pristimantis* sp. 1 from related species (Table 6), we describe it as a new species (see below).

PRISTIMANTIS SP. 2.

Pristimantis sp. 2 is morphologically distinguishable from all species of the *Pristimantis conspicillatus* Group, but remains cryptic in qualitative characters to *P. danae*, a member of the *P. unistrigatus* Group

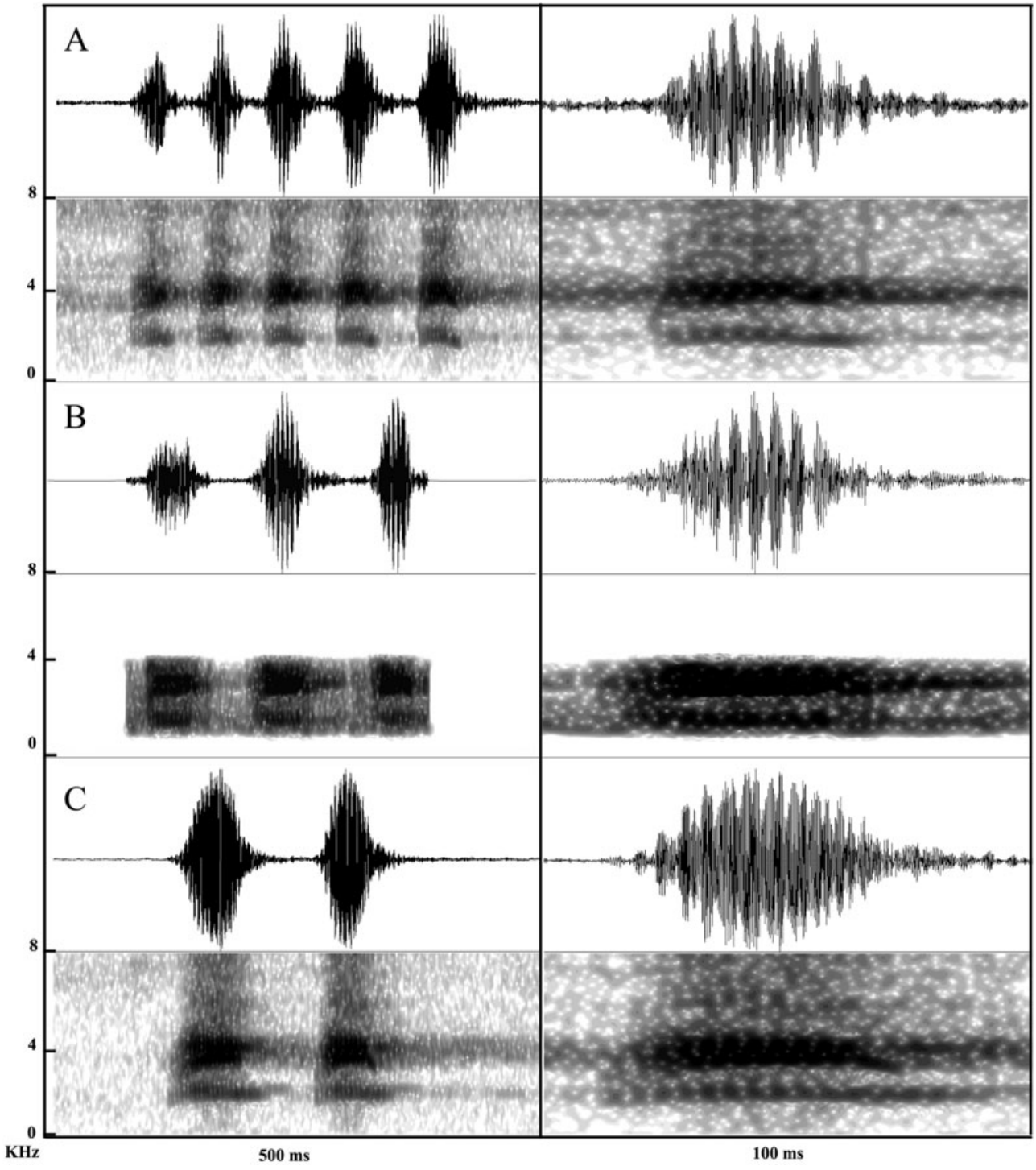

Figure 2. Oscillograms and audiospectrograms of the advertisement call of *Pristimantis* sp. 1 (A), *P. fenestratus* (B) and *P. samaipatae* (C).

(Table 3). PCA on morphometrics did not to separate *Pristimantis* sp. 2 from *P. danae*. DFA of male morphometrics separated both taxa through a model including EL ($F = 121.2$, $P < 0.001$) and HW ($F = 55.1$, $P < 0.001$) that resulted in two misclassifications (3.6%, $N = 56$). These correspond to specimen MNK-A 4743 from Serranía de Chepite (79% probability for *P. danae*) and specimen MNK-A 3705 from Serranía Beu

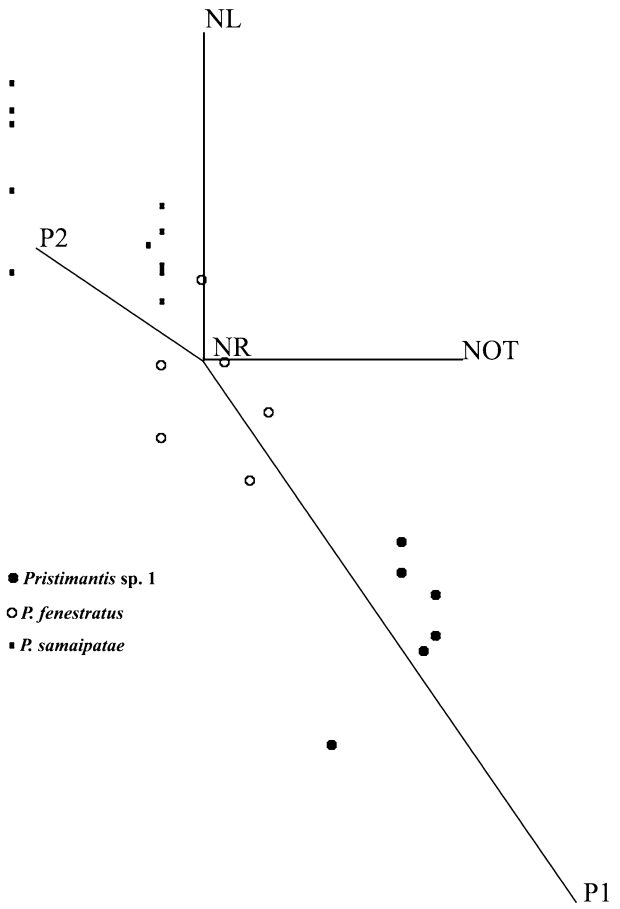

Figure 3. Principal component analysis (PCA) of bioacoustic characters for *Pristimantis* sp. 1, *P. fenestratus* and *P. samaipatae*. Abbreviations are: P, principal component; NOT, numbers of notes; NL, note length; NR, note rate.

(62% probability for *P. danae*), both from the Andean slopes of the Pilón-Lajas Biosphere Reserve, Bolivia.

Qualitative structural characters of advertisement calls allow a clear distinction of *Pristimantis* sp. 2 and *P. danae* (Fig. 5). The call of *Pristimantis* sp. 2 consists of 2–3 highly pulsed (4–11 pulses per note) amplitude-modulated notes (Table 1), while the call of *P. danae* consists of a very short note usually composed of two pulses (single-pulse notes sometimes emitted). The first pulsed note of the call of *Pristimantis* sp. 2 is generally shorter, while the second may show some modulation in frequency. PCAs allow a clear distinction of both taxa (Fig. 6). The first component explains 81.8% of variance and the second 18%. Both components explain the variation of the four variables (note length, number of pulses, dominant frequency and fundamental frequency). In the second component, frequency variables are inversely related to dominant frequency and number of pulses. DFA resulted in a model including only NL ($F = 261.1$, $P < 0.0001$) that significantly distinguished both taxa without misclassifications.

Pristimantis sp. 1 is reciprocally monophyletic to *P. danae* in MP and NJ analyses. Both taxa fall within a main clade that includes members of the *P. unistrigatus* Group. *Pristimantis* sp. 2 and *P. danae* appear as sister taxa in the NJ tree with no support, while in the MP tree *Pristimantis* sp. 2 is the sister taxon to *P. rhabdolaemus*. The Bayesian phylogenetic analysis places *Pristimantis* sp. 2 as paraphyletic to *P. danae*. Additionally, uncorrected pairwise distances for the pair *P. danae–Pristimantis* sp. 2 are comparatively high, 8.9–10.8 (9.7 ± 0.6), in relation to other amphibians (Vences *et al.*, 2005).

In summary, qualitative or quantitative morphological characters do not support the independence of *Pristimantis* sp. 2 from *P. danae*. It is supported, however, by qualitative and quantitative differences in bioacoustic and molecular characters (Table 6). As different independent lines of evidence support the independence of *Pristimantis* sp. 2 we describe it as a new species (see below).

TAXONOMY

PRISTIMANTIS KOEHLERI SP. NOV. (Fig. 7A)

Holotype: MNK-A 6626 (field number JMP 033), an adult male from km 6 of Angostura–Samaipata road, Departamento Santa Cruz, Bolivia (18°11′S, 63°34′W), collected by J. M. Padial, 03.i.2003.

Paratopotypes: MNCN 42990-1, MNK-A 6627 (adult males, field numbers JMP 031–3), same data as the holotype.

Paratypes: Bolivia, Departamento Santa Cruz: 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 (17°50′S/63°25′W), collected by J. M. Padial and E. Ávila, 26.xi.2003; MNCN 42983, 42985–6, 43014 (subadult females, field numbers JMP 152, 173, 184 and 153, respectively) from La Chonta, Amboró National Park (17°39′36″S, 63°42′6.6″W) collected by J. M. Padial and R. de Sá, 21–22.iv.2003, and MNCN 43040 (adult male, field number JMP 377) collected at the same locality by J. M. Padial & E. Ávila, 05.xi.2003; ZFMK 80005–6 (adult males), ZFMK 80007 (adult female) from Macuñucú, Amboró National Park collected by J. Köhler and S. Lötters, 2.xii.1998; ZFMK 79991 and 79993 (adult females) and 79992 (juvenile) from Mataracú, Amboró National Park, collected by J. Köhler and S. Lötters, 14.i.1999.

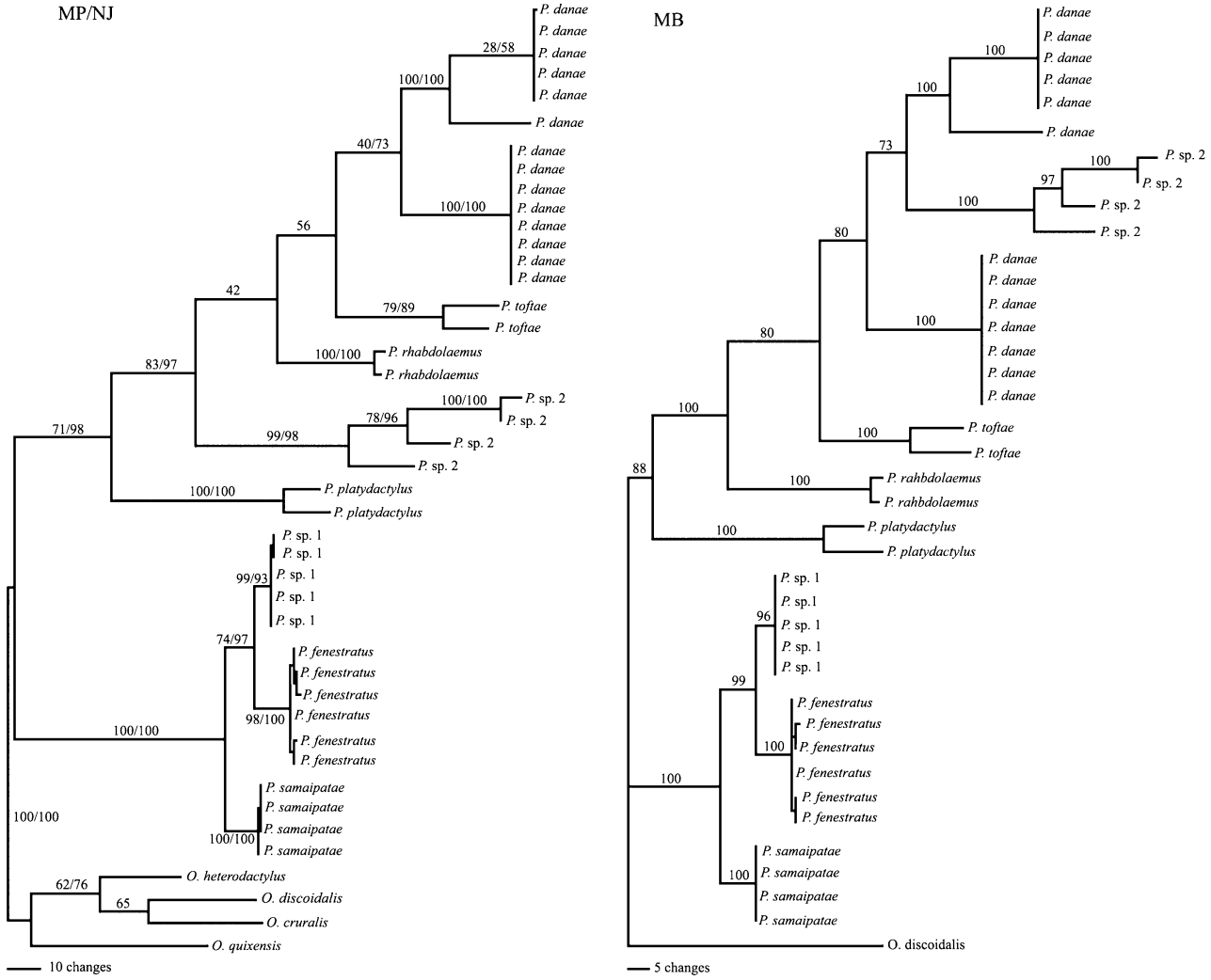

Figure 4. Majority rule consensus trees based on maximum parsimony (MP) and Bayesian (MB) phylogenetic analyses of partial 16S rDNA (*c*. 590 bp) of several *Pristimantis* selected for this study. The outgroup is composed of members of the genus *Oreobates*. Single values on the MP tree (left) correspond to MP boostrap values. When the MP topology coincides with neighbour-joining (NJ) topology (not illustrated) two values are shown (the second representing NJ boostrap values). Values in the MB tree are Bayesian posterior probabilities.

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 *Pristimantis conspicillatus* Group, as defined by Lynch & Duellman (1997), characterized by: (1) skin on dorsum coarsely shagreen, flanks with larger granules; venter finely 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 tubercles round, smaller than subarticular tubercles; terminal discs of inner

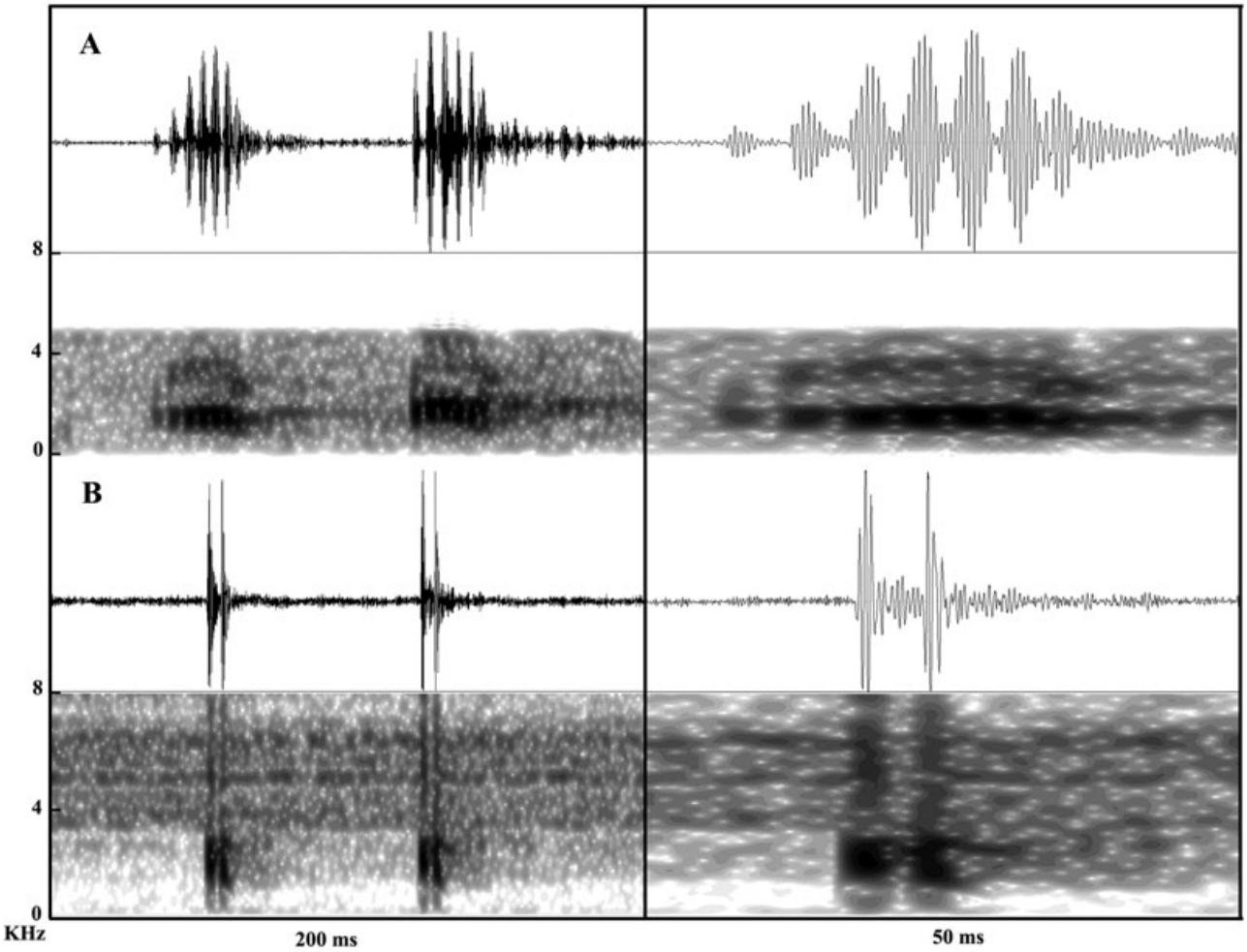

Figure 5. Oscillograms and audiospectrograms of the advertisement call of *Pristimantis* sp. 2 (A) and *P. danae* (B).

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 *Pristimantis fenestratus* (Fig. 7B), *P. dundeei* and *P. samaipatae* in qualitative characters (Table 3). It differs, however, from these three species as follows. From *P. dundeei* by lacking warts on dorsal skin, by lacking basal toe webbing and by having less developed finger fringes. From *P. fenestratus* by lacking basal toe webbing and having smaller size (SVL) of adult males (23.8–29.4 vs. 26.0–34.7) and females (34.0–39.5 vs. 37.8–57.2). From *P. samaipatae*, by having granular skin on belly (smooth), weak finger fringes, and smaller size (SVL) of adult males (23.8–29.4 vs. 30.1–40.0) and females (34.0–39.5 vs. 44.4–51.4). For additional differences among these three species see Table 3 and results of morphological, bioacoustic and molecular analyses above. For differences of *P. koehleri* and other members of the group see Table 3.

Description of the holotype: Head longer than wide (head length/head width = 1.2); snout acuminate in dorsal view and subacuminate in lateral profile; nostrils slightly protuberant, orientated posterolaterally; canthus rostralis straight in dorsal view, sharp in

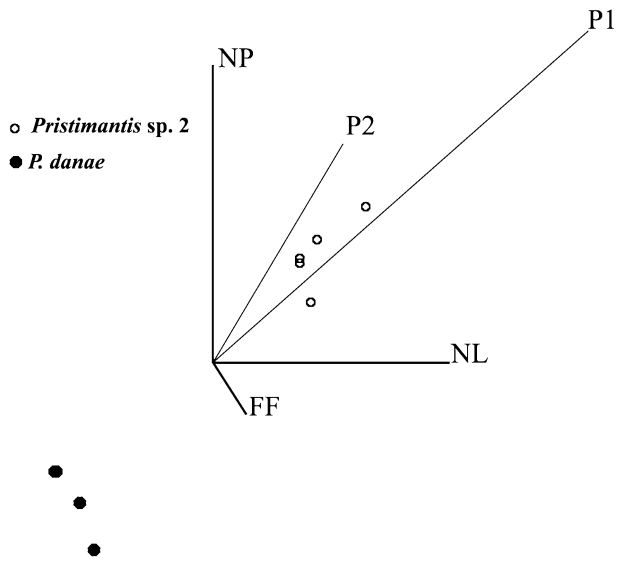

Figure 6. Principal component analysis (PCA) of bioacoustic characters for *Pristimantis* sp. 2 and *P. danae*. Abbreviations are: P, principal component; NP, number of pulses; NL, note length; FF, fundamental frequency.

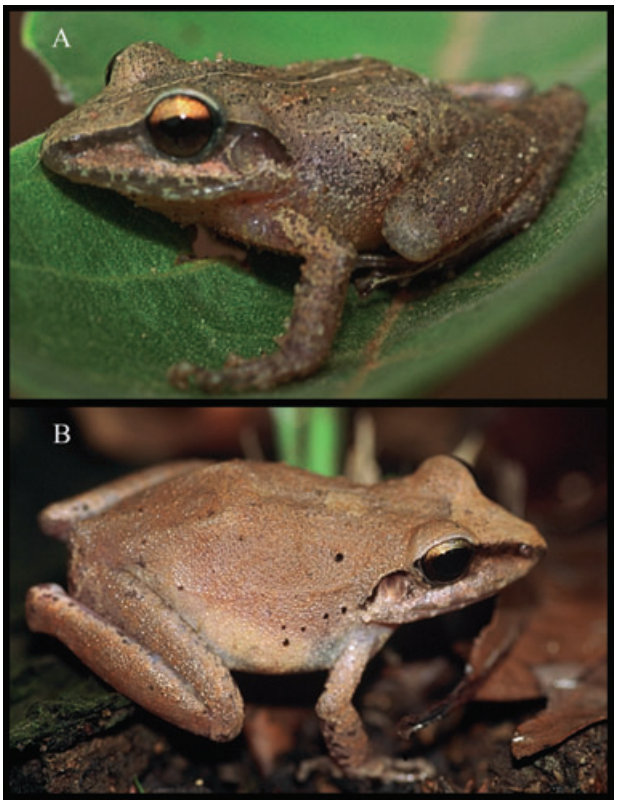

Figure 7. A, adult male of *Pristimantis koehleri* from Km 6 of Angostura–Samaipata road, Departamento Santa Cruz, Bolivia (one from the type series MNK-A 6626–7, MNCN 42990–1); B, adult female of *Pristimantis fenestratus* from Chalalán, Departamento La Paz, Bolivia (one from the series MNCN 43239–44).

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 to 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, nonspinous 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; 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 greyish-brown mottling; posterior and anterior surfaces of hind limbs brown without spots. The colour pattern in life is very similar, but greyish 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.

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, e.g. MNCN 42986, 43054 or MNK-A 7170, 7172, present a fine mid-dorsal stripe from tip of snout to vent. Some dark dorsal marks, such 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 inter-Andean dry valleys of the Departamento de Santa Cruz extending to the northwest along the humid forests of the Andean slopes (see Fig. 8). De la Riva (1994) cited this species as *Pristimantis peruvianus* from Amboró National Park (Departamento Santa Cruz), Bulo Bulo and Valle de Sajta (Departamento Cochabamba). Köhler (2000a) cited this species as *P. dundeei* for Macuñucú and Mataracú, along 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 *Pristimantis dundeei*). His data for the calls are similar to those provided by us, although they differ by a longer note length reported by Köhler (2000a). Reichle's (2002) recording for *P.* cf. *peruvianus* corresponds to *P. koehleri*. Specimens cited as *P. peruvianus* by De la Riva (1994) also correspond to *P. koehleri* as well as Bolivian specimens cited as *P.* cf. *peruvianus* by Padial & De la Riva (2005a). With the description of *P. koehleri*, *P. dundeei* would no longer occur in Bolivia, according to previous data. However, specimens from Noel Kempff Mercado National Park, Departamento Santa Cruz, Bolivia (see Appendix S1) represent the first country record of *P. dundeei*.

PRISTIMANTIS REICHLEI SP. NOV. (Fig. 9A)

Holotype: MNK-A 6620 (field number JMP 286), an adult female from Los Guácharos 500 m asl, Carrasco National Park, Provincia Chapare, Departamento Cochabamba, Bolivia (17°03′51.5″S/65°28′34.7″W) collected by J. M. Padial and D. Embert, 9.vii.2003.

Paratopotypes: MNCN 43012 (adult female, field number JMP 295), 43024 (adult female, field number JMP 303), 43028 (adult male, field number JMP 313), MNK-A 6621 (adult female, field number JMP 296), collected by J. M. Padial and D. Embert, 10–14.vii.2003, CBG 327 (adult male), 328 (adult female), 329 (adult male), collected by R. Aguayo.

Paratypes: BOLIVIA: Departamento Cochabamba: ZFMK 72587–9, 72564–5, 72537 a locality between Paractito and El Palmar, Carrasco National Park, collected by J. Köhler and S. Lötters, 16–18.xii.1998; ZFMK 66973–6, 66988, from a point between Parajti and El Palmar, Carrasco National Park, collected by J. Köhler and S. Lötters, 3–6.ii.1998; ZFMK 59574, from Villa Tunari, collected by P. Ibisch, 22.viii.1991; Departamento La Paz: MNCN 43071–2 (adult males, field numbers 596–7), MNK-7193 (adult male, field number 595), from Arroyo Huactaya, Madidi National Park (14°20′12.1″S, 68°05′57.3″W) collected by D. Embert, 16.xii.2003; MNK-A 7273 (adult male, field number JMP 952) from Serranía de Bella Vista, road between Caranavi and Palos Blancos, collected by J. M. Padial and C. Ureña, 07.iii.2004; MNK-A 7178, from Chalalán, Area Natural de Manejo Integrado Madidi (14°25′28.4″S, 67°55′14.4″W), collected on 13.xii.2003 by J. 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, 25.i.2005; MNCN 43151, Florida, Reserva Nacional de Vida Silvestre Manuripi (immature female) collected by M. 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, 22.ii.2006. Departamento Huánuco: NMW 28966 (ten specimens, two adult females and eight juveniles) from Río Llullapichis, Panguana, 220 m, collected by M. Aichinger;

Table 4. Morphometrics of adult specimens of *Pristimantis koehleri*, *P. fenestratus* and *P. samaipatae*

	Adult females			Adult males		
	P. koehleri ($N = 5$)	*P. fenestratus* ($N = 44$)	*P. samaipatae* ($N = 9$)	*P. koehleri* ($N = 10$)	*P. fenestratus* ($N = 44$)	*P. samaipatae* ($N = 20$)
SVL	34.0–39.5 (36.9 ± 2.2)	37.8–57.2 (43.7 ± 4.6)	44.4–51.4 (49.1 ± 2.2)	23.8–29.4 (27.0 ± 1.7)	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)	15.0–22.8 (17.5 ± 2.0)	17.4–20.8 (19.4 ± 1.2)	9.6–12.0 (10.6 ± 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)	13.0–21.8 (15.8 ± 2.0)	16.9–19.6 (18.5 ± 0.9)	8.8–10.3 (9.4 ± 0.5)	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)	4.4–7.4 (5.4 ± 0.7)	5.3–6.5 (5.9 ± 0.5)	3.1–4.3 (3.6 ± 0.4)	3.5–4.6 (4.0 ± 0.3)	3.9–5.1 (4.4 ± 0.3)
EN	4.2–5.0 (4.7 ± 0.4)	4.8–7.7 (5.8 ± 0.7)	5.6–6.9 (6.3 ± 0.5)	3.0–4.2 (3.6 ± 0.4)	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)	2.9–4.9 (3.7 ± 0.5)	3.5–4.7 (4.0 ± 0.4)	2.0–2.7 (2.3 ± 0.2)	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)	6.4–10.4 (7.9 ± 0.8)	8.0–9.4 (8.7 ± 0.4)	4.0–5.4 (4.8 ± 0.4)	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)	2.6–4.7 (3.1 ± 0.4)	2.8–3.6 (3.3 ± 0.3)	1.8–2.5 (2.2 ± 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)	2.3–4.2 (2.8 ± 0.4)	2.7–3.4 (3.1 ± 0.2)	1.5–2.2 (1.9 ± 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)	1.2–2.6 (1.8 ± 0.3)	1.4–2.5 (2.0 ± 0.3)	0.9–1.4 (1.1 ± 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)	1.1–2.6 (1.7 ± 0.3)	1.7–2.5 (2.0 ± 0.3)	0.9–1.2 (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)	7.2–11.6 (8.9 ± 1.1)	8.7–10.0 (9.4 ± 0.5)	4.6–5.5 (5.1 ± 0.3)	5.3–7.8 (6.3 ± 0.6)	4.9–7.2 (6.1 ± 0.6)
TL	20.4–22.0 (21.2 ± 0.8)	20.6–32.0 (25.4 ± 2.5)	27.5–31.1 (28.9 ± 1.3)	13.8–19.9 (15.6 ± 1.8)	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)	19.2–28.0 (23.0 ± 2.3)	24.5–28.3 (26.3 ± 1.2)	12.5–15.8 (13.6 ± 1.0)	13.5–18.2 (15.8 ± 1.2)	154–19.9 (17.0 ± 1.0)
FL	18.1–18.9 (18.5 ± 0.4)	17.8–29.0 (22.4 ± 2.6)	24.5–27.8 (26.1 ± 1.4)	11.9–15.2 (13.4 ± 0.9)	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)	1.2–2.7 (1.6 ± 0.3)	1.41–2.1 (1.7 ± 0.2)	1.0–1.4 (1.2 ± 0.1)	0.9–1.7 (1.2 ± 0.2)	0.9–1.7 (1.4 ± 0.2)
HL/HW	1.1–1.1 (1.1 ± 0.0)	1.0–1.2 (1.1 ± 0.0)	1.0–1.1 (1.0 ± 0.0)	1.1–1.3 (1.1 ± 0.1)	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)	0.5–0.7 (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)	0.5–0.6 (0.5 ± 0.0)	0.5–0.6 (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)

Mean ± standard deviation in parentheses follows range (in mm).

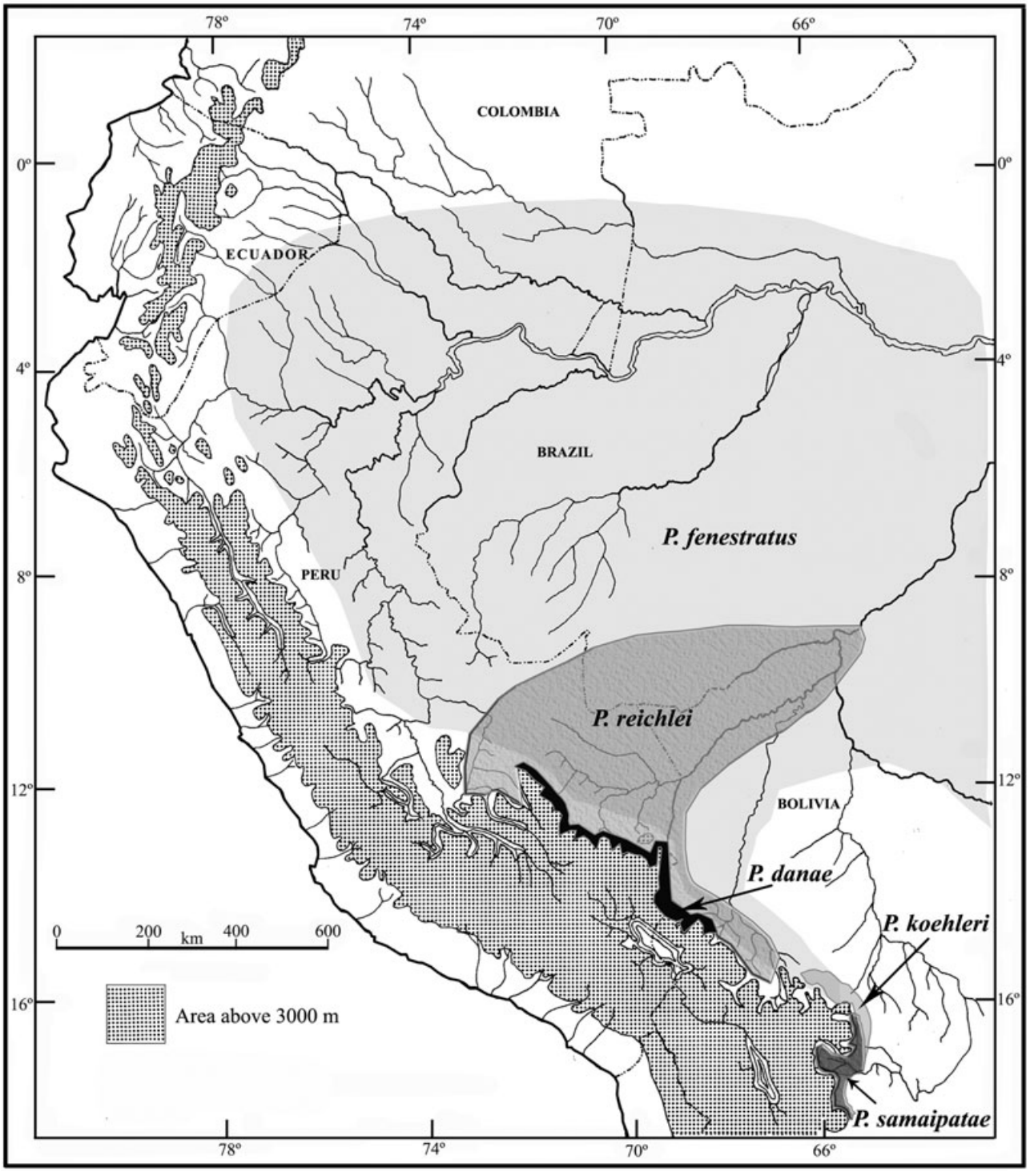

Figure 8. Map of part of South America depicting the approximate distribution of *Pristimantis danae*, *P. koehleri*, *P. fenestratus*, *P. reichlei*, and *P. samaipatae*.

Departamento Madre de Dios: KU 154856–57 from Cocha Cashu, Manu National Park, collected by C. A. Toft, 10 and 20.viii.1973, KU 205107 collected by T. A. Titus, 16.ii.1986, KU 205120 collected by P. A. Burrowes and R. de Sá, 2.ii.1986, KU 205132 collected by L. Trueb, n 09.i.1986, KU 205133 collected by T. Titus, 1.i.1986, KU 205134 collected 1986 by P. A. Burrowes and R. de Sá, 28.i, KU 205137 collected by R. de Sá,

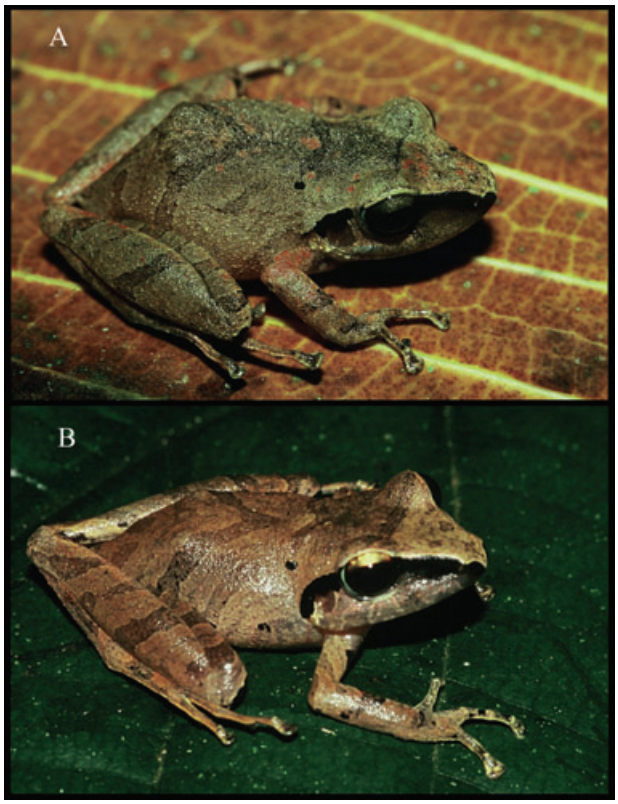

Figure 9. A, adult male of *Pristimantis reichlei* from Chalalán, Departamento La Paz, Bolivia (MNK-A 7178); B, adult male of *P. danae* from Huairuro, Departamento La Paz, Bolivia (one from the series MNCN 43054–64, 43067–8).

18.ii.1986, KU 205138 collected by P. A. Burrowes, 27.ii.1986, KU 205142 collected by P. A. Burrowes, 01.ii.1986, KU 207708 collected by A. Channing, 22.xi.1986, KU 207715, collected by W. E. Duellman, 16.xi.1986, KU 207716 collected by B. Quibell, 17.xi.1986, KU 207717 collected by B. Quibell, 24.xi.1986, KU 215481 collected by V. R. Morales, 15.i.1989, KU 215482 collected by E. R. Wild, 24.i.1989, KU 215483 collected by D. A. Kizirian, 26.i.1989, KU 215484 collected by W. R. Wild, 02.vii.1989, KU 215485 collected by D. A. Kizirian, 11.vii.1989, KU 215486 collected by H. R. Sisniegos, 12.vii.1989, KU 215487 collected by A. W. Salas, 25.i.1990, KU 215488 collected by L. A. Coloma, 16.ii.1990, all from Cuzco Amazónico, 15 km E of Puerto Maldonado; KU 154853–4 colected by C. A. Toft, 03.viii.1973, KU 154855 colected by C. A. Toft, 04.viii.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, 4–5.ii.1984; 342623–29 collected by R. McDiarmid, 14–22.ix.1988, USNM 342630–2 collected by R. McDiarmid and V. Morales, 24.i.1989, USNM 342854–55, 345174–76, collected by R. Reynolds and J. Icochea, 2.vii.1993, USNM 345177 collected by R. Reynolds and P. Sehgelmeble, 14.ii.1992, USNM 345278 collected by R. Reynolds, 21.ii.1992, USNM 345279 collected by P. Sehgelmeble, 29.ii.1996, USNM 345280–1 collected by R. Reynolds, 29.ii and 2.iii.1992, all from Pakitza, Reserve Zone, Manu National Park, *c*. 57 km (airline) NW of mouth of Río Manu, on Río Manu (11°52′S, 71°18′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-Sécure 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 Sécure 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 Pilón 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; CBF 2538, 2543–4, San Sebastián; PERU: Departamento Cusco: USNM 537903–34, San Martín-3, *c*. 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, *c*. 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, Tambopata Reserve, Explorer's Inn; USNM 346142, Atalaya, *c*. 3 km NW, on west bank of Río Alto Madre de Dios, Hacienda Amazonia; MHNSM 751–755, 1194, 9302–3, 9259–68; MHNSM 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, 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, Zona Reservada Tambopata–Candamo, Colpa de Guacamayos; 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 *Pristimantis 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 grooves 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 *P. peruvianus*. However, it differs from *P. peruvianus* by having first finger shorter than second, coarsely granular belly and lacking dorsolateral folds. For differences with other members of the *P. conspicillatus* Group see Table 3. *Pristimantis reichlei* is most similar to *P. danae*, from which it cannot be distinguished by qualitative characters (Table 3, Fig. 9B). Nevertheless, differences in morphometrics, advertisement call and 16S rDNA allow a clear separation (see above). This species is readily distinguished from other members of the group by the combination of: canthus rostralis and loreal region bold, dorsum finely shagreen, and presence of orange spots on posterior part of thighs. Other species of the *P. 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: *P. altamazonicus*, *P. carvalhoi* and *P. 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; *P. diadematus*, *P. eurydactylus* and *P. ventrimarmoratus* present bold black reticulation and spots on belly and limbs and have warty skin; *P. rhabdolaemus*, *P. toftae* and *P. sagittulus* have conspicuous dorsolateral folds; *P. salaputium, P. martiae* and *P. platydactylus* all have warty dorsal skin, poorly evident tympanic membrane and lack orange or yellow spots on posterior surfaces of thighs; finally, *P. ockendeni* presents a conspicuous dark subocular vertical bar, has light brown canthal and loreal regions, and lacks pale 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, orientated 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 a row of around ten 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.

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, twice length of 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; 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; 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.

Colour: In preservative, dorsal surfaces tan with dark brown chevrons, flanks lighter. Bold black colour 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 hind limbs; plantar surfaces dark brown; ventral surfaces cream with inconspicuous fine greyish-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 colour pattern in life is similar, but the dorsum is greyish-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, greyish or yellowish-brown colorations. Some dark dorsal marks, as an interocular stripe, a W-shaped occipital mark, an X-shaped middorsal mark or sacral chevrons, can be present. The brown mottling on ventral surfaces also varies in intensity. In life, the colour of the spots of the posterior surface of thighs varies from yellow to intense orange; the spots 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. In contrast to the holotype, some specimens have enlarged granules on the 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 5.

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 from the Departamento Huánuco, in Amazonian Peru, along the Andean slopes and adjacent lowlands of Peru, Brazil and Bolivia. The southernmost record lies in the Chapare region of central Bolivia (Fig. 8). It has been recorded in lowland Amazonian forest and humid montane forest of the Andean foothills up to 1500 m (Chaquisacha, Carrasco National Park, Bolivia). The parapatric altitudinal distribution of *P. danae* and *P. reichlei*, along most of their distributional ranges, makes some identifications uncertain. Doubtful records should be tested by means of morphometric, bioacoustic or molecular analyses. However, *P. danae* has been recorded from higher altitudes and seems to be restricted to southern Peru and northern Bolivia.

Table 5. Morphometrics of adult specimens of *Pristimantis reichlei* and *P. danae*

	Adult females		Adult males	
	P. reichlei (*N* = 23)	*P. danae* (*N* = 2)	*P. reichlei* (*N* = 32)	*P. 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)

Mean ± standard deviation in parentheses follows range (in mm).

Table 6. Summary of results of different comparative analyses applied to solve taxonomic problems of two species of *Pristimantis*

		Pristimantis koehleri	*Pristimantis reichlei*
		Central Bolivian Andes populations formerly assigned to *P. fenestratus* (De la Riva, 1993), *P. peruvianus* (De la Riva, 1994) and *P. dundeei* (Köhler, 2000a)	Southern Peruvian and Bolivian lowland populations formerly assigned to *P. peruvianus* or *P. danae* (Padial & De la Riva, 2005a)
Step 1: reduction of taxon sampling	Qualitative morphology	Distinguished from *P. dundeei* and *P. peruvianus*, barely distinguished from *P. samaipatae*, cryptic with *P. fenestratus*.	Distinguished from all members of *P. conspicillatus* Group including *P. peruvianus*, cryptic with *P. danae*
Step 2: comparative analyses of different lines of evidence	Morphometrics (PCA)	Distinguished from *P. samaipatae*, partially from *P. fenestratus*.	Cryptic with *P. danae*
	Advertisement call (PCA)	Distinguished from *P. fenestratus* and *P. samaipatae*.	Distinguished from *P. danae*
	Phylogenetics (16S rDNA)	Reciprocally monophyletic and sister to *P. fenestratus*, distinguished from *P. samaipatae*.	Reciprocally monophyletic to *P. danae* for MP and NJ analyses, paraphyletic to *P. danae* for Bayesian analysis
	Distribution	Sympatric to *P. samaipatae*, parapatric to *P. fenestratus*.	Parapatric to *P. danae*

The southernmost record of *P. danae* corresponds to Valle de Zongo, Departmento La Paz, Bolivia (16°11′47.5″S, 68°07′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 *P. peruvianus* from Panguana (Peru) by Schlüter (1980), Cocha Cashu (Peru) by Rodríguez (1994) and Tambopata by Duellman (2005) correspond to *P. reichlei*. The last of these was reanalysed herein (USNM tape 265/17; Table 1). The Bolivian record of *P. danae* by Köhler & Jungfer (1995) and the advertisement call of *P. danae* from Chapare 1250 m by Köhler (2000a) correspond to *P. reichlei*. The illustration and call of *P. danae* by Köhler & Lötters (2002) correspond to *P. reichlei*. The illustration of *P. danae* by De la Riva *et al.* (2000: 141) corresponds to *P. reichlei*. The illustration of *P. peruvianus* by De la Riva *et al.* (2000: 145) corresponds to *P. danae*. Specimens from Tambopata reported by Doan & Arizábal (2002) as *P. peruvianus* correspond to *P. reichlei*. Specimens cited by Padial *et al.* (2004) as *P. danae* for different localities in Bolivia correspond to *P. reichlei* and are now included herein as referred specimens (see above). Peruvian specimens reported by Padial & De la Riva (2005a) as *P.* cf. *peruvianus* (except KU 154863–5) also correspond to *P. reichlei*. Specimens identified by Padial, Bielskis & Castroviejo (2000) as *P.* cf. *peruvianus* from the Andean slopes of Department La Paz are *P. fenestratus*. The diagnosis and redescription provided by Köhler (2000a) for *P. peruvianus* matches qualitative characters of many Colombian, Ecuadorian and northern Peruvian populations assigned to this species. However, the taxonomic status of *P. peruvianus* remains uncertain because characters proposed by Lynch (1980) to separate it from *P. conspicillatus* seem invalid due to variability. 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.

DISCUSSION

The two new species taxa described herein, *Pristimantis koehleri* and *P. reichlei*, inhabit the forests of the Amazonian versant of the Andes and adjacent lowlands of Central Bolivia and southern Peru. The origin of both species seems to be related to habitat conditions different from those occupied by their sister taxa. For example, *P. koehleri* is the sister species of *P. fenestratus*. The latter has a broad distribution along the humid lowland Amazonian forests and humid forests of the Andean hills. The former is mainly restricted to the semi-deciduous forests (longer dry seasons and cooler temperatures; see Köhler, 2000a) of the Andean hills, at the southern edge of the distribution of *P. fenestratus*. Both species share a small area of overlap along the humid Andean slopes of Central Bolivia (Fig. 8). The situation also is applicable to *P. samaipatae*, which is the sister taxon to the latter pair. It is restricted to semi-deciduous forests, sharing localities only with *P. koehleri*. Furthermore, morphological and bioacoustic analyses indicate a close relationship of the Cerrado inhabitant *P. dundeei* with *P. koehleri* (Köhler, 2000a). This suggests a biogeographical connection between the Cerrado and the central Andes, as hypothesized for members of the genus *Oreobates* (Padial *et al.*, 2008a). According to Bayesian and NJ analyses, *P. reichlei* and *P. danae* also seem to be closely related, 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). Given the allopatry and/or parapatry of all these taxa in habitats with different ranges of precipitations, temperature and humidity, recent divergence in isolation in humid refugia (Funk *et al.*, 2007) for the pair *P. danae–P. reichlei*, and dry refugia (Pennington, Prado & Pendry, 2000; Killeen *et al.*, 2007) for *P. koehleri* and *P. samaipatae*, might have contributed to this speciation process.

During the 250 years of Linnaean 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 evidence of species limits (Mayr, 1942). For example, the existence of reproductive barriers was explicitly or implicitly assumed by many taxonomists when describing species, as lack of interbreeding was the basis for the most broadly accepted species definition. In more recent times, the utility of molecular and morphological phylogenetic methods was tested to delineate species limits (e.g. Sites & Marshall, 2004). Classical species taxa were corroborated by some phylogenetic methods while rejected by others (e.g. Wiens & Penkrot, 2002). However, the broad concordance of morphological species boundaries and species boundaries delineated by phylogenetic analyses of specific molecular markers for some groups led to the proposal of DNA taxonomy (Tautz *et al.*, 2003). Some problems emerged parallel to this proposal. For example, species formerly accepted were shown to be not necessarily monophyletic (Funk & Omland, 2003) and different molecular markers (e.g. mitochondrial vs. nuclear genes) turned out to disagree in the results and accuracy for each group of organisms

(see review of Vogler & Monaghan, 2006). Although some biologists were inclined toward the use of molecular methods to define species limits (e.g. Blaxter, 2003), others pleaded for the use of an integrative approach that included different independent lines of evidence to support more stable species taxa hypotheses (Dayrat, 2005; Will, Mishler & Wheeler, 2005). This proposal was timely for two main reasons. First, some biologists interpreted the steady increase in species discoveries using molecular phylogenetics in recent decades as a symptom of taxonomic instability and failure in the identification of true species (Isaac *et al.*, 2004; and responses of Köhler *et al.*, 2005; Padial & De la Riva, 2006). Second, the species problem might have been solved (Hey, 2006) by recognizing that species equate to lineages of metapopulations evolving independently (De Queiroz, 2005a). Under this perspective, none of the previously proposed criteria to define species is considered necessary or sufficient, and any line of evidence can be applied to propose equally valid species taxa hypotheses (De Queiroz, 2005b). Therefore, an integrative taxonomy considering several independent but concordant lines of evidence to test and support species taxa hypotheses should converge in a more reliable and stable taxonomy.

This study exemplifies how different 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 group of frogs. As different lines of evidence converged in the recognition of two new species taxa, we consider each of them as stable taxonomic hypotheses. A corollary of this study is that similar results are expected when applying this approach to a broader scale revision of *Pristimantis*. Indeed, the use of bioacoustics (e.g. Köhler & Lötters, 1999; Köhler, 2000b; Reichle, Lötters & La Riva, 2001; Padial, González & La Riva, 2005) and molecular phylogenetics (Fouquet *et al.*, 2007; Lehtinen *et al.*, 2007) to infer species limits is contributing to the discovery of cryptic lineages of *Pristimantis* and other tropical frogs. Within *Pristimantis*, representing the largest Neotropical vertebrate genus, with around 400 species (Heinicke *et al.*, 2007), most species have been described by the classical morphological approach. Many cryptic species and putative synonyms could be hidden under these species names. Hence, an integrative taxonomic approach might lead to an important increase in species number (as new, stable taxonomic hypotheses), and to the resolution of many taxonomic problems.

ACKNOWLEDGEMENTS

Most of J.M.P.'s fieldwork in Bolivia was financed by a grant of the Mutis programme of the MAE-AECI (Spain); his trip to visit the museums in the USA was funded by an Ernst Mayr Travel Grant in Animal Systematics (Museum of Comparative Zoology, Harvard University), and his trip to visit the British Museum was funded by the 'European Commission's Research Infrastructure Action' via the 'SYNTHESYS Project'. We are grateful to the following for the loan of specimens, support and/or space provided at their institutions: R. Bain, D. Frost, T. Grant and D. Kizirian (AMNH), B. Clarke and M. Wilkinson (BM), J. Aparicio (CBF), R. Aguayo and A. Muñoz (CBG), R. Márquez, M. Pérez, G. Solís and L. González (Fonozoo-MNCN), G. Nilson (GNM), W. E. Duellman, J. Simmons and L. Trueb (KU), J. Hanken and J. Rosado (MCZ), J. C. Chaparro and O. Aguilar (MHNC), C. Aguilar (MHNSM), B. Álvarez, J. E. González-Fernández and I. Rey (MNCN), L. González, A. Justiniano, R. Montaño, M. Suárez and R. Vespa (MNK), P. E. Vanzolini and H. Zaher (MZUSP), Jirí Moravec (NMP), F. Tiedeman, H. Grillitsch and R. Gemel (NMW), D. Cannatella and T. LaDuc (TNHC), R. A. Nussbaum and G. Schneider (UMMZ), K. De Queiroz, W. R. Heyer, R. McDiarmid and R. V. Wilson (USNM), J. A. Campbell (UTA), W. Böhme, S. Lötters and J. Köhler (ZFMK), and J. B. Rasmussen and M. Andersen (ZMUC). R. Aguayo, E. Ávila, S. Castroviejo, J. C. Chaparro, P. Comas, R. de Sá, D. Embert, J. M. Gil-Sánchez, M. González-Osto, J. Heredia-Fernández, P. Macuapa, M. Moleón, J. Pérez-Marín, D. Ramos, C. Rojas, S. Ten and C. Ureña provided valuable help in the field. We are grateful to S. Castroviejo-Fischer, P. Flores, A. Machordom and M. García-París for their perennial disposition to help in the lab or with the phylogenetic analyses. We thank to F. Palacios for his decisive support and to J. Köhler and S. Reichle for their constructive discussions over many years. R. Heyer (USNM) kindly provided advertisement call recordings. G. Mamani and the personnel at the 'eco-lodge' Chalalán kindly facilitated our fieldwork there. This work was partially funded by projects REN/GLO 2001-1046 and CGL2005-03156 of the Spanish Ministry of Education and Science (I. De la Riva, Principal Investigator). We thank Miguel Vences for his constructive criticism on previous drafts of the manuscript.

REFERENCES

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transaction Automatic Control* **19:** 716–723.

Angulo A, Cocroft RB, Reichle S. 2003. Species identity in the genus *Adenomera* (Anura: Leptodactylidae) in southeastern Peru. *Herpetologica* **59:** 490–504.

Arroyo SB, Sánchez PM, Ramírez-Pinilla MP, Suárez HA, Miranda-Esquivel DR. 2005. Morphometric analysis to differentiate taxonomically seven species of *Eleutherodac-*

tylus (Amphibia: Anura: Leptodactylidae) from an Andean cloud forest of Colombia. *Zootaxa* **1018:** 1–14.

BIAS. 2002. *Peak 3.2*. Petaluma: Berkley Integrated Audio Software Inc.

Bickford D, Lohman DJ, Navjot SS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22:** 148–155.

Blaxter M. 2003. Counting angels with DNA. *Nature* **421:** 122–124.

Boersma P, Weenick D. 2006. Praat: doing phonetics by computer. Version 4.5.02. Published by the authors.

Bosch J, De la Riva I. 2004. Are frog calls modulated by the environment? An analysis with anuran species from Bolivia. *Canadian Journal of Zoology* **82:** 880–888.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85:** 407–415.

De la Riva I. 1993. Sinopsis del género *Eleutherodactylus* (Amphibia, Anura, Leptodactylidae) en Bolivia y adición de tres especies nuevas para el país. *Revista Española de Herpetología* **7:** 97–105.

De la Riva I. 1994. Geographic distribution. *Eleutherodactylus peruvianus* (NCN). *Herpetological Review* **25:** 159.

De la Riva I, Köhler J, Lötters S, Reichle S. 2000. Ten years of research on Bolivian amphibians: updated checklist, distribution, taxonomic problems, literature and iconography. *Revista Española de Herpetología* **14:** 19–164.

De Queiroz K. 2005a. Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences* **102:** 6600–6607.

De Queiroz K. 2005b. A unified concept of species and its consequences for the future of taxonomy. *Proceedings of the California Academy of Sciences* **56:** 196–215.

De Queiroz K. 2005c. Different species problems and their resolution. *Bioessays* **27:** 1263–1269.

Doan TM, Arizábal W. 2002. Microgeographic variation in species composition of the herpetofaunal communities of Tambopata region, Peru. *Biotropica* **34:** 101–117.

Duellman WE. 2005. *Cusco Amazónico: the lives of amphibians and reptiles in an Amazonian rainforest*. Ithaca: Cornell University Press.

Duellman WE, Hedges SB. 2005. Eleutherodactylinae frogs (Anura: Leptodactylidae) from the Cordillera Yanagacha in Central Peru. *Copeia* **2005:** 526–538.

Duellman WE, Lehr E. 2007. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in the Cordillera Occidental in Peru with descriptions of three new species. *Scientific Papers of the Natural History Museum of the University of Kansas* **39:** 1–13.

Duellman WE, Pramuk JB. 1999. Frogs of the genus *Eleutherodactylus* (Anura: Leptodactylidae) in the Andes of northern Peru. *Scientific Papers of the Natural History Museum of the University of Kansas* **13:** 1–78.

Duellman WE, Trueb L. 1986. *The biology of amphibians*. New York: McGraw Hill Book Company.

Fouquet A, Vences M, Salducci MD, Meyer A, Marty C, Blanc M, Gilles A. 2007. Revealing cryptic diversity using molecular phylogenetics and phylogeography in frogs of the *Scinax ruber* and *Rhinella margaritifera* species groups. *Molecular Phylogenetics and Evolution* **43:** 567–582.

Free Software Foundation, Inc. 1991. Audacity: a free, cross-platform digital audio editor. Version 1.2.6. Free Software Foundation Inc.

Frost DR. 2006. *Amphibian species of the world 4.0, an online reference*. New York: American Museum of Natural History. Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html (accessed 17 September 2006).

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, De Sa RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* **297:** 1–370.

Funk CW, Caldwell JP, Peden CE, Padial JM, De la Riva I, Cannatella DC. 2007. Tests of biogeographic hypotheses for diversification in the Amazonian forest frog, *Physalaemus petersi*. *Molecular Phylogenetics and Evolution* **44:** 825–837.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics* **34:** 397–423.

Grant T, Kluge AG. 2004. Transformation series as an ideographic character concept. *Cladistics* **20:** 23–31.

Hayek LA, Heyer WR, Gascon C. 2001. Frog morphometrics: a cautionary tale. *Alytes* **18:** 153–177.

Hedges SB, Duellman WE, Heinicke MP. 2008. New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* **1737:** 1–182.

Hey J. 2006. On the failure of modern species concept. *Trends in Ecology and Evolution* **21:** 447–450.

Heyer RW, García-López JM, Cardoso AJ. 1996. Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species. *Amphibia-Reptilia* **1996:** 7–31.

Heyer WR, Muñoz AM. 1999. Validation of *Eleutherodactylus crepitans* Bokermann, 1965, notes on the types and type locality of *Telatrema heterodactylum* Miranda-Ribeiro, 1937, and description of a new species of *Eleutherodactylus* from Mato Grosso, Brazil (Amphibia: Anura: Leptodactylidae). *Proceedings of the Biological Society of Washington* **112:** 1–18.

Hillis DM, Moritz C, Mable BK, eds. **1996.** *Molecular systematics*. Sunderland, MA: Sinauer Associates.

Huelsenbeck JP, Ronquist F. 2001. MrBAYES: Bayesian inferences of phylogenetic trees. *Bioinformatics* **8:** 754–755.

Isaac NJ, Mallet J, Mace GM. 2004. Taxonomic inflation: its influence on macroecology and conservation. *Trends in Ecology and Evolution* **19:** 464–469.

Killeen TJ, Douglas M, Consiglio T, Jørgense PM, Mejía J. 2007. Dry spots and wet spots in the Andean hotspot. *Journal of Biogeography (Special issue)* 1–17.

Köhler J, Jungfer KH. 1995. Eine neue Art und ein Erst-

nachweis von Fröschen der Gattung *Eleutherodactylus* aus Bolivien. *Salamandra* **31:** 149–156.

Köhler J, Lötters S. 1999. New species of the *Eleutherodactylus unistrigatus* group (Amphibia: Anura: Leptodactylidae) from montane rain forest of Bolivia. *Copeia* **1999:** 422–427.

Köhler J, Lötters S. 2002. *Eleutherodactylus danae*. In: Marquez R, De la Riva I, Bosch J, Matheu E, eds. *Sounds of the frogs and toads of Bolivia*. Madrid: ALOSA, AHE, MNCN, track 38.

Köhler J, Vieites DR, Bonett RM, Hita–García F, Glaw F, Steinke D, Vences M. 2005. Boost in species discoveries in a highly endangered vertebrate group: new amphibians and global conservation. *BiosScience* **55:** 693–696.

Köhler J. 2000a. Amphibian diversity in Bolivia: a study with special reference to montane forest regions. *Bonner zoologische Monographien* **48:** 1–243.

Köhler J. 2000b. New species of *Eleutherodactylus* (Anura: Leptodactylidae) from cloud forest of Bolivia. *Copeia* **2000:** 516–520.

Lehr E, Duellman WE. 2007. Two new species of *Eleutherodactylus* (Anura: Leptodactylidae) from the Cordillera Occidental in Peru. *Copeia* **2007:** 140–149.

Lehr E, Torres C, Suárez J. 2007. A new species of arboreal *Eleutherodactylus* (Anura: Leptodactylidae) from the Amazonian lowlands of central Peru. *Herpetologica* **63:** 94–99.

Lehtinen RM, Nussbaum RA, Richards CM, Cannatella DC, Vences M. 2007. Mitochondrial genes reveal cryptic diversity in plant-breeding frogs from Madagascar (Anura, Mantellidae, Guibemantis). *Molecular Phylogenetics and Evolution* **44:** 1121–1129.

Leviton AER, Gibbs H, Heal E, Dawson CE. 1985. Standards in herpetology and ichthyology: part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia* **1985:** 802–832.

Lynch JD. 1975. The identity of the frog *Eleutherodactylus conspicillatus* (Günther), with description of two related species from northwestern South America (Amphibia, Leptodactylidae). *Los Angeles County Museum of Natural History Contributions in Science* **272:** 1–19.

Lynch JD. 1980. A taxonomic and distributional synopsis of the Amazonian frogs of the genus *Eleutherodactylus*. *American Museum Novitates* **2696:** 1–24.

Lynch JD. 1986. The definition of the Middle American clade of *Eleutherodactylus* based on jaw musculature (Amphibia: Leptodactylidae). *Herpetologica* **42:** 248–258.

Lynch JD, Duellman WE. 1997. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: systematics, ecology, and biogeography. *University of Kansas Natural History Museum Special Publications* **23:** 1–236.

Márquez R, De la Riva I, Bosch J. 1995. Advertisement calls of Bolivian Leptodactylidae (Amphibia, Anura). *Journal of Zoology* **237:** 313–336.

Mayr E. 1942. *Systematics and the origin of species from the viewpoint of a zoologist*, 3rd edn. 1999. Cambridge, MA: Harvard University Press.

Moravec J, Aparicio J, Köhler J. 2006. A new species of tree frog, genus *Dendropsophus* (Anura: Hylidae), from the Amazon of northern Bolivia. *Zootaxa* **1327:** 23–40.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403:** 853–858.

Padial JM, Bielskis S, Castroviejo J. 2000. Sobre los anfibios del pie de monte del Departamento de La Paz (Bolivia). *Boletín de la Asociación Herpetológica Española* **11:** 59–62.

Padial JM, Castroviejo-Fischer S, Köhler J, Domic E, De la Riva I. 2007a. Systematics of the *Eleutherodactylus fraudator* species group (Anura: Brachycephalidae). *Herpetological Monographs* **21:** 214–241.

Padial JM, Chaparro JC, De la Riva I. 2007b. A new species of the *Eleutherodactylus discoidalis* group (Anura: Brachycephalidae) from cloud forests of Peru. *Herpetologica* **63:** 114–122.

Padial JM, Chaparro JC, De la Riva I. 2008a. Systematics of *Oreobates* and the *Eleutherodactylus discoidalis* species Group (Amphibia, Anura) based on two mtDNA genes and external morphology. *Zoological Journal of the Linnean Society* **152:** 353–365.

Padial JM, De la Riva I. 2005a. Rediscovery, redescription and advertisement call of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937) (Anura: Leptodactylidae), and notes on other *Eleutherodactylus*. *Journal of Herpetology* **39:** 372–379.

Padial JM, De la Riva I. 2005b. The taxonomic status of *Eleutherodactylus skydmainos* Flores & Rodríguez, 1997 and *E. karcharias* Flores & Rodríguez, 1997 (Anura: Leptodactylidae). *Amphibia-Reptilia* **26:** 515–521.

Padial JM, De la Riva I. 2006. Taxonomic inflation and the stability of species lists: the perils of ostrich's behavior. *Systematic Biology* **55:** 859–867.

Padial JM, González L, De la Riva I. 2005. A new species of the *Eleutherodactylus discoidalis* group (Anura: Leptodactylidae) from Andean humid montane forest of Bolivia. *Herpetologica* **61:** 318–325.

Padial JM, Gonzales L, Reichle S, Aguayo R, De la Riva I. 2004. First records of five species of the genus *Eleutherodactylus* Duméril and Bibron, 1841 (Anura: Leptodactylidae) for Bolivia. *Graellsia* **60:** 167–174.

Padial JM, Köhler J, Muñoz A, De la Riva I. 2008b. Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement call in the *Eleutherodactylus discoidalis* species group (Anura: Brachycephalidae). *Zoological Journal of the Linnean Society* **152:** 737–773.

Padial JM, Reichle S, McDiarmid R, De la Riva I. 2006. A new species of arboreal Chaunus (Anura: Bufonidae) from Madidi National Park, Bolivia. *Zootaxa* **1278:** 57–68.

Parra-Olea G, García-París M, Wake D. 2004. Molecular diversification of salamanders of the tropical American genus Bolitoglossa (Caudata: Plethodontidae) and its evolutionary and biogeographical implications. *Biological Journal of the Linnean Society* **81:** 325–346.

Pennington RT, Prado DE, Pendry CA. 2000. Neotropical

seasonally dry forest and Quaternary vegetation changes. *Journal of Biogeography* **27:** 261–273.

Pfenninger M, Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* **7:** 121.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43:** 304–311.

Reichle S. 2002. *Eleutherodactylus* cf. *peruvianus*. In: Márquez R, De la Riva I, Bosch J, Matheu E, eds. *Sounds of frogs and toads of Bolivia*. Barcelona: ALOSA-AHE-MNCN, Track 43.

Reichle S, Lötters S, De la Riva I. 2001. A new species of the *discoidalis* group of *Eleutherodactylus* (Anura, Leptodactylidae) from inner-Andean dry valleys of Bolivia. *Journal of Herpetology* **35:** 21–26.

Rodríguez LO. 1994. A new species of the *Eleutherodactylus conspicillatus* group (Leptodactylidae) from Peru, with comments on its call. *Alytes* **12:** 49–63.

Sambrook E, Fritsch F, Maniatis T. 1989. *Molecular cloning*. New York: Cold Spring Harbor Press.

Schlüter A. 1980. Bio-akustische Untersuchungen an Leptodactyliden in einem begrenzten Gebiet des tropischen Regenwaldes von Peru. *Salamandra* **16:** 227–247.

Sites JW Jr, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution and Systematics* **35:** 199–227.

Stuart S, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306:** 1783–1786.

Swofford DL. 1998. *PAUP*. Phylogenetic analysis using parsimony* and other methods*. Version 4b10. Sunderland, MA: Sinauer Associates.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends in Ecology and Evolution* **18:** 70–74.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876–4882.

Vences M, Thomas M, Bonnett R, Vieites D. 2005. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society B* **360:** 1859–1868.

Vences M, Wake D. 2007. Speciation, species boundaries and phylogeography of amphibians. In: Heatwole H, ed. *Amphibian biology*, vol. 7. Chipping Norton, NSW: Surrey Beatty & Sons, 2613–2671.

Vogler AP, Monaghan MT. 2006. Recent advances in DNA taxonomy. *Journal of Zoological Systematics and Evolutionary Research* **45:** 1–10.

Wiens JJ, Penkrot TL. 2002. Delimiting species based on DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* **51:** 69–91.

Wiley EO. 1981. *Phylogenetics. The theory and practice of phylogenetic systematics*. New York: John Wiley & Sons.

Will KW, Mishler BD, Wheeler QD. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54:** 844–851.

Yang Z, Rannala B. 1997. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo method. *Molecular Biology and Evolution* **14:** 717–724.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Specimens of *Pristimantis* examined.
Appendix S2. Sound collection reference numbers, locality data and specimen vouchers for analysed call recordings.
Appendix S3. Sample sounds of the advertisement call of *Pristimantis* species compared in this study.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.