

Acoustic signals, species diagnosis, and species concepts: the case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia

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Overall morphological similarity and intrapopulation variation of morphological features make species identification in cryptic species complexes challenging. In such cases, additional features, such as acoustic and/or genetic characters, are being used to aid species identification. The use of acoustic signals as a suite of diagnostic features can be especially rewarding in certain taxonomic groups that rely on acoustic communication. Such is the case of the Neotropical leaf litter frogs of the *Leptodactylus marmoratus* group (formerly *Adenomera*). Recent studies using different suites of features for field identification have revealed a previously undetected species richness for this group. It is in the light of acoustic signals that we identify a new species for this group, and describe it herein. We also discuss the systematics of populations associated with the Amazonian realm. We further explore the use of acoustic signals in species identification and diagnosis in anurans and other taxa that communicate acoustically (e.g. lacewings, orthopterans, and birds), and the relevance and importance of the use of species concepts in species diagnosis and descriptions. © 2008 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2008, 152, 59–77.

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INTRODUCTION

Overall morphological similarity and intrapopulation variation of morphological features make species identification in certain cryptic species complexes (*sensu* Bickford *et al.*, 2007) a challenging task. Species delimitations and identification have implications for both estimating species richness and assessing conservation needs. Given the difficulties associated with cryptic species, the use of different suites of diagnostic features, such as molecular (Hebert *et al.*, 2003; Ron, Santos & Cannatella, 2006;

Bickford *et al.*, 2007) and/or behavioural data (Heyer & Reid, 2003; Kokubum & Giaretta, 2005), are being increasingly used to aid the determination of biological identities in taxonomically problematic groups. One such group is constituted by the former leptodactylid genus *Adenomera* Steindachner, 1867.

Resulting from the great degree of morphological variation both within and between populations (De la Riva, 1996; Kwet & Angulo, 2002; Ponssa & Heyer, 2007), and from an overall similarity in morphological traits, it is generally difficult to tell some of these frog species apart. Thus, the taxonomy of these small Neotropical leaf litter frogs is regarded as chaotic (Duellman, 2005), and their intrageneric relationships have been poorly understood. In addition, there is very limited information available on their natural history, biogeography, reproductive biology, and ecology,

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making these small frogs rather poorly known amphibians despite their ubiquitous distributions and common occurrence in many urban and pristine localities over South America, east of the Andes.

A recent systematic revision of living amphibians has proposed many substantial changes in current amphibian taxonomy (Frost *et al.*, 2006). These proposed changes also pertain to the genus *Adenomera* Steindachner, 1867. *Adenomera* was revalidated as a genus by Heyer (1974) to include those members of the *Leptodactylus marmoratus* group *sensu* Heyer (1973). Frost *et al.* (2006), however, recommend that *Adenomera*, *Lithodytes* Fitzinger, 1843, and *Vanzolinius* Heyer, 1974 (Anura, Leptodactylidae) be placed into *Leptodactylus* Fitzinger, 1826, to render the genus *Leptodactylus* monophyletic. Evidence from other studies (Angulo, 2004; Kokubum & Giaretta, 2005) supports this recommendation. Herein we adopt Frost *et al.*'s (2006) nomenclatural recommendation of including *Adenomera* within *Leptodactylus*. However, there is also independent evidence pointing to species of the former genus *Adenomera* comprising a monophyletic group (Angulo, 2004). In order to distinguish these taxa from other *Leptodactylus*, we consider that it is appropriate to use the term 'marmoratus group' to refer to them. Thus, taxa formerly considered as *Adenomera* can be identified as *Leptodactylus* of the *marmoratus* group *sensu* Heyer (1973). It is worth mentioning that the nomenclatural recommendation of including *Adenomera* within *Leptodactylus* proposed by Frost *et al.* (2006) has not been adopted by all researchers working with this group (see Ponssa & Heyer, 2007; Kwet, 2007), an element that may influence the way species are recognized or tallied for the group (e.g. AmphibiaWeb, 2007).

Given that morphological features alone generally do not provide the kind of resolution needed to assign species identities within this group, other characters also need to be used to this end. Heyer (1984) suggested that advertisement calls should be rewarding in resolving the systematics of the group. Advertisement calls are important in species and mate recognition (Blair, 1964; Ryan, 1988; Ryan & Rand, 1993), and constitute important premating isolating mechanisms in sympatric conditions (Blair, 1964, 1974; Fouquette, 1975); thus, they may play an important role in speciation events. Recent and ongoing research suggests that advertisement calls indeed provide us with useful features to untangle species identity within the group (see Kwet & Angulo, 2002; Angulo & Icochea, 2003; Angulo, Cocroft & Reichle, 2003; Almeida & Angulo, 2006; Kwet, 2007). Molecular data are also being used to untangle species identities in other anuran taxa (e.g. Dawood, Channing & Bogart, 2002), and they have recently been successfully used in the *L. marmoratus* group (Angulo, 2004).

There are currently 11 recognized species for the *L. marmoratus* group [Almeida & Angulo, 2006; Frost, 2007; including the recently revalidated *Leptodactylus nanus* Müller, 1922 (Kwet, 2007)], although this number is bound to change quickly as newly identified species are described (see, e.g. Angulo *et al.*, 2003; Angulo, 2004; Kokubum & Giaretta, 2005; Kwet, 2007). Of these recognized species, *Leptodactylus andreae* Müller, 1923, *Leptodactylus diptyx* Boettger, 1885, and *Leptodactylus hylaedactylus* (Cope, 1868) have been reported to occur in Bolivia (De la Riva *et al.*, 2000). In comparing calls from a previously unassigned population in the region of Chapare with calls from other populations in the Upper Amazon Basin, and elsewhere, we found that the calls from Chapare comprise a distinct set of vocalizations not previously known for this group. Acoustic evidence thus suggests that the Chapare population is a different species, which is herein described. We also discuss the systematic status of Amazonian and associated populations.

In view of the growing number of studies that use alternative or complementary approaches in species identification and diagnosis, it would seem appropriate to specify exactly how we determine a species to be a new, undescribed form. In doing so, we are referring to a conceptual basis often used, but hardly covered or explained in species descriptions: the species concept.

The age-long question of what is a species has been the subject of ongoing debate and controversy, as any glimpse through the evolutionary literature will quickly reveal. Although efforts have been made to develop a unified species concept (de Queiroz, 1998, 1999, 2005), there is currently no consensus on what would be a satisfactory universal concept; the all-applicable species concept remains elusive, and hence difficulties arise from the use of multiple concepts. Difficulties in reaching a consensus on a universal species concept are not all that surprising in view of the inherently diverse, variable, and dynamic nature of biological systems, which makes it difficult to identify clear-cut boundaries. In addition, there are both biological and philosophical aspects that are inherent to the discussion (see, e.g. Pigliucci, 2003), which provide different perspectives on the 'species problem'.

In terms of concept usage, it has been pointed out that the Biological Species Concept (BSC) was the dominant concept in herpetology (Frost & Hillis, 1990) and ornithology (Dillon & Fjeldså, 2005) for decades (in the BSC, species are defined as 'groups of interbreeding natural populations that are reproductively isolated from other such groups'; Mayr, 1984: 533). It is also possible that this was the underlying concept in many relatively recent species descrip-

tions. Nevertheless, species descriptions for a variety of taxa have traditionally concentrated primarily on morphological traits, so it is also likely that the Morphological Species Concept (MSC, and see Mayden, 1997 for synonym concepts) may be a popular choice of concept in species descriptions. Historically, at least, it was the most likely species concept used, given that the MSC predates the BSC (Bickford *et al.*, 2007). The MSC, as defined by Cronquist (1978, cited in Mayden, 1997) considers that 'species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means'.

It is beyond the scope of the present paper to review the issue of the 'species problem', species concepts, and definitions; rather, we would like to refer to a conceptual framework based on species concept usage for which the issue of identifying morphologically cryptic species in the field can be addressed. It is in this light that we discuss the use of acoustic signals in species identification and diagnosis in different taxa that use such signals to communicate (e.g. lacewings, orthopterans, birds, and anurans), and the relevance of the use of species concepts in species definitions and descriptions.

MATERIAL AND METHODS

Field work was conducted along the Carretera Antigua, just above the village of El Palmar on the old road from Villa Tunari to Cochabamba, Province of Chapare, Department of Cochabamba, Bolivia, c. 800 m above sea level, during September 1997. Three adult specimens were collected, fixed in 10% formalin, and preserved in 70% ethyl alcohol, and were later deposited at the Colección Boliviana de Fauna (CBF) and the Museo Noel Kempff Mercado (NKA), both in Bolivia.

Although the limited sample size and the morphological variation observed in these specimens may raise questions regarding their identity, we believe that they do belong to the same species given that: (1) the two voucher males have advertisement calls that are a close match to each other in important acoustic parameters and features, and both differ from other calls recorded for the *L. marmoratus* group; and (2) there was only one species of the *L. marmoratus* group found in this area, and only one type of advertisement call was heard and recorded in the field. The single female was also located in the general area where males were found calling (c. 200–300 m away). It is also relevant to point out that obtaining call vouchers in many species of the *L. marmoratus* group can at times be quite challenging, given their often shy nature and their capacity to conceal themselves well within leaf litter and grasses. Call vouchers can

be considered twice as valuable: they are valuable not only as a repository of DNA and morphological data, but also for the acoustic data that can be directly associated with them. Finally, and against the backdrop of ongoing amphibian declines, it is important to describe newly identified species in a relatively quick fashion (provided that there is compelling evidence to support specific status) to better address current conservation needs.

Specimens of the new species were compared with other species of the group; materials used for comparative purposes are deposited at the Academy of Natural Sciences of Philadelphia (ANSP), Philadelphia, USA; CBF, La Paz, Bolivia; NKA, Santa Cruz de la Sierra, Bolivia; Museu de Biologia Mello Leitão (MBML), Santa Teresa, Brazil; Museu de Ciências e Tecnologia da PUCRS (MCP), Porto Alegre, Brazil; Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil; Museum national d'Histoire naturelle (MNHN), Paris, France; National Museum of Natural History (USNM), Washington, DC, USA; Royal Ontario Museum (ROM), Toronto, Canada; and Zoologische Staatssammlung München (ZSM), München, Germany. Measurements of the new species were made to the nearest 0.1 mm and were taken following the method of Heyer *et al.* (1990). The following measurements were taken with a stereoscopic microscope: head length (HL), head width (HW), eye diameter (ED), tympanum diameter (TYD), eye/nostril distance (END), interorbital distance (IOD), internarial distance (IND), forearm length (FAL), hand length (HDL), thigh length (THL), shank length (SL), tarsus length (TSL), and foot length (FL). Snout–vent length (SVL) was measured using digital calipers. Individuals were sexed according to the method of Heyer (1973).

Two males, including the holotype, were recorded and collected: (1) on September 25, 1997, at 19:10 h, air temperature 21.1 °C, microphone positioned at 180° from male when recording, and (2) on September 25, 1997, at 20:00 h, air temperature 20.8 °C, male calling with his back turned to the microphone. Acoustic signals were recorded using a Sony Walkman Professional WM D6C tape recorder and a Sennheiser ME-80 directional microphone. Signal acquisition and analyses were conducted using a combination of acoustic signal analysis software. Signal acquisition was performed with the Macintosh-based digital signal analysis program, Signalyze 3.12 (Keller, 1994), at a sampling frequency rate of 44.1 kHz and with 16-bit precision. For signal analyses we used Signalyze, and call figures of the temporal and spectral domains of signals were produced with the PC-based DADiSP (DSP Development Corporation, 1999), except for the spectrogram, which was produced with Signalyze.

We used the following call parameters to describe vocalizations: call length, call rate, call rise time,

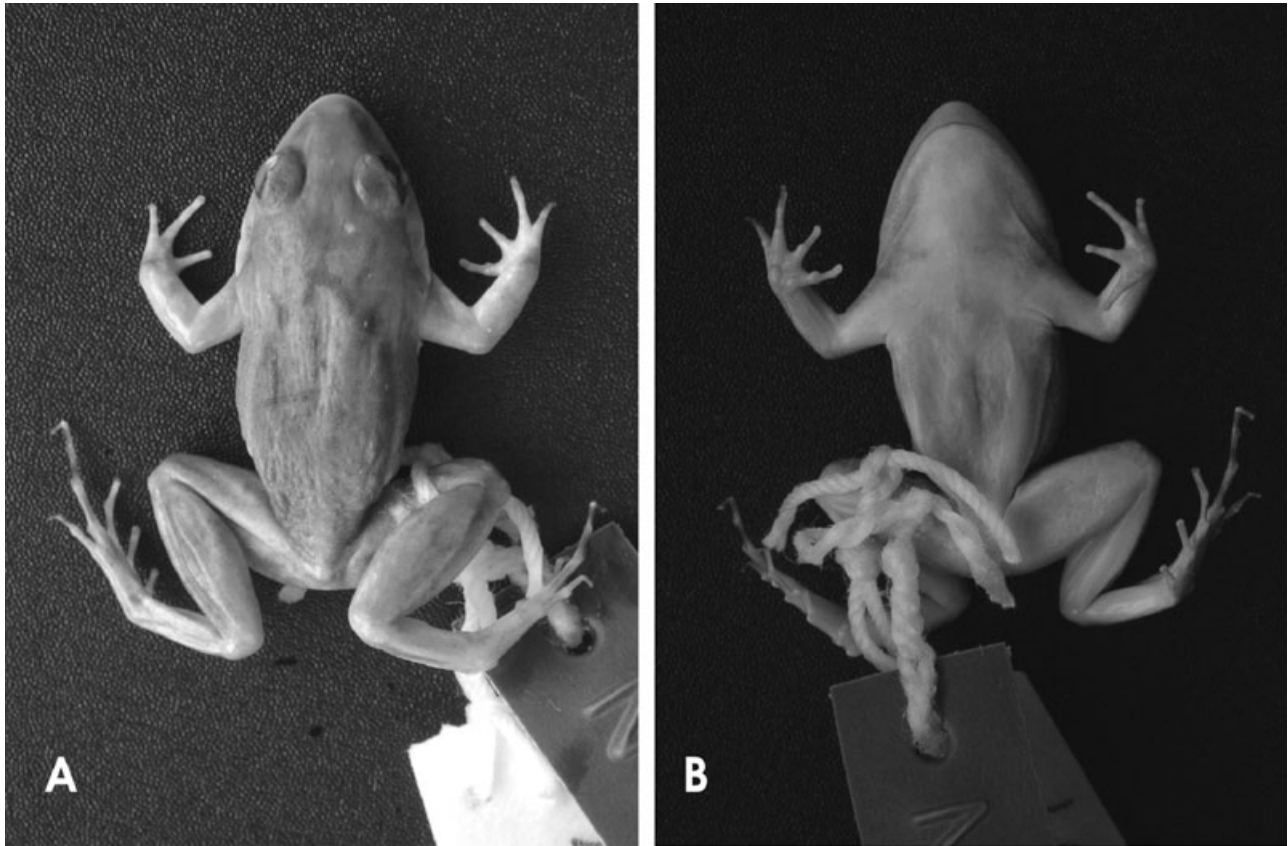


Figure 1. *Leptodactylus coca* sp. nov. Dorsal (A) and ventral (B) view of holotype male NKA 3630, snout–vent length (SVL) = 24.1 mm.

number of pulses per call, pulse rate, fundamental frequency, dominant frequency, and change in dominant frequency. We also took note of the number of harmonically related frequencies observed: even though these are a function of recording distance, it is important to note their presence where possible. Terminology for call variables follows that of Cocroft & Ryan (1995), except for fundamental frequency, which follows Ewing (1989) and change in dominant frequency (dominant frequency at the end of the call minus dominant frequency at the beginning of the call, modified from Márquez, De la Riva & Bosch, 1995). Frequency measurements were taken at the peak amplitude of each signal.

RESULTS

LEPTODACTYLUS COCA SP. NOV. (FIGS 1, 2)

Holotype: Adult male, NKA 3630, field number SR 99.134, collected near Carretera Antigua from Villa Tunari to Cochabamba (c. 800 m above sea level), just above the Village of El Palmar, Province of Chapare, Department of Cochabamba, Bolivia, on

September 25, 1997 by S. Reichle and J. Aparicio. The holotype is also a call voucher for the species, recorded by S. Reichle.

Paratypes: Two additional specimens were collected at the type locality: a gravid female (CBF 4298), and a male, which is also a call voucher (CBF 2619), recorded by S. Reichle and collected by S. Reichle and J. Aparicio on September 25, 1997.

DIAGNOSIS

Leptodactylus coca differs in its advertisement call from all other known members of the *L. marmoratus* group for which acoustic data are available. *Leptodactylus araucaria* (Kwet & Angulo, 2002) and *L. nanus* can be distinguished from *L. coca* by their small size [maximum SVL in *L. araucaria* males, 18.8 mm (Kwet & Angulo, 2002); maximum SVL in *L. nanus* males, 19.4 mm (Kwet, 2007)], whereas *Leptodactylus lutzi* (Heyer, 1975) differs from the new species by its large size [SVL in males, 25.7–33.5 mm (Kok *et al.*, 2007)], the presence of a dark triangular seat patch, and distinct spotting on the posterior face of the thigh

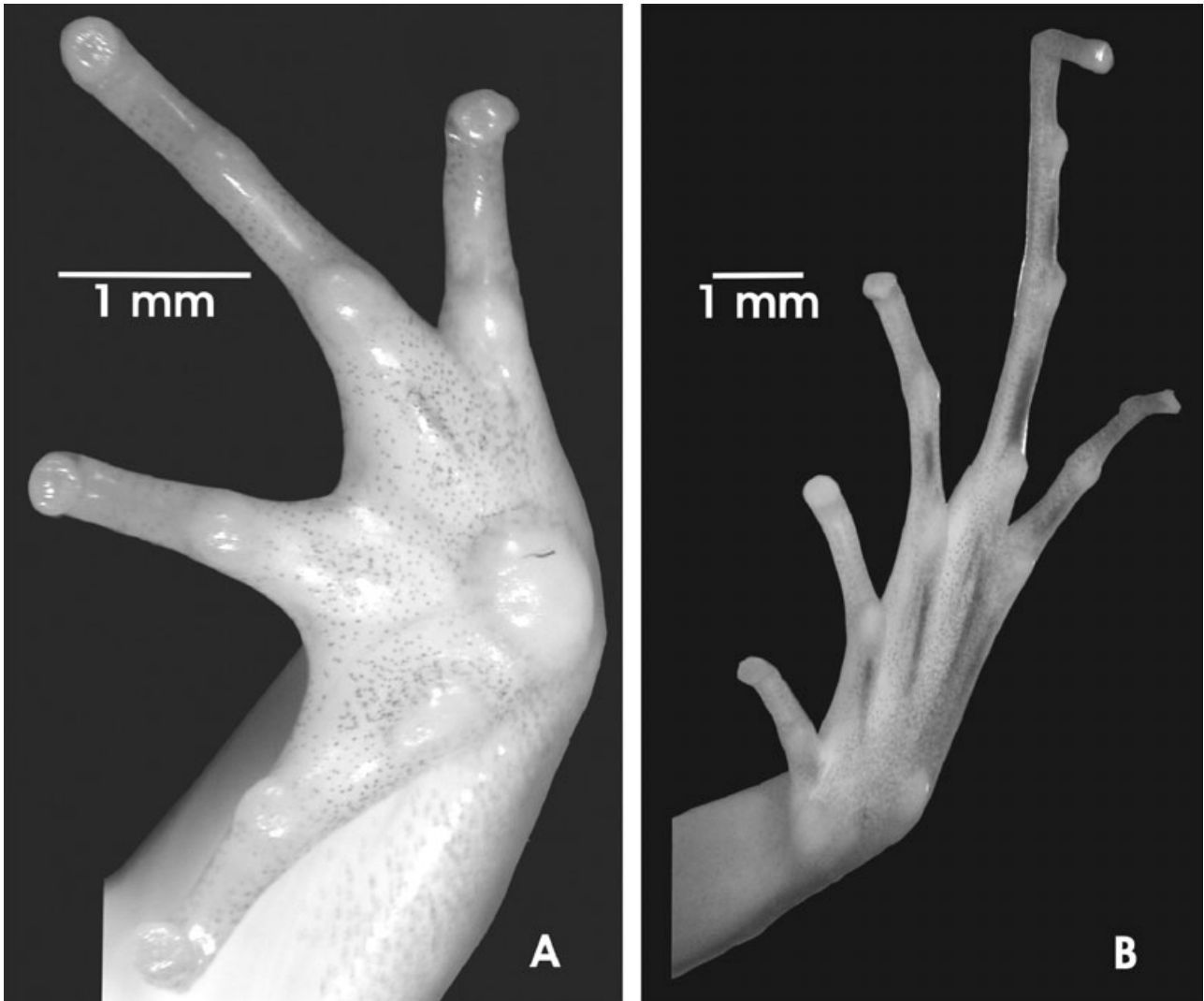


Figure 2. *Leptodactylus coca* sp. nov. Ventral view of left palm of hand (A) and left sole of foot (B) of holotype male NKA 3630.

(Heyer, 1975). Individuals of *Leptodactylus bokermanni* Heyer, 1973 have not been found to possess a light mid-dorsal stripe extending from above the vent to the middle of the body, whereas this is a possible pattern for individuals of *L. coca*. The new species differs from *Leptodactylus martinezi* Bokermann, 1956 in lacking four longitudinal series of dark glands on the dorsum (Bokermann, 1956). *L. andreae*, *L. diptyx*, *L. hylaedactylus*, *Leptodactylus marmoratus* Steindachner, 1867, and *Leptodactylus thomei* Almeida & Angulo, 2006 all have distinct, different advertisement calls to those of *L. coca*. There is a recently described species of *Leptodactylus* of the *marmoratus* group from French Guiana, *Leptodactylus heyeri* (Boistel, de Massary & Angulo, 2006), which has a similar call to that of *L. coca*. However, even with similar calls, there are differences in acous-

tic patterns. For example, even if the call rate is often influenced by the motivation of callers, the call of *L. heyeri* is issued at a much lower call rate than that of *L. coca*, and the call of *L. heyeri* does not show evidence of pulse structure, whereas it is present in *L. coca*. The species also differ morphologically: *L. heyeri* has a relatively broader head in proportion to the body; the dorsal coloration is very distinct, with stripes and bands, and well-defined black lumbar glands; and males of this species have a yellow throat and belly.

DESCRIPTION OF HOLOTYPE

Body small, robust, with relatively short limbs. Dorsal outline of snout rounded (Fig. 1), in profile snout nearly acuminate; head wider than long; nos-

trils positioned dorsolaterally, closer to tip of snout than to corner of eye; internarial distance about a quarter of head width. Tympanum distinct, slightly more than half the diameter of eye; tympanic membrane translucent, possible to observe tympanic canal; supratympanic fold weakly developed, extending from back of eye to arm insertion, dark, contouring outline above fold extending from back to eye to about half or two thirds of the way down to the arm; oval cream-coloured gland below angle of jaw, and supratympanic fold present and distinct; canthus rostralis indistinct. Vocal sac single and internal, paired elongate vocal slits present. Vomerine teeth posterior to choanae in transverse series parallel to choanae, separated from each other by approximately the length of one vomerine tooth row. Arms short and robust; fingers slender, finger lengths III > I = II > IV; finger tips rounded, without fringes or expansions; palms of hand with two large ovoid-shaped cream-coloured metacarpal tubercles; smaller inner metacarpal tubercle a little less than half the size of larger metacarpal tubercle; fingers with conspicuous, distinct cream-coloured rounded subarticular tubercles, absence of nuptial asperities. Hindlimbs robust, shank slightly longer than thigh; toe lengths IV > III > V > II > I; toe tips very slightly flattened or not, with very minor expansions (character state B of Heyer, 1973); toes without fringes. Metatarsal tubercles conspicuous, distinct; inner tarsal fold weakly developed, lined with scant, small white tubercles; tarsus also with small white tubercles, although not profuse; sole of foot with very sparse, scant white tubercles. Texture on dorsal surface mostly smooth, becoming tuberculate around the sacral area; thighs mostly smooth, shanks with very small, mostly scant, tubercles. Dorsolaterally, a dark longitudinal glandular fold starting just behind shoulder and running discontinuously posterior to sacral region; beneath it a second cream coloured glandular lateral fold extending along the same length as the darker fold. Surface of venter smooth.

COLOUR IN PRESERVATIVE

Preserved in 70% alcohol, the dorsum is a light brown colour, with the discontinuous upper dorsolateral glandular lines a darker shade of brown. There is a dark marking in the shape of an inverted U on the dorsum between the shoulders. Tips of glands on lower back dark brown; dorsal surface of limbs, a uniform light brown, except for the white-tipped tubercles and a dark bar on left foot. Tip of snout to back of head at about tympanum level a darker shade of brown, with a slight milky/foggy aspect to it. Venter white, immaculate; skin on edges of lower lip sparsely dotted with melanophores.

Table 1. Morphometric measurements for *Leptodactylus coca* sp. nov. All measurements are expressed in mm

Specimen	*NKA	CBF	CBF
	3630 ♂	2619 ♂	4298 ♀
Snout–vent length (SVL)	24.1	23.6	25.6
Head length (HL)	8.0	7.5	7.6
Head width (HW)	8.7	8.4	8.3
Eye diameter (ED)	2.6	2.7	2.7
Tympanum diameter (TYD)	1.4	1.3	1.5
Eye–nostril distance (END)	1.6	1.6	1.8
Interorbital distance (IOD)	2.4	2.3	2.4
Internarial distance (IND)	2.0	1.7	1.9
Forearm length (FAL)	4.3	4.7	4.5
Hand length (HDL)	5.0	5.0	5.5
Thigh length (THL)	9.7	9.6	9.9
Shank length (SL)	10.3	10.3	11.0
Tarsus length (TSL)	6.5	6.5	6.5
Foot length (FL)	11.5	11.0	11.8

*Holotype.

COLOUR IN LIFE

The coloration in life does not differ much from the colour described in preservative, just more intense.

MEASUREMENTS OF HOLOTYPE (IN MM)

SVL, 24.1; HL, 8.0; HW, 8.7; ED, 2.6; TYD, 1.4; END, 1.6; IOD, 2.4; IND, 2; FAL, 4.3; HDL, 5; THL, 9.7; SL, 10.3; TSL, 6.5; FL, 11.5.

MORPHOLOGICAL VARIATION

Female slightly larger than males (Table 1). The dorsal pattern of the male specimen CBF 2619 is similar to that of the holotype, except that it lacks the fogginess observed in the snout and head of the holotype; it does not have the inverted U-shaped dark marking between the shoulders; the two dark brown dorsolateral discontinuous glandular lines are ventrally flanked by broad cream lateral stripes that run parallel to them. In addition, it appears to have an inverted triangular-shaped interorbital spot, which, although fainter on the left side of the specimen, is dark brown from its base between the eyes to the back of the head, with a lighter spot within the triangle close to the right eye. Furthermore, specimen CBF 2619 has two symmetrical inguinal dark blotches on either side of the lower dorsum, and another two symmetrical dark blotches lower down, flanking the base of the hairline cream stripe that extends from the base of the vent to the lower back, and very sparse dark spots on the upper dorsum. The supratympanic fold is more pronounced from just behind the eye to behind the tympanum; it is weaker

Table 2. Measurement of advertisement call parameters for *Leptodactylus coca* sp. nov. Numbers in the first line are means \pm standard deviation; numbers in brackets are ranges

Recording	*NKA 3630	CBF 2619
Number of calls	23	18
Temperature (°C)	20.8	21.1
Call length (ms)	117.96 \pm 3.2 (110.14–123.45)	141.87 \pm 2.51 (136.03–145.22)
Call rate (calls/min)	84	66
Call rise time (ms)	52.08 \pm 22.17 (23.63–88.62)	41.71 \pm 20.25 (11.56–96.05)
Pulses/call	12 \pm 1.14 (10–15)	Strong amplitude modulation, but no distinct pulses
Pulse rate (/s)	120 \pm 12.85 (105.78–146.88)	Strong amplitude modulation, but no distinct pulses
Fundamental frequency (Hz)	1731.9 \pm 33.11 (1694.6–1794.3)	1855.2 \pm 26 (1814.2–1913.9)
Dominant frequency (Hz)	3599.03 \pm 68.5 (3449.1–3668.4)	3678.3 \pm 29.2 (3628.5–3748.1)
Change in dominant frequency (Hz)	254.65 \pm 173.2 (0–775.2)	445.02 \pm 99.10 (258.4–602.99)
Total number of harmonics detected	Up to 4	Up to 5 or 6

*Holotype.

from the back of tympanum to the arm. An accompanying dark brown bar follows the outline of the supratympanic fold only to the back of the tympanum. In contrast with the holotype, this specimen shows distinguishable irregular crossbars on the hindlimbs. The toe tips are expanded (character states C-D or D *sensu* Heyer, 1973) and flattened.

Female specimen CBF 4298 is morphologically the most different of the three specimens collected. It has a cream-coloured patch on the dorsum between shoulders; an inverted triangular-shaped interorbital spot that is dark brown from its base between the eyes to the level of the posterior edge of the tympanum, with the exception of a couple of lighter-coloured irregular spots within the triangle that take the appearance of eyes on a mask; it then takes a lighter shade from the apex of this spot posteriorly until it reaches the anterior edge of the shoulder-level cream patch. The 'mask' is flanked by two lighter coloured trapezoidal-shaped spots, one on either side of the head, behind the eyes. The supratympanic fold is well developed from the back of the eye to the arm, with a dark brown bar following the contour of the fold nearly to the arm. There is a cream-coloured mid-dorsal hair-line stripe running from above the vent to the cream-coloured shoulder patch, becoming progressively thinner anteriorly, and two lateral thin cream-coloured stripes run from the back of the tympanum to the groin, one on either side of the body. Two symmetrical inguinal dark blotches are located on either side of the lower dorsum. The remainder of the dorsum is marked with an irregular pattern of darker spots against a lighter brown background, which roughly resembles broken discontinuous lines running along the anterior–posterior axis. Irregular

crossbars are present on both arms and hindlimbs. The toe tips are slightly expanded (character state C *sensu* Heyer, 1973) and mildly flattened or not flattened.

In contrast to the holotype, both paratypes have profuse white-tipped tubercles on the soles of the feet. All specimens have rounded snouts in dorsal outline, although in profile the snout of the female appears to be more rounded than that of the males; all specimens have white-tipped tubercles on the dorsal surfaces of shanks and thighs (although these are more profuse on the shanks than they are on the thighs), and they all have immaculate creamish-coloured venters.

ADVERTISEMENT CALL

Acoustic parameter measurements for the two recorded voucher males are listed in Table 2. Figure 3 depicts both temporal and spectral patterns of the call. The advertisement call of *L. coca* has been previously described in Angulo (2004) as *Adenomera* 'Chapare'; it is here directly associated to the species name *L. coca*. The description is based on the combined calls from both vouchers, which were recorded at 21.1 °C and 20.8 °C, respectively. The advertisement call of the new species can be heard as a sound file on track 6, CD 2 of Márquez *et al.* (2002).

In comparison with other species of the *L. marmoratus* group (see Kwet & Angulo, 2002; Angulo *et al.*, 2003; and references within; Kok *et al.*, 2007; and this study, for acoustic parameters for other species) the advertisement call of *L. coca* is intermediate in duration, ranging from 110 to 145 ms, emitted at an intermediate to low call rate (66 and 84 calls/min). Call

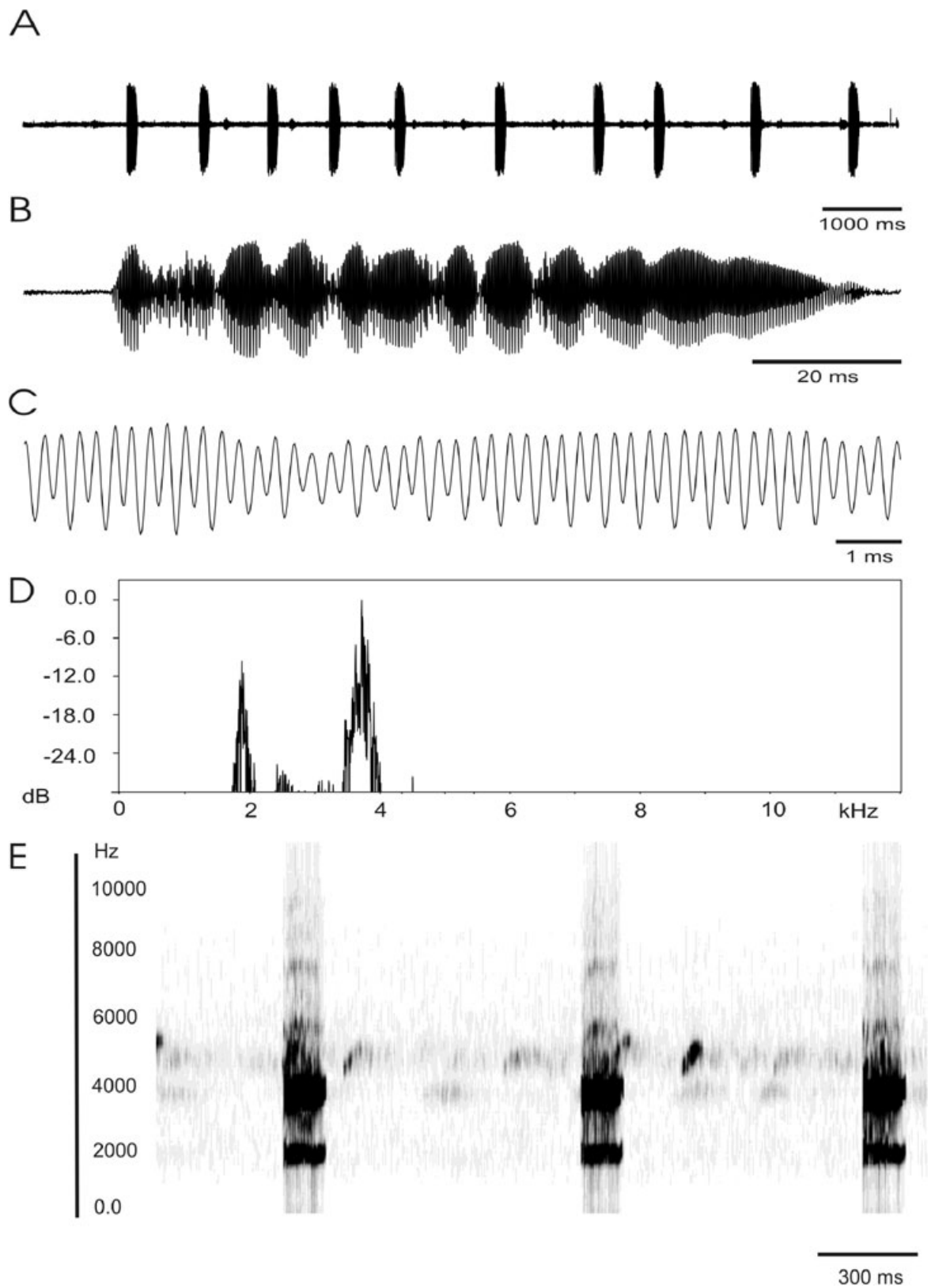


Figure 3. Advertisement call of *Leptodactylus coca* sp. nov. A, sequence of ten calls. B, one of these calls at higher resolution. C, section of call in (B) showing detail of waveform. D, power spectrum of (B). E, spectrogram of the first three calls in (A). Modified from Angulo (2004).

rise time is highly variable (11.6–96 ms), a fact reflected by associated standard deviations for both call vouchers. The signal undergoes quite strong amplitude modulation for most of its duration, before going into its final decay phase. This amplitude modulation creates some pulse structure, but because these pulses tend to meld together, it is difficult to specify an amplitude pattern. In one of the vouchers these modulations vary between 10 and 15 per call, emitted at an average rate of 120 pulses/s. The fundamental frequency ranges between 1695 and 1914 Hz, and a harmonically related second frequency, ranging between 3449 and 3748 Hz, is the main carrier. Up to five or six frequency bands can be detected at any time (this is also a function of recording conditions); nevertheless, given the fact that the energy peaks of upper harmonics are considerably lower than that of the main carrier, these frequency peaks are not depicted in the spectrum image of Figure 3, as they fall below the cut-off point of –30 dB. There is either no or some frequency modulation of the main carrier (0–775 Hz).

NATURAL HISTORY

The new species was found on open ground and at the base of dense, grassy vegetation in montane rainforests at about 800 m above sea level in the coca-growing region of Chapare, Bolivia. Voucher males were recorded in the evening after a heavy downpour during the day; individuals vocalized from dense clusters of grass as well as from open areas. Many individuals were heard calling, and some of them were very close (within 5 m) to each other. No water bodies were found close by, and we suspect that the species could display direct development (e.g. as has been reported for *L. marmoratus*; Lutz, 1931, 1947).

ETYMOLOGY

The new species is named after the coca plant, *Erythroxylon coca*, given its occurrence near coca plantations of the Chapare region, in Bolivia.

CONSERVATION STATUS

The three type specimens are the only known representatives of the new species. They were all collected on the same day, after heavy rain. Given the available information, we suggest that this species be considered as Data Deficient following the IUCN's Red List categories (IUCN, 2001).

DISCUSSION

One of the most difficult aspects of working with *Leptodactylus* of the *marmoratus* group is the resolu-

tion of species identities. This is made challenging not only because of the great morphological variation currently observed both within and among populations, but also because (1) great morphological variation occurs in most of the currently recognized species, and (2) locality data for several of the nominal species are at best vague (e.g. *L. diptyx*, *L. hylaedactylus*, and *L. marmoratus*). Of the 11 nominal species currently recognized for the group, three have been described in the last 6 years, two in the 1970s, one in the 1950s, two in the early 1920s, and three in the mid to late 1800s. Only the recent species descriptions encompass advertisement call data; the other species descriptions rely on morphological features (see Table 3 for a summary of morphometric measurements for all described species).

In his systematic revision of the *L. marmoratus* group, Heyer (1973) examined the geographical distribution patterns of several morphological features. With the data available at the time, he concluded that there were five species in the group. As there were 12 names available for members of the group, some had to be synonymized. Seven of the names were synonymized under *L. hylaedactylus* (Heyer, 1973), the nominal Amazonian species with the greatest number of synonyms (currently at a total of 11, *sensu* Frost, 2007). Given recent research pointing to greater species richness in the group, it would not be unlikely that any one of the recently identified taxa in the Amazon Basin and associated environments could be one of these junior synonyms. More recently, De la Riva (1996) removed *L. diptyx* from the synonymy within *L. hylaedactylus* to accommodate populations from Bolivia and Paraguay with a distinct reproductive mode. Unfortunately, however, the lectotype of *L. diptyx* was not examined when this change was effected (I. De la Riva, pers. comm.), and we have not been able to obtain the specimen on loan. We have, however, compared the calls and morphotypes of those populations that have become associated with the nominal *L. diptyx*, with those of *L. coca*, and the differences support the notion that these populations pertain to different taxa (see Comparison of advertisement calls).

In the specific case of the population assigned to the new species *L. coca*, we have not resurrected any former name synonymized under *L. hylaedactylus* for the following two reasons: (1) the type localities of the synonyms under this nominal species are geographically distant from the location where *L. coca* occurs, and there is evidence of the occurrence of other call types not assigned to a nominal species that are closer to these type localities, thus being better candidates for resurrection of a name in the absence of topotypic calls; (2) W. R. Heyer had kindly loaned photographic copies of type material of the *marmoratus* group to

Table 3. Morphometric measurements for adult specimens of described species of the *Leptodactylus marmoratus* group. Single numbers refer to holotype measurements when only these are available (except for the case of *Leptodactylus diptyx*, where five undesignated specimens were listed in the species description, but for which no ranges were provided); numbers in brackets refer to ranges derived from type series measurements, except for *Leptodactylus nanus* (measurements provided in Kwet, 2007). All measurements are expressed in mm. Measurements for *Leptodactylus hylaedactylus* were converted from lines (units of measurement equivalent to one-twelfth of an inch)

Species	SVL	HL	HW	ED	TYD	END	IOD	IND	THL	FL	Source
<i>Leptodactylus andreae</i> *	(20–21)	(8–8.5)	(7.5–9)	NA	½ of ED	NA	NA	NA	NA	NA	Müller (1923)
<i>Leptodactylus araucaria</i> (n = 11)	(17.1–19.9)	(5.7–6.9)	(5.5–6.7)	(1.7–2.0)	(1.1–1.3)	(1.4–1.7)	3.4–3.9	(1.8–2.2)	(7.3–8.2)	(9.0–10.1)	Kwet & Angulo (2002)
<i>Leptodactylus bokermanni</i> †	24.0	9.0	7.9	2.7	1.3	2.0	2.2	NA	9.7	12.3	Heyer (1973)
<i>Leptodactylus diptyx</i> (n = 5)	22	NA	NA	NA	½ of ED	NA	NA	NA	NA	NA	Boettiger (1885)
<i>Leptodactylus heyeri</i> (n = 4)	(22.5–25.8)	(9.1–11.0)	(9.2–10.7)	(2.3–3.0)	(1.5–2.0)	(1.9–2.0)	(2.5–3.3)	(2.1–2.7)	NA	NA	Boistel <i>et al.</i> (2006)
<i>Leptodactylus hylaedactylus</i> (n = 1)	24.6	NA	8.5	NA	NA	NA	NA	NA	NA	NA	Cope (1868)
<i>Leptodactylus lutzi</i> ‡ (n = 3)	(30.1–34.1)	11.1	11.2	3.0	1.7	2.8	2.4	NA	13.4	14.9	Heyer (1975)
<i>Leptodactylus marmoratus</i> (n = 1)	21.6	7.7	7.4	NA	NA	NA	NA	NA	9.2	NA	Kwet & Angulo (2002)
<i>Leptodactylus martinezi</i> (n = 2)	(23–23)	NA	(7–7)	(2.6–2.7)	(1.0–1.0)	(2.2–2.3)	NA	(2.0–2.1)	NA	NA	Bokermann (1956)
<i>Leptodactylus nanus</i> (n = 25)	(16.3–20)	(5.5–6.8)	(5.4–6.4)	(1.4–2.0)	(0.9–1.4)	(1.4–2.1)	(3.2–3.9)	(1.7–2.3)	(7.4–9.6)	(8.5–10.4)	Kwet (2007)
<i>Leptodactylus thomei</i> (n = 25)	(19.18–23.21)	(5.19–6.38)	(5.50–6.75)	(1.92–2.40)	(0.92–1.20)	(1.52–1.88)	(1.69–2.06)	(1.88–2.36)	(6.25–8.63)	(7.13–9.50)	Almeida & Angulo (2006)

*While there are several specimens in the type species, the author provides measurements only for the holotype and the largest female.

†Although there are other type specimens for *Leptodactylus bokermanni*, measurements for these were not included as they come from different localities and could comprise different species.

‡A more recent study provides new data on morphological variation (Kok *et al.*, 2007).

A. Angulo in 2000. We have compared these images to the specimens of the new population. Although it is true that morphological features vary widely within and among populations of the *marmoratus* group, we were at least able to get an idea of how different or similar these morphotypes could be from the new population. The photographic image of the holotype of *Leptodactylus hololius* Boulenger, 1918 (BMNH 1915.3.9.13) shows a similar body form and dorsal patterning to that of one of the specimens of *L. coca*, specimen CBF 4298. The type locality of *L. hololius*, however, is in northern Peru (Marañón River, Pebas), which, again, is geographically closer to other call types.

Currently, Amazonian drainage populations assigned to *L. andreae*, *L. diptyx*, and *L. hylaedactylus* are a major taxonomic challenge, as are those populations assigned to *L. bokermanni* and *L. marmoratus* in south-eastern Brazil. Populations initially thought to be the nominal *L. andreae* are at least three different species in south-eastern Peru, based on advertisement call data (Angulo *et al.*, 2003). Audio recordings of populations in south-eastern Peru and in Santa Cruz de la Sierra, however, indicate that *L. hylaedactylus* and *L. diptyx* have uncannily similar calls, with only subtle variations. In the case of this species pair, molecular profiles combined with detailed acoustic features and observations on reproductive modes may help to resolve species identities. Nevertheless, advertisement call data at the resolution that has been used in recent studies are still among the most valuable data for untangling species identity in most *Leptodactylus* of the *marmoratus* group.

COMPARISON OF ADVERTISEMENT CALLS

There are several published advertisement calls available for *Leptodactylus* of the *marmoratus* group of the Amazon Basin, Guianas, and their associated environments (Heyer, 1973; Straughan & Heyer, 1976; Schlüter, 1980; Zimmerman & Bogart, 1984; Schneider, Joermann & Hödl, 1988; Márquez *et al.*, 1995; Angulo & Icochea, 2003; Angulo *et al.*, 2003; Boistel, Massary & Angulo, 2006; Kok *et al.*, 2007); although the taxonomic status of several of these populations still awaits resolution, the distinctive advertisement calls of most of these populations are indicative of their species-specific nature, and can be used as a means of comparing other populations in the region. Herein we briefly compare the call of *L. coca* with calls from Amazon-associated populations (see Table 4 for acoustic parameters of published calls). Given that the call of the recently described *L. heyeri* from French Guiana is very similar to that of *L. coca*, we also compare the calls of *L. heyeri*.

Angulo (2004) described several of the calls discussed herein, and assigned certain names to undescribed or unresolved species. These names are provisional; following the Fourth Edition of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 2000: 6), these names are disclaimed for nomenclatural purposes until additional material can be further examined.

The call of *L. andreae* is described in Zimmerman & Bogart (1984) and Boistel *et al.* (2006). The calls from both the Amazonas and Saint-Eugène populations are shorter in duration and have higher fundamental and dominant frequencies than the call of *L. coca*. Likewise, the call of *L. diptyx*, described by Márquez *et al.* (1995) as that of *Adenomera* (= *Leptodactylus*) *andreae* (De la Riva *et al.*, 2000), is also shorter and has higher fundamental and dominant frequencies than the call of *L. coca*.

The call of *L. hylaedactylus* is provided in Heyer (1973), Straughan & Heyer (1976), Schlüter (1980), Schneider *et al.* (1988), Márquez *et al.* (1995), Angulo *et al.* (2003), and Boistel *et al.* (2006). Based on differences in the advertisement call, there is reason to believe that some of these populations may in fact represent different species (see Angulo *et al.*, 2003 for a more detailed discussion). The advertisement call of *L. coca* differs in call length from those populations described by Schlüter (1980), Schneider *et al.* (1988), Márquez *et al.* (1995), Angulo *et al.* (2003), Boistel *et al.* (2006), and the population of Chapada dos Guimarães, Mato Grosso, described by Straughan & Heyer (1976), in having a longer call; there is an overlap in this parameter for populations from Jataí, Goiás, and Santo Antônio do Leverger, Mato Grosso (Straughan & Heyer, 1976). There are also overlaps with these latter two populations with regards to call rate and some frequency values in the Jataí and Santo Antônio populations (see Table 4). However, both these populations present a lower frequency component (below 500 Hz), which is absent in *L. coca*, and the population from Santo Antônio do Leverger appears to lack intensity modulation. The population studied by Angulo *et al.* (2003) has a much lower number of pulses per call than *L. coca*.

The call of *L. heyeri* (Boistel *et al.*, 2006) is similar to that of *L. coca*. It can be, however, marginally longer, with a slightly higher main carrier frequency, and a lower call rate. The most outstanding difference between these calls is the amplitude pattern: there is no apparent pulse structure in the call of *L. heyeri*, whereas this structure is present in *L. coca*, although in one of the call vouchers it is difficult to determine the number of pulses. These differences in the advertisement calls, in addition to morphological differences and ecological associations (e.g. *L. heyeri* is

Table 4. Summary of acoustic parameters for species of the *Leptodactylus marmoratus* group of the Amazon Basin derived from published literature. *Leptodactylus heyeri* from French Guiana and *Leptodactylus lutzi* from Guiana are also included. F_0 represents the fundamental frequency

Species	Temp (C)	Call length (ms)	Call rate (calls/min)	F_0 (Hz)	Dom. Freq. (Hz)	Other freq. (Hz)	Pulses	Source
<i>Leptodactylus andreae</i> Brazil, Amazonas	N/A	80 (60–100)	79.59 (48.99–144.65)	2210 (2190–2230)	4100–4430	?	?	Zimmerman & Bogart (1984)
<i>L. andreae</i> French Guiana, Saint-Eugène	25.5	66.5 (44.5–85.7)	67.8*	2438 (2316–2692)	4871 (4557–5493)	Yes, up to 7 harmonics, although values are not specified	1	Boistel <i>et al.</i> (2006)
<i>Leptodactylus cf. andreae</i>	26–27	29 (21.2–37.7)	60.5 (30–78)	2370.06 (2247.7–2466.5)	2370.06 (2247.7–2466.5)	4682.59 (4475.6–4873.4) 7118.85 (6663.7–7304.6)	1	Angulo & Icochea (2003)
<i>L. diptyx</i> (as <i>L. andreae</i> in source) Bolivia, Santa Cruz, Buenavista	N/A	66.5 (56.6–88.3)	82.5 (52.6–128.4)	2228.6 (2180.8–2281.7)	4322.2 (4200–4502.9)	0	?	Márquez <i>et al.</i> (1995)
<i>L. heyeri</i> French Guiana, Saint-Eugène	25	154.2 (136.87–184.5)	33.6*	1856 (1815–1878)	3657 (3568–3844)	Yes, up to 6 harmonics, although values are not specified	1	Boistel <i>et al.</i> (2006)
<i>L. hylaedactylus</i> Bolivia, Santa Cruz, Puerto Almacén	N/A	56.8 (43.9–69.2)	132.2 (117.8–149.6)	2240.2 (2079.8–2463.5)	4448.2 (4200–4785.6)	0	1	Márquez <i>et al.</i> (1995)
<i>L. hylaedactylus</i> Brazil, Amazonas	23.9 & 23.8	59.87 & 73.11	–	< 2000 (1800–1900)	3200–4000	5000–6000	7–10	Schneider <i>et al.</i> (1988)

<i>L. hylaedactylus</i> Brazil																			Heyer (1973); Straughan & Heyer (1976)
a) Goiás, Jataí	a) 26	a) 130	a) 82	a) < 500Hz, 1860–2600	a) 3700–4833	a) 5966–6600; 8000													
b) Mato Grosso, Sto. Antonio de Leverger	b) 29	b) 110	b) 82	b) < 500Hz	b) 3833–5133	b) 5925–7300; 8000													
c) Mato Grosso, Chapada dos Guimarães	c) 19	c) 80	c) 190	c) < 500Hz, 2000–3270	c) 4240–5380	c) 6375–7725													
<i>L. hylaedactylus</i> Peru, Panguana	26	80	150	1700–2200 1800–2300	3500–4500 3400–4500	0													Schlüter (1980)
<i>L. hylaedactylus</i> Peru, Tambopata	25.5	49.7 (45.0–52.7)	259.2*	2107 (2091–2128)	4341 (4282–4407)	Yes, up to 5 harmonics, although values not specified	4												Boistel <i>et al.</i> (2006), for Other freq see in Angulo, Massary & Boistel (2006)
<i>L. hylaedactylus</i> Peru, Tambopata	24.2–27	46.02 (34.77–62.27)	210 (162–252)	2069.11 (1949.4–2208)	4248.43 (3958.4–4475.6)	6146.48 (5728.8–6743.2)	4–6												Angulo <i>et al.</i> (2003)
<i>L. lutzi</i>	c. 25	54.3 (41–64)	17–23	1722 (1636–1808)	3455 (3273–3617)	0	1												Kok <i>et al.</i> (2007)
<i>Leptodactylus</i> 'Forest Call I' Peru, Tambopata	22.2–26	208.5 (155.5–259.25)	12 (6–36)	2372.29 (2128.4–2585.9)	4771.1 (4415.9–5052.4)	6891.65 (6126.6–7614.4)	20–39												Angulo <i>et al.</i> (2003)
<i>Leptodactylus</i> 'Forest Call II' Peru, Tambopata	22–23.2	281.35 (191.98–505.75)	22.8 (15–31.2)	2213.54 (2028.9–2466.5)	4520.93 (4057.9–4953)	6450.23 (5171.8–7399.6)	10–17												Angulo <i>et al.</i> (2003)
<i>Leptodactylus</i> 'Forest Call III' Peru, Tambopata	25.9–26.6	48.61 (37.05–89.98)	52.22 (48–60)	2506.7 (2208–2679.5)	5106.58 (4654.6–5338.8)	–	1												Angulo <i>et al.</i> (2003)

*Calls/min were calculated from Boistel *et al.*'s (2006) note rate in number of notes per second [NR (NN/s)].

found in low elevations and on the summit of small hills up to 120 m elevation), support recognition of these two populations as distinct, different taxa.

Leptodactylus coca differs from Tambopata forest-associated populations (Angulo *et al.*, 2003) in the following features: fundamental and dominant frequencies (all higher in the three Tambopata forest call types); a longer call than in *Leptodactylus* 'Forest Call III' and a shorter one than *Leptodactylus* Forest Calls I and II; and a higher call rate than in these other populations. The call of *L. coca* has a pulse structure, whereas the call of *Leptodactylus* 'Forest Call III' does not, and although *Leptodactylus* Forest Calls I and II have a pulse structure, they differ from *L. coca* in the number of pulses (*Leptodactylus* 'Forest Call I') and the pulse rate (*Leptodactylus* 'Forest Call II'). The call of another unassigned population (*Leptodactylus* cf. *andreae*, Angulo & Icochea, 2003) differs from that of *L. coca* in having a much shorter call, without pulse structure, and in having higher frequency values for the first and second harmonics than in *L. coca*; furthermore, the fundamental frequency in this species is its dominant frequency, whereas in *L. coca* it is the second harmonically related frequency band.

ACOUSTIC SIGNALS AS DIAGNOSTIC FEATURES

Although morphological differences have traditionally been used to define and describe new species for almost all taxonomic groups, there are instances where morphological features alone do not suffice to identify and define new species. Typically, the cases in question involve morphologically cryptic species complexes and polymorphic species. Both these phenomena abound in the amphibian world, and *Leptodactylus* of the *marmoratus* group is but one of such groups. More and more different types of data, such as acoustic signals (Heyer, García-López & Cardoso, 1996; Angulo *et al.*, 2003), species ecology, or molecular sequences (Dawood *et al.*, 2002; Fu *et al.*, 2005; Ron *et al.*, 2006), which are characteristic of each taxon, are being used, either in combination or on their own, to help clarify the identities of otherwise cryptic or taxonomically difficult species groups.

The use of acoustic signals as diagnostic features in identifying species is common practice in several taxonomic groups that communicate acoustically, such as lacewings, orthopterans, birds, and anurans (frogs and toads). Differences among these signals, both within and between populations, have been supported by genetic differences in the past (Otte, 1989; references therein).

An example of the use of acoustic signals in the form of vibrational cues transmitted over a substrate to identify green lacewings can be found in Wells &

Henry (1998). The courtship songs of three species of the genus *Chrysoperla* were found to differ in both temporal and spectral characteristics; additional data gathered from playback experiments performed on females and hybridization experiments supported the hypothesis that different song-type populations are distinct biological species (Wells & Henry, 1998).

Notable examples of the use of acoustic signals in the identification of orthopteran species include Otte & Alexander's (1983) study of the cricket fauna of Australia: these authors state that 'most of our information was secured by . . . listening for the species-specific songs of male crickets'. They further explain how this approach affects treatment of data:

Utilizing songs as a principal method of initially distinguishing species, securing specimens, and amassing distribution records has a number of effects on the subsequent analysis and interpretation of taxonomic data. Perhaps the most important is that for the most part one knows before he begins to examine specimens microscopically which of them belong to the same and different species, and in many cases he knows even which are more likely related to one another. The morphological analysis then becomes a matter largely of locating differences and similarities between the species, rather than discovering how many species were taken. (Otte & Alexander, 1983)

Another important orthopteran example involves the Hawaiian cricket genus *Laupala*. Although these crickets are morphologically cryptic, taxonomic distinctions were made within species groups based on differences in male calling song, which were later corroborated with genetic data (Shaw, 1996a, b). The discoveries of two new species of *Laupala* crickets were initially based on differences in the song (*Laupala melewiki*), or on a combination of morphology, song, and natural history (*Laupala makaio*), and subsequent analyses on partial mitochondrial DNA sequences provided further evidence for the specific status of these crickets (Shaw, 2000).

Other crickets, such as members of the North American genus *Gryllus*, were also initially identified using their calling songs, and a subsequent phylogenetic analysis using mitochondrial DNA sequences supported a taxonomic reassignment that was already suspected based on song differences (Huang *et al.*, 2000).

Similar approaches are employed in certain groups of birds, where vocalizations are either initially used to infer species identity, and are later associated with other (e.g. genetic, ecological, or morphological) data (see, e.g. Cuervo *et al.*, 2005; Gebauer, Kaiser & Wassmann, 2006), or they are used in combination with other data (Johnson & Jones, 2001) to infer species identity. However, a key consideration in bird studies is the impact of song learning (Slabbekoorn &

Smith, 2002a; Päckert, 2006); this may be especially important in particular bird groups.

Acoustic signals were also considered useful in earlier species diagnosis of anurans (Barrio, 1964), but it was not until more recently that they have become more widespread in the diagnosis of morphologically cryptic anuran species (see, e.g. Heyer *et al.*, 1996; Reichle, Lötters & De la Riva, 2001; Kwet & Angulo, 2002; Angulo *et al.*, 2003; Brown *et al.*, 2006), and their use is becoming even more frequent as their potential to help untangle species identity in taxonomically difficult groups is realized, and as the cost of recording devices becomes more affordable. It is worth mentioning that although anuran calls are diverse, they are not generally considered to be as diverse or as complex as some bird songs are, and they also appear to be genetically determined (Rand, 1985), so that they do not need to have the same degree of consideration of song learning as some groups of birds do.

The combined evidence from all these studies thus supports the inference that giving attention to acoustic variation can be highly rewarding in identifying species and resolving species-level taxonomy in a number of cryptic taxa that use acoustic signals to communicate. Furthermore, morphologically cryptic species that are identified primarily by their acoustic signals would be good candidates for speciation studies, in view of the potential for speciation events that come with the divergence of mating signals (Wells & Henry, 1998).

SPECIES CONCEPTS

The species concept is paramount in the definition and understanding of these fundamental and natural units of evolution: species. Yet, at least 23 different species concepts have been identified (22 are examined in Mayden, 1997; an additional, more recent concept can be found in Stamos, 2003, the Biosimilarity Species Concept). Given the great diversity of life forms and the often overwhelming variation displayed by individual groups of organisms, as well as their biology and dynamic nature, it is hardly surprising to find a diversity of concepts and views on the issue of the 'species problem' to match this diversity of life. Although in recent times there has been a certain consensus on the idea that species comprise lineages or evolving populations (see, e.g. de Queiroz, 1998, 1999; Hey *et al.*, 2003; and references therein; this can be in part explained by the recognition of the importance of a lineage perspective, and also the great increase in the number of phylogenetic studies that are conducted and published), species diagnosability in the field is still something greatly desired, but may be extremely difficult to attain under lineage-orientated species concepts.

Although we recognize that there is no ideal species concept, we do believe that it is important to use a conceptual framework to structure empirical studies. Mayden (1997) proposed a hierarchical system whereby he identified primary and secondary concepts as a function of how well they performed according to three traditional criteria: theoretical significance, generality, and operationality. Primary concepts would be those that satisfy the need for a theoretical framework, and help to structure ideas and perceptions on species, whereas secondary concepts would be subordinate to primary concepts and would work as operational tools in identifying species. We take Mayden's (1997) approach in selecting a primary and a secondary species concept to identify a new species. Furthermore, we concur with Mayden's (1997) choice for a primary concept.

As a primary concept, we choose the Evolutionary Species Concept (ESC), which refers to a 'lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies' (Simpson, 1961). The concept thus emphasizes the historical lineage of populations (temporal and dimensional aspects), or what can be regarded as a primary feature of biological organisms. It is, however, vaguely defined (i.e. 'unitary evolutionary role and tendencies'), which can make application difficult. Hence, there is a need to complement the ESC with auxiliary (secondary) concepts.

In practice, we have observed the difficulties associated with attempting to identify leaf litter frogs of the *L. marmoratus* group on the basis of morphology alone. The traditional MSC is unfortunately insufficient on its own to identify most members of this group in the field, given their subtle morphological variations and lack of discreteness in this suite of features. *Leptodactylus* of the *marmoratus* group, however, like most other anuran species, is an ensemble that uses acoustic signals to communicate between members of the same species. For communication to be successful there must be an efficient exchange between signals and receiver/sender systems. Paterson (1993) referred to a Specific Mate Recognition System (SMRS), which is a co-adapted signal-response reaction chain. This system is fundamental to Paterson's Recognition Species Concept (RSC), where a species is 'that most inclusive population of individual biparental organisms which share a common fertilization system' (Paterson, 1993: 147). We choose this primary definition of the RSC as a secondary, operational species concept, given that acoustic communication systems are extremely important SMRSs in most anurans, including morphologically conserved groups.

In an SMRS, adaptive changes can occur in a step-wise fashion, so co-adaptation between sender and

receiver can thus be re-established with each new step. According to Paterson (1993), adaptive characters and those of the fertilization system are maintained under stabilizing selection when a species occupies its normal habitat. There are, however, some issues with the RSC, given that it was burdened with assumptions that did not follow directly from the primary definition (Templeton, 1989). One of these assumptions is that the SMRS is maintained by stabilizing selection as long as the species lives in its natural habitat; if the habitat changed, then the SMRS could be affected by directional selection, giving rise to new species. This assumption is an unnecessary requirement of the primary definition of the RSC.

There are several studies that support the hypothesis that the environment plays a role in acoustic signal divergence between populations and closely related species (e.g. birds, Slabbekoorn & Smith, 2002b; Baker, 2006; Nicholls *et al.*, 2006; and cricket frogs, Ryan, Cocroft & Wilczynski, 1990). However, this does not preclude the possibility that change can also occur within the same habitat. A natural habitat has local selection pressures brought about by species that coexist in the same habitat and the processes that occur within this habitat, which may also have an influence on the evolution of the SMRS of a species (see Gerhardt, 1982; Otte, 1994, cited in Wells & Henry, 1998), without any need of changing habitats.

Although we find the application of a secondary concept (in this particular instance, the RSC) helpful, its utilization does not preclude the use of other types of data. It is a tool to initially identify separate groups among morphologically similar organisms in the field. Once an initial separation is performed, a second level may include the examination of the morphological differences among these organisms, a third level may comprise examination of habitat use and ecological segregation, and so on. Therefore, in using a combination of concepts (ESC as a primary concept, the primary definition of the RSC as a secondary concept, in this specific case, although this is likely to change with the biological system under study), and integrating other available data in addition to those already exposed in these concepts, we may be able to apply a theoretical framework as well as a working guide to better inform us in our decisions on the status of populations of morphologically cryptic groups.

Given, then, the application of the concepts discussed above in light of the available bioacoustical, morphological, and ecological evidence for the population of Chapare, and in comparing this evidence with that of other populations of the region, we conclude that the differences observed justify the allocation of specific status to the population of the *L. marmoratus* group in Chapare, Bolivia.

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- Leptodactylus araucaria type series*: Brazil, Rio Grande do Sul, São Francisco de Paula, Potreiro Novo, Pró-Mata PUCRS, MCP 2421 (holotype), MCP 1794, MCP 3463, MCP 3672–73, MCP 3677; Brazil, Rio Grande do Sul, Bom Jesus, Encruzilhada das Antas, MCP 3346.
- Leptodactylus cf. bokermanni*: Brazil, São Paulo, Serra da Bocaina, São José do Barreiro, USNM 318183–318184.
- Leptodactylus diptyx*: Bolivia, Santa Cruz de la Sierra, NKA 3624–29.
- Leptodactylus heyeri*: French Guiana, Saint Eugène, MNHN 1997.2273, MNHN 1999.8301, MNHN 1999.8303.
- Leptodactylus hylaedactylus*: South America, Ecuador or Peru, Napo or Upper Marañon, ANSP 2240 (holotype).
- Leptodactylus lutzi*: Guyana, north-east Plateau of Mount Ayanganna (1490 m), ROM 40165, ROM 40167.
- Leptodactylus marmoratus*: Brazil, Rio de Janeiro, Jacarepaguá, Parque Estadual da Pedra Branca, Pau da Fome, MNRJ 27652–53; Brazil, Rio de Janeiro, Itaguaí, Hórto Florestal Santa Cruz, ROM 11683.
- Leptodactylus martinezi*: Brazil, Goiás, Ilha do Bananal, Mato Verde, USNM 200619–21; Brazil, Goiás, Rio São Miguel, MNRJ 5415; Brazil, Goiás, Veadeiros, MNRJ 5832–33; Brazil, Goiás, Catalão, MNRJ 24799.
- Leptodactylus nanus type series*: Brazil, Santa Catarina, Corupá (Rio Novo, Colonia Hansa), ZSM 661/1920 (three specimens).
- Leptodactylus thomei type series*: Brazil, Espírito Santo, Povoação, MBML 2521 (holotype), MBML 2297, MBML 2300, MBML 2302–14, MBML 2515–2520, MBML 2522–2527.

APPENDIX

OTHER SPECIMENS EXAMINED

Leptodactylus andreae type series: Brazil, Peixeboi, ZSM 145/1911/1–4 (four specimens).