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# Description of a new cryptic southwestern Amazonian species of leaf-gluing treefrog, genus *Dendropsophus* (Amphibia: Anura: Hylidae)

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**Abstract**. The western Amazonian treefrog *Dendropsophus bifurcus* (Andersson, 1945) is not as widespread as previously thought. Specimens from Bolivia and southern Peru, previously assigned to that species, are distinct and differ in calls, colouration and behaviour. The new species described here can be most easily distinguished from *D. bifurcus* from the northwestern part of the Amazon Basin in Colombia, Ecuador and northern Peru by its tibial colour pattern, and from the sympatric *D. leucophyllatus* by adult size and ventral colouration. Molecular data (12S and 16S rRNA) indicate that this Amazonian species is not even closely related to the Amazonian *D. bifurcus*, but has its closest relative, *D. elegans*, in the Atlantic Forest region of southeastern Brazil. *Dendropsophus bifurcus* appears to be only one of various species believed to be widespread in Amazonia, but actually consisting of groups of cryptic species that may not even, as in *D. bifurcus* and the new species, be closely related. We also discuss the inclusion of *D. anceps* in the *D. leucophyllatus* species group.

Key words. Bolivia, Brazil, *Dendropsophus bifurcus*, *Dendropsophus leucophyllatus* group, *Dendropsophus salli* sp. n., molecular genetics, Peru, vocalizations.

## Introduction

Small Neotropical treefrogs with 30 chromosomes and usually less than 40 mm in snout-to-vent length are widespread, especially in the lowlands of Amazonia, the Guiana Shield, the Atlantic Forest and Central America. They were formerly all placed in the genus *Hyla* Laurenti, 1768, and associated with six species groups, i.e., the *Hyla columbiana, H. leucophyllata, H. microcephala, H. minima, H. minuta,* and *H. parviceps* groups (Cochran & Goin 1970, Duellman 1974, 1982, 2001, Duellman & Crump 1974, Duellman & Fouquette 1968, Duellman & Trueb 1983). Several species were only tentatively placed within one of these groups or remained unallocated (e.g. Kaplan 1994). Faivovich et al. (2005) transferred them all to the genus *Dendropsophus* Fitzinger, and, based mostly on DNA sequence data, recognized nine species groups, but still left several species unassigned.

Among the most easily recognizable frogs as a phenetic group are those of the leaf-gluing treefrogs, the *Dendropsophus leucophyllatus* group, because they share a number of conspicuous characters, especially a wide body, bold dorsal colouration (light markings on dark ground or vice versa), a well-developed axillary membrane, flesh-coloured thighs, a reproductive mode of gluing their eggs onto leaves or twigs above the surface of a more or less stagnant body of water, and hatching tadpoles that drop into the water below. This mode of reproduction (Type E18 of Duellman & Trueb 1986) has been used to coin the name "leaf gluers" (German: *Laubkleber*) as early as in 1892 (Boettger & Pechuel-Loesche 1892). Furthermore, the tadpoles have violin-shaped bodies, in most cases one row of papillae on the posterior labium, and no labial tooth rows (Duellman 2001, Duellman & Trueb 1983, Gomes & Peixoto 1991). Currently, the group is comprised of eight species: *Dendropsophus bifurcus* (Andersson, 1945), from the western Amazon Basin of Colombia to Bolivia, *D. ebraccatus* (Cope, 1874), from Central America and western Colombia, *D. elegans* (Wied, 1824), from southeastern Brazil, *D. leucophyllatus* (Beireis, 1873), from the Guyanas and the Amazon Basin, *Dendropsophus rossalleni* (Goin, 1957), from central and western Amazonia, and *D. sarayacuensis* (Shreve, 1935) and *D. triangulum* (Günther, 1869), both from western Amazonia (Frost 2009). Faivovich et al. (2005) added another species from southeastern Brazil, *Dendropsophus anceps* (A. Lutz, 1929), which lacks almost all the mentioned morphological characters shared by the other members of the group.

While the frogs of the *D. leucophyllatus* group can be recognized morphologically as such fairly easily (with the one exception mentioned), some have proven difficult to assign to a certain species, because they are polymorphic with respect to their colour patterns. Thus, *D. triangulum* was described as a new species four times subsequent to its first description in 1869 (Duellman 1974), because different colour morphs were thought to be new species. *Dendropsophus elegans* was considered a synonym of *D. leucophyllatus* for more than a century (Caramaschi & Jim

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1982), and a frog described as *Hyla favosa* Cope, 1886 was shown to be merely a colour variant of *D. leucophyllatus* (Titus et al. 1989). Chek et al. (2001) provided molecular evidence that *D. leucophyllatus* (as *Hyla leucophyllata*) is a composite of more than one species, when they found that some populations of *D. leucophyllatus* were more closely related to *D. triangulum* than to other populations of *D. leucophyllatus*.

Two similar species, *D. bifurcus*, originally described from the Río Pastaza, Ecuador, and *D. leucophyllatus*, with "Surinam" as its type locality, occur sympatrically in parts of the western Amazon Basin from Colombia to Bolivia. Duellman (1974) distinguished *D. bifurcus* from northern Peru and Ecuador from *D. leucophyllatus* by its smaller size and tibial colouration. In *D. bifurcus* the tibia is tan except for a light (during the day) fleck on the heel. In *D. leucophyllatus* there are usually two light flecks (sometimes fused) on the tibia.

In the Bolivian departments of Pando, Beni and Santa Cruz, frogs were collected that frequently lived in sympatry with *D. leucophyllatus* and were repeatedly assigned to *D. bifurcus* because of their smaller size (e.g., de la Riva 1990), although they did not have one, but several large light flecks on the tibia. The different colouration and some behavioural inconsistencies between northern and southern populations observed by us (see Discussion) led us to investigate the possibility that these frogs did not represent southern morphs of *D. bifurcus*, but a distinct species. Surprisingly, it did not only prove to be a distinct species, but also that it is not even closely related to the other Amazonian species.

## Materials and methods

### Morphology

Measurements of frogs were taken with callipers or, if less than 5 mm, with the ocular micrometer of a dissecting microscope. Morphological terminology follows Duellman (2001). We use the webbing formula established by Savage & Heyer (1967), as modified by Myers & Duellman (1982). Abbreviations are as follows: ED: eye diameter; EN: distance from eye to naris; FD: diameter of finger disc on Finger III; FL: foot length from the tibiotarsal articulation to the tip of Toe IV; HL: head length; HW: head width; IN: internarial distance; SVL: snout-to-vent length; TD: tympanum diameter; TE: distance between tympanum and eye; TL: tibia length. Museum abbreviations are as follows: MNK-A: Museo de Historia Natural Noel Kempff Mercado (Amphibian Collection), Santa Cruz de la Sierra, Bolivia; SMNS: Staatliches Museum für Naturkunde, Stuttgart, Germany; ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

### Bioacoustics

Calls were recorded using a Sony WM D6C cassette recorder and a Sennheiser Me-66 directional microphone. They were analysed with Cool Edit 2000 software, and digitalised at 22500 Hz. Frequency information was obtained through Fast Fourier Transform (FFT) at 1024 points window width. A window width of FFT 256 was used for the production of spectrograms. Terminology in call descriptions follows Heyer et al. (1990).

### Molecular genetics

Total genomic DNA from tissue samples (preserved in 99% ethanol) of *Dendropsophus bifurcus* specimens sampled in Ecuador (near Jatun Sacha Biological Station, Ecuador) and Bolivia (sampled on Río Chevejecure, Bolivia, 14°52'58"S, 65°57'59"W) were isolated by phenol/chloroform extraction according to Blin & Stafford (1976) and stored at 4°C. GenBank sequences of the following treefrogs were added to the data set (accession numbers in parentheses): *Dendropsophus marmoratus* (AF308082, AF308114), *Dendropsophus minutus* (AF308081, AF308112), *Dendropsophus anceps* (AY843597), *Dendropsophus bifurcus* (AF308073, AF308098), *Dendropsophus sarayacuensis* (AE308076, AF308104), *Dendropsophus triangulum* (AY326053), *Dendropsophus leucophyllatus* (AF308072, AF308097), *Dendropsophus ebraccatus* (AF308074, AF308101), and *Dendropsophus elegans* (AF308075, AF308103). See also supporting information S1.

Genomic DNA was amplified by a polymerase chain reaction (PCR), using universal primers specific for 12S- and 16S rRNA mitochondrial genes (Palumbi 1996) named 12Sfor (AAACTGGGATTAGATACCCCACTAT) and 16Srev (CCGGTCTGAACTCAGATCACGT). PCR reactions were performed with a Taq Polymerase Kit (Quiagen) in a Biozym PTC 200 cycler under the following conditions: 120 s at 92 °C predenaturation, 30 cycles consisting of 40 s at 92 °C denaturation, 60 s at primer specific annealing temperature, and 60 s per 1 kb at 72 °C elongation. A fragment of approximately 2000 bp was amplified. Additionally, internal primers were designed to amplify smaller fragments in order to simplify the cloning and sequencing process: 16Sfor (GAAAGATTAAAAGAAAAAGAAGGAACTCG) and 16Srev2 (CGATGTTTTTGGTAAACAGGC). The PCR fragments were purified by an agarose gel electrophoresis, ligated into pGEM-T vector system I (Promega), and electroporated into *Escherichia coli* TOP 10 cells (Invitrogen). DNA was isolated from positive clones, using the QIAprep Spin Miniprep Kit (Qiagen). Three positive clones were sequenced on both strands for every primer set with universal primers using an automated LI-COR DNA sequencer 4200. Nucleotide sequence data have been deposited at GenBank (Accession numbers: AY362975-AY362977).

Computer-generated alignments were initially constructed using Clustal X (vers. 1.81 ; Thompson et al. 1997). In a manual realignment, sequences were carefully adjusted to known secondary structure models of *Lithobates catesbeianus* (Nagae 1988) and *Xenopus laevis* (Gutell & Fox 1988). Conformation and improvement of the alignment was followed by separation of rDNA sequences in loop and stem regions. To recognize and eliminate poorly alignable sequence parts for phylogenetic analyses, GBLOCKS software with default settings for rDNA alignments was applied (Castresana 2000). See supporting information S2.

Phylogenetic reconstructions were performed applying three methods: maximum-parsimony (MP) and neigh-

bour-joining (NJ) included in PAUP (Swofford 2000) and maximum likelihood (ML) as implemented in TREE-PUZZLE (Schmidt et al. 2002) and PhyML (Guindon & Gascuel 2003). Heuristic parsimony analyses were performed with random taxon addition and tree bisection-reconnection (TBR) branch swapping. Neighbour-joining analyses were carried out with the optimal criterion set to distance (p-distances). The K2P model was used (equal base frequencies; unequal TS/TV). Maximum likelihood analyses were based on the HKY model (Hasegawa et al. 1985) and the GTR model, using the discrete gamma distribution (eight categories) for site heterogeneity (Yang et al. 1996) and by assuming that a certain fraction of sites are evolutionarily invariable (Tamura et al. 2007). Support of internal branches was determined either by bootstrap analyses (MP and NJ) based on 10000 replicates or by quartet puzzling support values based on 10000 puzzling steps (ML).

## Results

### Molecular data

A mitochondrial region of 2000 nucleotides (approximately 490 bp of 12S rRNA, 1420 bp of 16S rRNA, and 69 bp of tRNA-Valin) was sequenced for each of the species collected for this study and combined with previously available data (S1). The totally sequenced region of the aligned data set corresponds to position 2509-4551 of the mitochondrial genome of *X. laevis*. This rDNA alignment was separated in loop and stem regions, but also analysed as complete data set (S2). Based on the analyses of Chek et al. (2001), *D. marmoratus* and *D. minutus* are appropriate outgroup species to analyse phylogenetic relationships of the *D. leucophyllatus* group. Recently, *D. anceps* was proposed to be a member of the *D. leucophyllatus* group (Faivovich et al. 2005). However, this species consistently clustered with the two outgroup taxa and was therefore omitted from the phylogenetic data set. MP, NJ, and ML analyses of the complete data set produced the same topology (Fig. 7). The species from Bolivia known as *D. bifurcus*, which we are describing as a new species here, appears to be closely related to *D. elegans* (bootstrap and puzzle values of 90 – 99% and not to be a sister group of *D. bifurcus* from Ecuador. Other relationships of the *D. leucophyllatus* group calculated with the complete data set are similar to the 12S and 16S rDNA analyses of Chek et al. (2001). The basal position of *D. elegans* and *D. salli* in the *D. leucophyllatus* group is supported by bootstrap and puzzle values of 94 – 96%. Branching patterns are far less resolved if stem regions are used for phylogenetic analyses. Only two clades are always confirmed by MP, NJ, and ML calculations based on rDNA stem sequences. Namely, the sister relationship between *D. bifurcus* sequenced by Chek et al. (2001) and *D. bifurcus* sequenced in this work, as well as the sister relationship between *D. elegans* and *D. salli*. If rDNA loop sequences are the underlying source for systematic analyses of the *D. leucophyllatus* group, branching patterns are nearly as resolved as if the complete data set is used. The relative position of *D. bifurcus* (in close relation *to D. sarayacuensis*) and *D. salli* (in close relation to *D. elegans*) was consistent across all analyses that were carried out with the rDNA loop data set. Thus, *D. bifurcus* and *D. salli* are clearly paraphyletic in our study. A high sequence divergence supports the observation that *D. bifurcus* and *D. salli* differentiated into two monophyletic lineages. We detected 336 nucleotide substitutions when comparing the sequence of *D. bifurcus* from Ecuador with the one of *D. salli* from Bolivia. This accounts for a sequence divergence of 16.9%.

### Systematics and bioacoustics

#### ***Dendropsophus salli* sp. n.**

(Figs. 1–3)

*Hyla bifurca* (non Andersson, 1945) – De la Riva 1990
*Hyla bifurca* – Márquez et al. 1993
*Hyla bifurca* – De la Riva et al. 2000
*Hyla bifurca* – Moravec & Aparicio 2000
*Hyla bifurca* – Reichle 2002, 2003
*Hyla bifurca* – Moravec & Aparicio 2005
*Dendropsophus bifurcus* – Faivovich et al. 2005 (partim)
*Dendropsophus bifurcus* – Embert & Reichle 2008
*Dendropsophus bifurcus* – Schulze et al. 2009
*Dendropsophus leucophyllatus* – von May et al. 2010

Holotype: MNK-A 8445 (Fig. 1), an adult male, collected about 7 km ESE of Rurrenabaque near the road to Yucumo (14°26'11"S, 67°29'35"W), 340 m a.s.l., Provincia Ballivian, Departamento Beni, Bolivia, by S. Reichle and K.-H. Jungfer on 29 December 2000.

Paratypes: Thirteen specimens (Fig. 2), all from Bolivia: MNK-A 8446–8447 (adult males), MNK-A 8448 (subadult female), ZFMK 62828 (adult male) from Río Chevejecure (14°52'58"S, 65°57'59"W), Departamento Beni, collected by S. Reichle and C. Cortez in January 1996; MNK-A 6574–6576, ZFMK 88037–88038 (adult males) from about 5 km ENE Palos Blancos, 452 m a.s.l., Departamento Beni (15°36'18"S, 67°12'36"W), collected by S. Reichle and D. Embert in March 2003; SMNS 13165–13166 (adult females), SMNS 13167–13168 (adult males) from San Sebastián/Tahuamanu, Departamento Pando (11°24'26"S, 69°01'04"W), collected by S. Reichle, M. Guerrero and G. Calderón in November 1999.

Diagnosis: A small species in the *Dendropsophus leucophyllatus* group with the following characters: (1) Males attaining 30.1 mm SVL, and females 32.5 mm SVL. De la Riva (1993, as *Hyla bifurca*) noted 37 mm SVL as the maximum

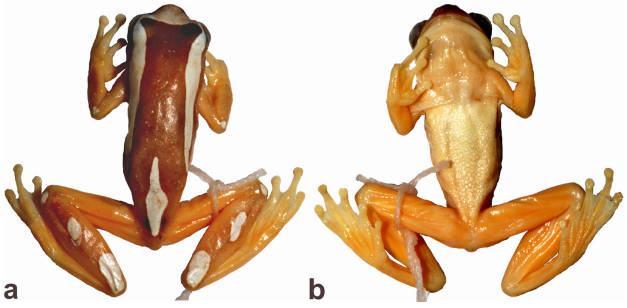


Figure 1. Dorsal (a) and ventral (b) views of the preserved male holotype of *Dendropsophus salli* sp. n. (MNK-A 8445).

size of females. (2) Axillary membrane reaching halfway to elbow. (3) Palmar tubercle single. (4) Pectoral patches moderate. (5) Dorsal light markings consisting of a triangular head blotch connected to broad dorsolateral bands along the anterior two thirds of body; light sacral blotch discrete. (6) Light elbow blotch present. (7) Light knee, mid-shank and heel blotches present. (8) Webbing yellow or orange, venter yellow in life, lacking red colouration. (9) Advertisement call consisting of two notes, the first one with a duration of 71.4 ms on average, the second one with 10.6 ms on average, both distinctly pulsed with 16–23, and 1–3 pulses, respectively.

Comparisons: *Dendropsophus salli* sp. n. differs from all other Bolivian hylid frogs except *D. leucophyllatus* and *D. sarayacuensis* by the presence of large light blotches on the dorsal surfaces of the head, dorsum, lower arms, and shank. Other frogs in the *D. leucophyllatus* group east of the Andes can be distinguished from *D. salli* (in parentheses) as follows: *Dendropsophus triangulum* bears two large pectoral patches that are in contact or nearly so (weak, distinctly apart), a light dorsum with or without a varying number of dark blotches or spots (light blotches on tan ground) and red venter (lemon-yellow). *Dendropsophus rossalleni* and *D. sarayacuensis* have frayed light spots or blotches (smooth-rimmed). A triangular blotch on the head of *D. sarayacuensis* does not reach a sickle-shaped postocular fleck, which reaches no further than mid-body (blotches fused to form a U-shaped mark extending to sacral area). *Dendropsophus rossalleni* usually lacks a triangle on the head, but has a broad crossbar in the anterior area of the orbits, sometimes with a spike medially towards the tip of the snout, or an isolated spot may be present posterior to the internarial region. Posteriorly, the light colour pattern is highly variable, ranging from almost absent to broad dorsolateral bands fusing anterior to the sacrum, but the crossbar on the head and the dorsolateral bands do not fuse. *Dendropsophus leucophyllatus* is a relatively large frog. Males from adjacent southern Peru (Duellman 2005) reach 35.3 mm and females 40.2 mm SVL (30.1 mm/37 mm). The dorsal pattern is similar to *D. salli* with a pattern of light dorsal blotches, or with a light reticulum. A light knee spot usually is absent (present), as are light spots on the arms (present). The webbing in live frogs is red (yellow or orange). The dorsal surfaces of the hind limbs of *D. bifurcus* bear only one light heel blotch or very rarely another one on mid-shank (knee and heel spots and one or two blotches on mid-shank). The dorsum of *D. bifurcus* usually bears numerous minute light spots (absent), and dark spots are present in some specimens at night (absent). The advertisement call is distinctly different (Fig. 5, see below and Discussion). Most *D. elegans* have a large tan mid-dorsal rectangular blotch bordered by light colouration (usually a light dorsolateral band discontinuous with sacral blotch). The tibia is uniformly light in colour (blotched). There are *D. salli*-coloured specimens of *D. elegans* (Gomes & Peixoto 1991: Fig. 8), however. The latter has more extensive webbing that reaches the disc on Finger IV (to penultimate subarticular tubercle).

Description of holotype: An adult male of 27.7 mm SVL. Body about as wide as head, which is slightly longer than wide. Top of head flat. Snout short, round in dorsal aspect and truncate in profile. Nostrils slightly protuberant, open-

Figure 2. Dorsal views of the paratypes of *Dendropsophus salli* sp. n., depicting variation in light blotches on the upper surfaces of the body. Upper row, from left to right: MNK-A 8446, MNK-A 8447, MNK-A 8448, ZFMK 62828 from Chevejecure, Beni, Bolivia; middle row: MNK-A 6574-6, ZFMK 88037–88038 from 5 km ENE Palos Blancos, Beni, Bolivia; lower row: SMNS 13165–13168 from San Sebastián/Tahuamanu, Pando, Bolivia.

ing posterolaterally. Canthus rostralis straight, indistinct, round. Loreal region plain. Lips thin, barely flared. A faint supratympanic fold, extending posteriorly from the posterior corner of the eye to an area above the anterior part of the arm insertion. Tympanic annulus almost round, only slightly wider than high. Its size equals the diameter of the disc on Finger III and also the distance between the eye and the tympanum. Tympanic membrane not differentiated, tympanic annulus visible below skin. Axillary membrane reaches halfway to the elbow. Two glandular patches visible on the chest posterior to the clavicle, separated from each other by about half their width.

Finger discs large. Disc on Finger III about 1.6 times the width of the finger. Subarticular tubercles round; distal tubercle on Finger IV bifid. Supernumerary tubercles on the proximal segments of digits. Palmar tubercle elongate, single. Prepollex elliptical, enlarged, lacking nuptial pad. Webbing formula of the hand is $I2^{+} - 2½II1½ - 3^{-}$ II I2½ – 2½IV.

Hind limbs moderately long; the heels overlap only slightly when adpressed. A flap-like tarsal fold extends from the inner metatarsal tubercle to the tibiotarsal articulation. Inner metatarsal tubercle large, ovoid. Outer metatarsal tubercle indistinguishable. Subarticular tubercles round, moderate, subconical. Numerous supernumerary tubercles on proximal segments of digits. Webbing formula of the foot is $I1^{+} - 2⅓II1 - 2⅔III1^{+} - 3^{-}IV3^{-} - 1^{+}V$.

Cloacal opening situated at the level of upper edges of thighs, bearing a small cloacal flap. Skin smooth, except for the vocal sac in the gular area to anterior edge of clavicle, which is longitudinally wrinkled, the pectoral patches, which are finely granular, and the belly, which is coarsely granular.

Tongue round, slightly free behind. Vocal slits open below the rim of the central part of the tongue. Choanae small, rounded. Vomerine odontophores situated between choanae, straight, in a line, but not in contact with each other, about as wide as choanae, bearing three vomerine teeth each.

Measurements: SVL 27.7 mm, HL 8.9 mm, HW 8.7 mm, TL 14.6 mm, FL 20.5 mm, ED 3.3 mm, TD 1.3 mm, EN 2.1 mm, IN 1.6 mm, TE 1.3 mm, FD 1.3 mm.

In preservative, the dorsal ground colour is dark brown. It continues laterally and includes the upper lip and the lateral area above the arm insertion to the upper level of the thigh. On the head, the brown colouration is interrupted by a white triangle with its base in the interorbital area and its apex between the nostrils. It is connected by a supraocular line to a broad dorsolateral band on each side of the body that reaches to the anterior portion of the sacrum. Another broad white streak is present middorsally in the sacral area. There are a few small white spots scattered over the whole dorsum. Ventrally and ventrolaterally, including the lower lip, the body is pale yellow. The limbs are pale yellow ventrally, with the exception of the thighs that are pale pink on all surfaces. The dorsal surfaces of the arms are tan. There is a white fleck on the elbow, and both upper and forearm bear several minute white spots. The hands are pale yellow with numerous tan melanophores on the dorsal surfaces of Finger III and IV. The shank is tan dorsally and bears a small blotch on the knee, and two larger ones medially and distally towards the tibiotarsal articulation. The posterior part of the tarsus and the dorsal surfaces of Fingers III and IV bear numerous tan melanophores.

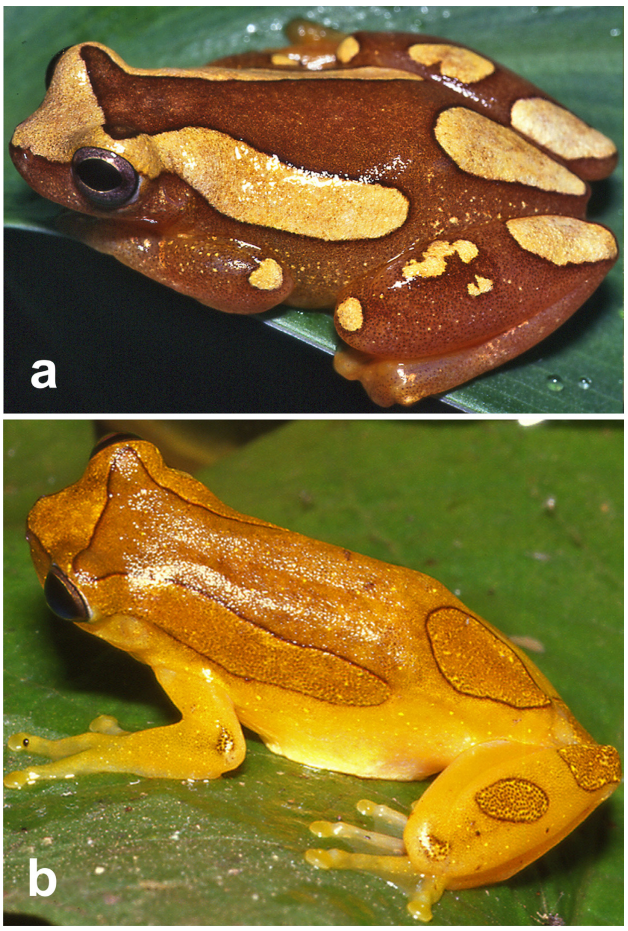


Figure 3. *Dendropsophus salli* sp. n. in life: (a) colouration during the day of a specimen from Chevejecure, Bolivia; (b) night colouration of a specimen from the type locality. Both specimens not collected.

In life (Fig. 3), during the day, the dorsal ground colour of the body and limbs was dark tan with bright yellow or sometimes creamy yellow blotches, narrowly outlined with black. The ventral areas were bright lemon-yellow. At night, the dorsal and lateral surfaces of head, body and dorsum were tan. The blotches on the dorsum and limbs were the same colour, but narrowly outlined with dark brown. The flanks and limbs were dark yellow. The iris was light silvery bronze (dark bronze at night) with a narrow golden ring around the pupil.

Variation: The eleven males of the type series have a mean SVL of 27.04 mm (± 1.63 mm SD). Two adult females are 32.0 and 32.5 mm in SVL. Proportions (means ± SD) for the males are as follows: HL/SVL 0.332 ± 0.013 SD, HW/SVL 0.326 ± 0.012 SD, TL/SVL 0.510 ± 0.017 SD, FL/SVL 0.728 ± 0.037 SD, TD/ED 0.421 ± 0.045 SD, HL/HW 1.017 ± 0.028 SD, TD/HL 1.032 ± 0.018 SD. Two adult females: HL/SVL 0.336, HW/SVL 0.327, TL/SVL 0.519, FL/SVL 0.750, TD/ED 0.506, HL/HW 1.028, TD/HL 0.189. Data based only on two females seem to indicate that the tympanum diameter in females might be wider than in males. The body is slightly wider than the head in some specimens, and HW

in some cases equals HL. Snouts vary in shape from round to truncate in dorsal aspect and from bluntly rounded to truncate in profile. Webbing on the hand varies slightly among specimens: I($2^{+}$–2½) – (2½–2⅔)II1½ – (2⅔–3) III(2½–2⅔) – ($2^{+}$–2½)IV. Variation of webbing between toes is I($1^{+}$–1 ½) – 2⅓II(1–$1^{+}$) – (2⅔–$3^{-}$)III($1^{+}$–1½) – (2⅔–$3^{-}$) IV(2⅔–$3^{-}$) – $1^{+}$V. Variation also exists with respect to the amount of light spots and blotches on the limbs. See Fig. 2 for variation in dorsal colouration of the type series.

Vocalizations: The advertisement call of *Dendropsophus salli* consists of two different notes, a longer Type I and a shorter Type II, which follows note Type I (Fig. 5a). In ten analysed calls (air temperature 23.7°C), the call consists of 3–4 notes (3.8 ± 0.42) and has a duration of 212–282 ms (261 ± 26). The dominant frequency is about 2.85–3.00 KHz (2.89 ± 0.07). Notes of Type I are much longer than those of Type II, and have durations of 61–80 ms (71.4 ± 8.6), while those of Type II have 5–15 ms (10.6 ± 2.4). Type I notes have 16–23 pulses/note (20 ± 2), Type II notes have 1–3 pulses/note (1.89 ± 0.4). The resulting pulse rates are higher in notes of Type I with 259–391 pulses/second (281 ± 18.8) than in those of Type II with 111–222 pulses/second (182 ± 38.1). Calls of *D. salli* (as *Hyla bifurca*) were published on CD by Reichle (2002).

Habitat and natural history: In Bolivia, *D. salli* is found in lowland moist forests, transitional forests between southwestern Amazonia and the Chiquitania Dry Forests (where they breed in temporary forest pools), but also in forest edge situations (De La Riva 1993, as *H. bifurca*, Reichle 2007, as *D. bifurcus*). De La Riva (1993) provides data on

Figure 4. An amplectant pair of *Dendropsophus bifurcus* at night near Pomona, Río Pastaza, Pastaza, Ecuador. Note the absence of a median light blotch on the tibia and on the heel. Dorsal markings are lighter than ground colour by day and night. Note the minute light dorsal spots. Some darker spots may also be present on the middorsum in some individuals at night. "Rio Pastaza", Ecuador, is the type locality of *Dendropsophus bifurcus* (Andersson 1945).

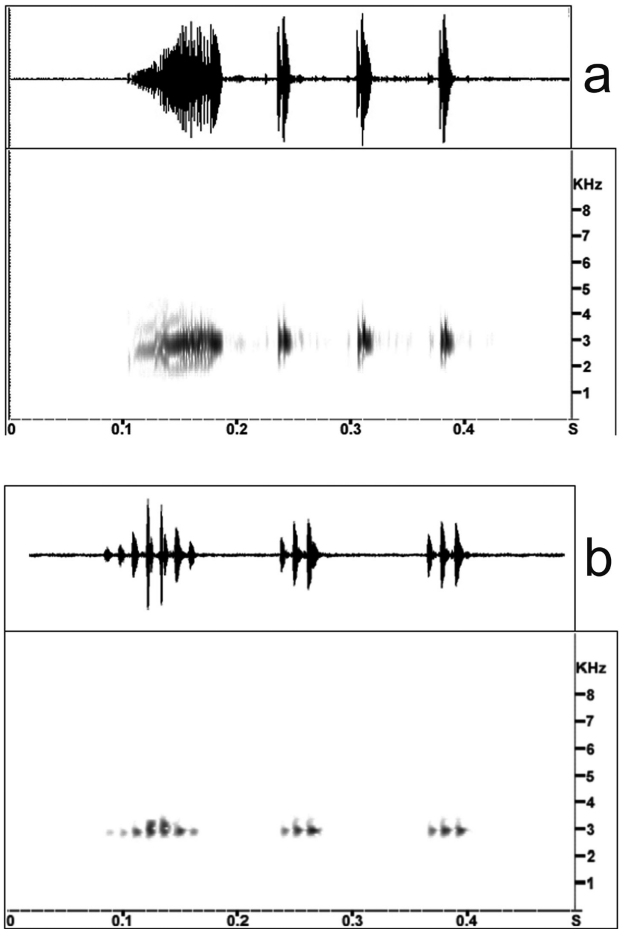


Figure 5. (a) Sound spectrogram and corresponding oscillogram (above) of an advertisement call of *Dendropsophus salli* sp. n., (Bolivia, Departamento Beni, 7 km ESE Rurrenabaque, 29 Dec. 2000, 23.7°C). One Type I note is followed by three Type II notes. (b) Sound spectrogram and corresponding oscillogram (above) of an advertisement call of *Dendropsophus bifurcus* (Ecuador, Provincia Napo, south slope Volcán Sumaco, Río Pucuno Valley, 1100 m a.s.l., 1 Jan. 1997, 20.4°C).

sites and perch heights of calling males. At the type locality, males were calling at the edge of an ephemeral pond. Other hylid frogs present were *Dendropsophus koechlini, D. leali*, *D. minutus*, *D. nanus*, *D. schubarti*, *Phyllomedusa camba*, *P. palliata* and *Pseudis paradoxa*. At Tahuamanu, in Departamento Pando, *D. salli* was found calling syntopically with *D. leucophyllatus*, *D. xapuriensis* and *Scinax garbei*. Differing from *D. leucophyllatus*, which calls rather consistently during the whole rainy season, *D. salli* is vocally active mainly after heavy rains. Typically, males call from broad-leaved plants or grassy vegetation 10–50 cm above the water's surface. A clutch of eggs referred to *D. salli* was found deposited on the upper surface of a floating water lily (Nymphaeaceae) leaf close to its margin.

Distribution: In Bolivia, *D. salli* ranges from the northern parts of the Departamento Pando southward to the northern parts of the Chiquitano Dry Forest near San Rafael. The easternmost record is from Noel Kempff National Park on the Brazilian/Bolivian border. A modelled map taken from Reichle (2007) indicates the localities where we have seen

specimens from or where specimens could be referred to as *D. salli* with certainty (Fig. 6). It also shows the potential distribution of the species in Bolivia (after Reichle 2007, as *D. bifurcus*). All Bolivian literature records fall into this range (e.g., de la Riva 1993). The potential distribution area reaches the Brazilian border. It is therefore highly likely that the species occurs in Brazil as well. In fact, Duellman (1977) includes Acre, Brazil, in the range of *D. bifurcus* (*Hyla bifurca* auct.). His data might refer to *D. salli*. The exact range of *D. salli* is unknown at the moment. Literature records such as Duellman (1977) or Rodríguez & Cadle (1990) not accompanied by descriptions or photos cannot therefore be evaluated. There are also reports on patterns "like those from Ecuador" or intermediate ones (de la Riva et al. 2000) for Bolivia that we are unable to refer to a species with certainty. Two definite photographic records of *D. salli* exist from Peru, one from the Tambopata River in southwestern Peru, Departamento Madre de Dios (MacQuarrie & Bärtschi 2001), and one from the same region (as *D. leucophyllatus*) (von May et al. 2010). The southernmost locality where we have found *D. bifurcus* is Santa María de Nieva in Departamento Amazonas, northern Peru.

Etymology: The new species is named after John Sall for his continuous generous contributions to forest conservation worldwide and especially in the Neotropical region.

## Discussion

We first took into consideration the idea that *Dendropsophus bifurcus* might be a composite of two species when we were keeping two males from Bolivia in a terrarium together with two females from Ecuador for more than two years. Although the males called, the females deposited unfertilized eggs without males. Small treefrogs in reproductive condition usually clasp anything similar to conspecific females (see Kwet 2001: 92–93). It was surprising that the Bolivian males, then believed to be *D. bifurcus*, did not even attempt to mate with the females from Ecuador. Thus, we assumed that some premating isolating mechanism prevented mating.

Despite the fact that *D. bifurcus* and the new species have been confused for many years, there are traditional taxonomic methods to distinguish them, and not only morphological ones. The calls of frogs of the *Dendropsophus leucophyllatus* group are structurally similar (Bastos & Haddad 1995, Márquez et al. 1993, Wells & Greer 1981, Wells & Schwartz 1984a, b). Nevertheless, the advertisement calls of *D. bifurcus* and *D. salli* are well distinguishable. The calls of *D. bifurcus* recorded on the southern slope of Volcán Sumaco, Río Pucuno Valley, 1100 m a.s.l., Provincia Napo, Ecuador (Fig. 5b) consist of two different note types of which Type I is longer and always emitted, Type II is shorter and, if at all, only emitted following Type

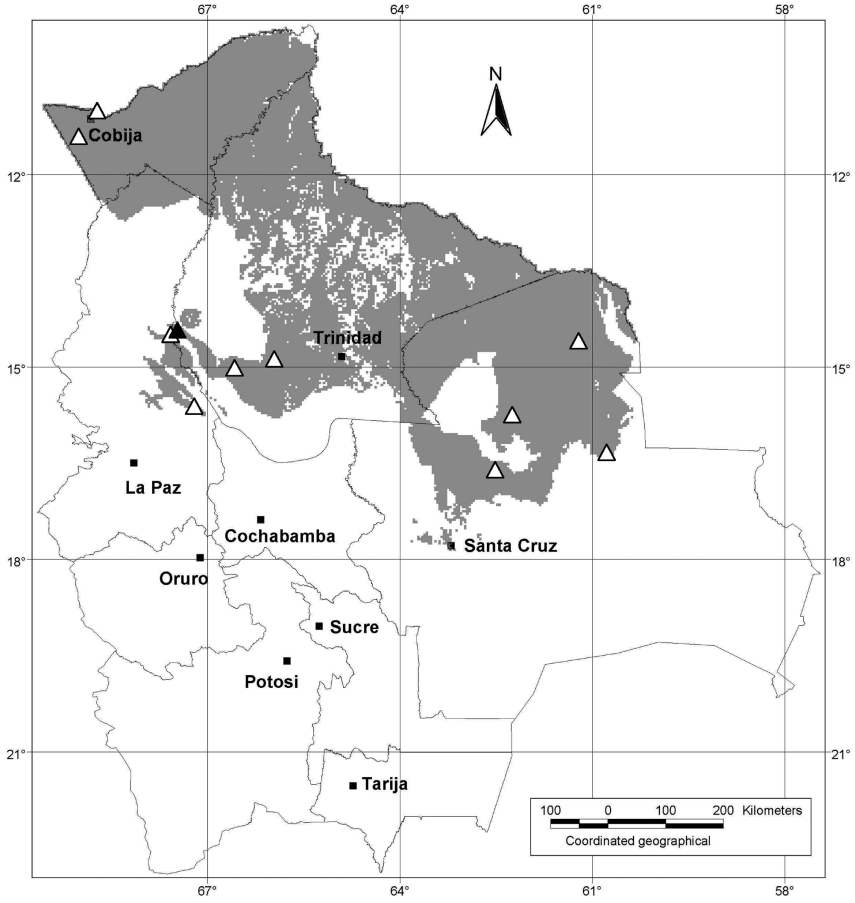


Figure 6. Map showing the distribution of *Dendropsophus salli* sp. n. in Bolivia. Triangles represent collection sites. The full triangle is the type locality. The shaded area is the potential range of the species in Bolivia based on abiotic parameters using BIOM software (see Reichle 2007, as *D. bifurcus*). See text for localities outside Bolivia.

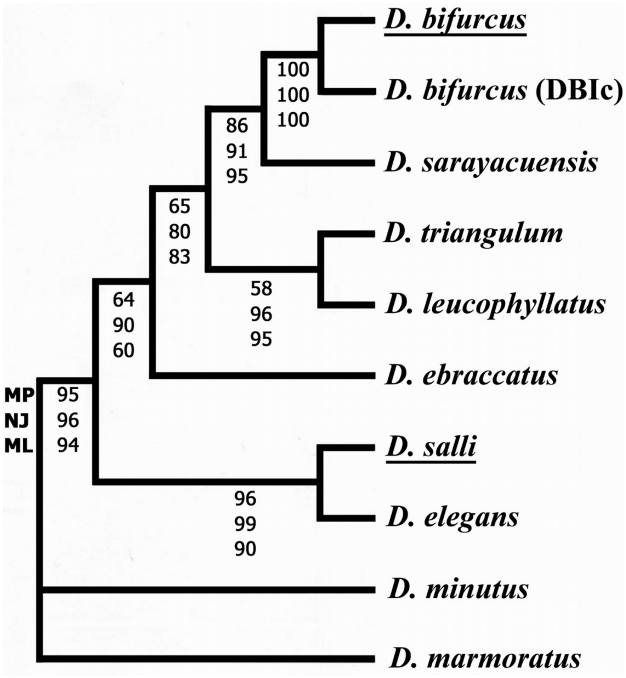


Figure 7. Strict consensus tree of species belonging to the *Dendropsophus leucophyllatus* group resulting from three different data sets. *Dendropsophus anceps* was omitted from the tree, because it clustered with the outgroup taxa. Branching patterns resulting from the complete rDNA data set including stem and loop regions. A single tree was calculated for MP, NJ, and ML methods. *Dendropsophus bifurcus* and *D. salli* sp. n. are clearly paraphyletic. DBIc represents the sequence obtained by Chek et al. (2001). Support values corresponding to internal nodes are top-down for MP, NJ, and ML analyses.

I. For ten advertisement calls analysed (air temperature 20.4°C), the following parameters were found: call length 92–305 ms (197.7 ± 51.1), number of notes per call 1–3 (2 ± 0.47), dominant frequency 2.89–2.98 KHz (2.96 ± 0.03). With its duration of 78–101 ms (90.1 ± 9.3), note Type I is always longer and with 7–9 (7.8 ± 0.79) pulses, it exhibits more pulses than note Type II with 10–33 ms (20.5 ± 8.4) and 1–3 pulses (2 ± 0.67). This results in different pulse rates. Type I presents a slightly slower pulse rate with 84.2–90.1 pulses/second (86.7 ± 3.5) than Type II with 76.9–111.1 (101.4 ± 14.4) pulses/second. No frequency modulation is present in the call. It is obvious that most of the parameters measured are variable, but the pulse rate of note Type I is consistent in all ten calls analysed.

In comparison, the advertisement call of both species exhibit the same general structure, namely a longer note Type I that is followed by one or several notes of a second, shorter note Type II. The dominant frequencies in both species are also very similar (2.89 KHz in *D. salli* and 2.96 KHz in *D. bifurcus*). Apart from some minor differences in call and note duration, the main differences are found in the pulse rates of both note types as well as in the different number of pulses for the first note type (20 pulses/note in *D. salli* vs. 7.8 pulses/note in *D. bifurcus*). Pulse rates are much higher in *D. salli* (note Type I 281 pulses/second, note Type II 182 pulses/second) than in *D. bifurcus* (note Type I 86.7 pulses/second, note type II 101.4 pulses/second). In *D. salli,* the pulse rate of note Type I is always higher than the pulse rate of note Type II. This situation is reversed in *D. bifurcus*. The last finding (as well as similar dominant frequencies) excludes the possibility of temperature-influenced artefacts affecting our data.

Márquez et al. (1993) published call data of *D. salli* (*Hyla bifurca* auct.) from Puerto Almacén, Bolivia. Both the spectrogram and oscillogram figured by the latter authors look much the same as ours, but the call descriptions differ in note durations and the resulting values for pulses per second. This might be due to the different call taxonomy used. Their longest note durations are within the range of our call durations, and their values of calls and notes per minute are almost identical to ours.

Mitochondrial genes have been widely used to infer phylogenetic relationships of amphibians (e.g., Hay et al. 1995, Vences et al. 2000, Chek et al. 2001, Hertwig et al. 2004, Faivovich et al. 2005). It is often the case that loop and stem regions of mitochondrial rRNA genes are not equally informative in phylogenetic studies. For instance, Wheeler & Honeycutt (1988) demonstrated that rRNA loop regions produce more reliable trees, whereas Dixon & Hillis (1993) showed that nucleotides in stem regions yield more phylogenetic information. Thus, it is important to take into consideration the different constraints caused by the secondary structure and recognize stem and loop regions in rRNA gene sequences as sources of different information.

Chek et al. (2001) investigated the evolutionary history of the 30-chromosome *Dendropsophus* and presented a general phylogeny for the *D. leucophyllatus* group that is identical to our tree topology (except for the newly recognized species *D. salli*). Phylogenetic relationships among species of the *D. leucophyllatus* group were mostly uncovered by investigating loop regions. The branching patterns resulting from the rDNA stem data set are weakly resolved instead. This was also discovered and discussed elsewhere (Wang & Lee 2002, Schmitz et al. 2005). The complete data set, consisting of stem and loop regions, however, lend strongest support to the phylogenetic relationships within the *D. leucophyllatus* group. Thus, larger sequence data sets are always superior for phylogenetic investigations as long as structural constraints are considered in cases where rRNA genes are the underlying source of the analyses. Faivovich et al. (2005) described a sister-group relationship of *D. anceps* and the *D. leucophyllatus* group. In our phylogenetic analyses, *D. anceps* clustered mostly with the outgroup taxa. Hence, we propose that the phylogenetic position of *D. anceps* needs further investigation, because not only our genetic, but also the following morphological characters indicate that *D. anceps* might not be a member of the *D. leucophyllatus* group (characters of the latter in parentheses): It has marbled dark brown dorsal colouration (contrasting light and dark markings), boldly barred thighs (uniform, light), only a short and inconspicuous axillary membrane (extensive), lacks glandular pectoral patches (conspicuous), and the tadpoles have 2/3 rows of labial teeth (lacking) and two rows of papillae on the posterior labium (one) (Lutz 1973, pers. obs. KHJ).

Our phylogenetic analyses of *D. salli* within the *D. leucophyllatus* group revealed that *D. salli* was not the closest relative of the Amazonian *D. bifurcus*, with which it had been confused, but with *D. elegans* of the Atlantic Forest

region of southeastern Brazil. This indicates a connection between the Bolivian amphibian fauna with the one from the Atlantic Forest and raises questions about the evolution and phylogenetic relationship of amphibians of the southwestern Amazon ecoregion (Dinerstein et al. 1995). It would not be surprising to find more widespread amphibian "species" that actually represent species pairs or complexes and that some of these elements in the southwestern Amazon Basin actually have closer relationships with the Atlantic Forest species than with their Amazonian look-alikes. Such a scenario seems rather likely in some species complexes such as *Dendropsophus "minutus"* ranging from southeastern Brazil to Trinidad, but may also be found, e.g., in *Hypsiboas crepitans* (Martins et al. 2009) or *Trachycephalus venulosus*.

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**References**

Andersson, L. G. (1945): Batrachians from East Ecuador collected 1937, 1938 by Wm. Clarke-Macintyre and Rolf Blomberg. – Arkiv för Zoologi, **37A**: 1–88.

Bastos, R. P. & C. F. B. Haddad (1995): Vocalizações e interações acústicas de *Hyla elegans* (Anura, Hylidae) durante a atividade reprodutiva. – Naturalia, **20**: 165–176.

Blin, N. & D. W. Stafford (1976): A general method of high molecular weight DNA from eukaryotes. – Nucleic Acids Research, **3**: 2303–2308.

Boettger, O. & E. Pechuel-Loesche (1892): Die Kriechtiere und Lurche. Brehms Tierleben. Vol. 7. Third edition. – Bibliographisches Institut, Leipzig and Wien, 737 pp.

Caramaschi, U. & J. Jim (1982): Revalidação de *Hyla elegans* Wied, 1824. – Ciência e Cultura Suplemento, **7**: 848.

Castresana, J. (2000): Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Molecular Biology and Evolution, **17**: 540–552.

Chek, A. A., S. C. Lougheed, J. P. Bogart & P. T. Boag (2001): Perception and history: molecular phylogeny of a diverse group of Neotropical frogs, the 30-chromosome *Hyla* (Anura: Hylidae). – Molecular Phylogenetics and Evolution, **18**: 370–385.

Cochran, D. M. & C. J. Goin (1970): Frogs of Colombia. – United States National Museum Bulletin, **288**: 1–655.

Darst, C. R. & D. C. Cannatella (2004): Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. – Molecular Phylogenetics and Evolution, **31**: 462–475.

De la Riva, I. (1990): Lista preliminar comentada de los anfibios de Bolivia con datos sobre su distribución. – Bollettino del Museo Regionale di Scienze Naturali Torino, **8**: 261–319.

De la Riva, I. (1993): Ecología de una comunidad neotropical de anfibios durante la estación lluviosa. – Ph.D. thesis, Universidad Complutense Madrid, 365 pp. (unpublished).

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

Dinerstein, E., D. Olson, D. Graham, A. Webster, S. Primm, M. Bookbinder & G. Ledec (1995): A conservation assessment of the terrestrial ecoregions of Latin America and the Caribbean. – The International Bank for Reconstruction and Development, World Wildlife Fund & World Bank, Washington, 135 pp.

Dixon, M. T. & D. M. Hillis (1993): Ribosomal RNA secondary structure: compensatory mutations and implications for phylogenetic analysis. – Molecular Biology and Evolution, 10: 256–267.

Duellman, W. E. (1974): A reassessment of the taxonomic status of some Neotropical hylid frogs. – Occasional Papers of the Museum of Natural History University of Kansas, **27**: 1–27.

Duellman, W. E. (1977): Liste der rezenten Amphibien und Reptilien. Hylidae, Centrolenidae, Pseudidae. – Das Tierreich, **95**: 1–225.

Duellman, W. E. (1982): A new species of small yellow *Hyla* from Perú (Anura: Hylidae). – Amphibia-Reptilia, **3**: 153–160.

Duellman, W. E. (2001): Hylid frogs of Middle America. – Society for the Study of Amphibians and Reptiles, Ithaca, 1159 pp.

Duellman, W. E. (2005): Cusco Amazónico. The lives of amphibians and reptiles in an Amazonian rainforest. – Cornell University Press, New York, 433 pp.

Duellman, W. E. & M. L. Crump (1974): Speciation in frogs of the *Hyla parviceps* group in the upper Amazon Basin. – Occasional Papers of the Museum of Natural History University of Kansas, **23**: 1–40.

Duellman, W. E. & M. J. Fouquette (1968): Middle American frogs of the *Hyla microcephala* group. – University of Kansas Publications of the Museum of Natural History, **17**: 517–557.

Duellman, W. E. & L. Trueb (1983): Frogs of the *Hyla columbiana* group: taxonomy and phylogenetic relationships. In Rhodin, A. G. J. & K. Miyata (eds.): Advances in herpetology and evolutionary biology. – Museum of Comparative Zoology, Cambridge, Mass.: 33–51.

Duellman, W. E. & L. Trueb (1986): Biology of amphibians. – McGraw-Hill, New York, 670 pp.

Embert, D. & S. Reichle (2008). Guía de anfibios y reptiles de la Chiquitania. – Fundación para la Conservación del Bosque Seco Chiquitano, Santa Cruz, 217 pp.

Faivovich, J., C. B. F. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell & W. C. Wheeler (2005): Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. – Bulletin of the American Museum of Natural History, **294**: 1–240.

Frost, D. R. (2009): Amphibian Species of the World: an Online Reference. Version 5.3 (12 February, 2009). – American Museum of Natural History. http://research.amnh.org/herpetology/amphibia/ (accessed 26 August 2009).

Gomes, M. D. R. & O. L. Peixoto (1991): Larvas de *Hyla* do grupo *"leucophyllata"* com a descrição da de *H. elegans* Wied, 1824 e notas sobre a variação do padrão de colorido do adulto nesta espécie (Anura, Hylidae). – Revista Brasileira de Biologia, **51**: 257–262.

Gutell, R. R. & G. E. Fox (1988): A compilation of large subunit RNA sequences presented in a structural format. – Nucleic Acids Research, **16**(Suppl): r175–r269.

Guindon, S. & O. Gascuel (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. – Systematic Biology, **52**: 696–704.

Hasegawa, M., H. Kishino & T. Yano (1985): Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. – Journal of Molecular Evolution, **22**: 160–174.

Hay, J. M., I. Ruvinsky, S. B. Hedges & L. R. Maxson (1995): Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. – Molecular Biology and Evolution, **12**: 928–937.

Hertwig, S., R. O. de Sá & A. Haas (2004) Phylogenetic signal and the utility of 12S and 16S in frog phylogeny. – Journal of Zoological Systematics and Evolutionary Research **42**: 2–18.

Heyer, W. R., A. S. Rand, C. A. G. Cruz, O. L.Peixoto & C. E. Nelson (1990). Frogs of Boracéia. – Arquivos de Zoologia, **31**: 231–410.

Kaplan, M. (1994): A new species of frog of the genus *Hyla* from the Cordillera Oriental in northern Colombia with comments on the taxonomy of *Hyla minuta*. – Journal of Herpetology, **28**: 79–87.

Kwet, A. (2001): Frösche im brasilianischen Araukarienwald. – NTV, Münster, 192 pp.

Lutz, B. (1973): Brazilian species of *Hyla*. – University of Texas Press, Austin and London, 265 pp.

MacQuarrie, K. & A. Bärtschi (2001): Where the Andes meet the Amazon. – Jordi Blassi, Barcelona, 336 pp.

Márquez, R., I. De la Riva & J. Bosch (1993): Advertisement calls of Bolivian species of *Hyla* (Amphibia, Anura, Hylidae). – Biotropica, **25**: 426–443.

Martins, L. B., W. R. Silva & A. A. Giaretta (2009): Distribution and calls of two South American frogs (Anura). – Salamandra, **45**: 106–109.

Moravec, J. & J. Aparicio (2000): Amphibians and reptiles recently recorded from the surroundings of Riberalta (Departamento Beni, Bolivia). – Časopis Národního muzea Řada přírodovědná Praha, 169: 1–15.

Moravec, J. & J. Aparicio (2005): Notes on the herpetofauna of Bioceanica and Bolpebra (Provincia Nicolas Suaréz, Departamento Pando, Bolivia). – Časopis Národního muzea Řada přírodovědná Praha, **174**: 95–113.

Myers, C. W. & W. E. Duellman (1982): A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. – American Museum Novitates, **2752**: 1–32.

Nagae, Y. (1988): The primary and secondary structures of the ribosomal RNAs of *Rana catesbeiana* mitochondrion. – Nippon Ika Daigaku Zasshi, **55**: 417–430.

Palumbi, S. R. (1996): Nucleic Acids II: The polymerase chain reaction. – pp. 205–247 in Hillis, D.M., C. Moritz & B.K. Mable (eds.): Molecular Systematics. – Sinauer, Sunderland.

Reichle, S. (2002): *Hyla bifurca*. – in Márquez, R., I. De la Riva, J. Bosch & E. Matheu (eds.): Guía sonora de las ranas y sapos de Bolivia. – ALOSA-AHE-MNCN, Barcelona, booklet 47 pp, 2 CDs.

Reichle, S. (2003): Anfibios. – in Ibisch, P. & G. Merida (eds.): Biodiversidad: La Riqueza de Bolivia. Estado de conocimiento y conservación. – Editorial FAN, Santa Cruz de la Sierra: 133–137.

Reichle, S. (2007): Distribution, diversity and conservation status of Bolivian amphibians. – Dissertation, Universität Bonn, 183 pp (unpublished).

Rodríguez, L. B. & J. E. Cadle. (1990): A preliminary overview of the herpetofauna of Cocha Cashu, Manu National Park, Peru. – pp. 410–425 in Gentry, A. H. (ed.): Four Neotropical rainforests. – Yale University Press, New Haven and London.

Savage, J. M. & W. R. Heyer (1967): Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. – Beiträge zur Neotropischen Fauna, **5**: 111–131.

Schmidt, H. A., K. Strimmer, M. Vingron & A. von Haeseler (2002): TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. – Bioinformatics, **18**: 502–504.

Schmitz, J., O. Piskurek & H. Zischler (2005): Forty million years of independent evolution: a mitochondrial gene and its corresponding nuclear pseudogene in primates. – Journal of Molecular Evolution, **61**: 1–11.

Schulze, A., M. Jansen & G. Köhler (2009): Diversity and ecology of anuran communities in San Sebastián (Chiquitano region, Bolivia). – Salamandra, **45**: 75–90.

Swofford, D. L. (2000): PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). – Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., J. Dudley, M. Nei & S. Kumar (2007): MEGA4 : Molecular evolutionary genetics analysis (MEGA) software version 4.0. – Molecular Biology and Evolution, 24: 1596–1599.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins (1997): The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. – Nucleic Acids Research, **25**: 4876–4882.

Titus, T. A., D. M. Hillis & W. E. Duellman (1989): Color polymorphism in Neotropical treefrogs: an allozymic investigation of the taxonomic status of *Hyla favosa* Cope. – Herpetologica, **45**: 17–23.

Vences, M., J. Kosuch, S. Lötters, A. Widmer, K.-H. Jungfer, J. Köhler & M. Veith (2000): Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. – Molecular Phylogenetics and Evolution, **15**: 34–40.

Wang, H.-Y. & S.-C. Lee (2002): Secondary structure of mitochondrial 12S rRNA among fish and its phylogenetic applications. – Molecular Biology and Evolution, **19**: 138–148.

Wells, K. D. & B. J. Greer (1981): Vocal responses to conspecific calls in a Neotropical hylid frog, *Hyla ebraccata*. – Copeia, **1981**: 615–624.

Wells, K. D. & J. J. Schwartz (1984a): Vocal communication in a Neotropical treefrog, *Hyla ebraccata*: advertisement calls. – Animal Behavior, **32**: 405–420.

Wells, K. D. & J. J. Schwartz (1984b): Vocal communication in a Neotropical treefrog, *Hyla ebraccata*: aggressive calls. – Behaviour, **91**: 128–145.

Wheeler, W. C. & R. L. Honeycutt (1988): Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. – Molecular Biology and Evolution, **5**: 90–96.

von May, R., J. M. Jacobs, R. D. Jennings, A. Catenazzi & L. O. Rodríguez (2010): Anfíbios de Los Amigos, Manu y Tambopata, Peru. – Field Museum Rapid Color Guide, 236, Version 2. http://fm2.fieldmuseum.org/plantguides/guide_pdfs/236%20Anfibios%20Amigos%201.2.pdf (accessed 19 August 2010).

Yang, Z. (1996): Among-site rate variation and its impact on phylogenetic analyses. – Trends in Ecology and Evolution, **11**: 367–372.

**Supporting information**

Additional information is available in the online version of this article at http://www.salamandra-journal.com.

**S1.** Taxon, source, GenBank accession numbers, and sequence information of *Dendropsophus* species used in the phylogenetic analyses.

**S2.** DNA alignment of partial mitochondrial 12S and 16S rRNA genes (including the intermediate tRNA-Valin region) separated into loop and stem regions.

## Supporting information

JUNGFER, K.-H., S. REICHLE & O. PISKUREK (2010): Description of a new cryptic southwestern Amazonian species of leaf-gluing treefrog, genus *Dendropsophus* (Amphibia: Anura: Hylidae). – Salamandra, **46**: 204–214.

**S1.** Information for *Dendropsophus* species used for the phylogenetic analyses.

| Species | Lineage | Sequence | GenBank accession numbers | Reference |
|---|---|---|---|---|
| *Dendropsophus anceps* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA + tRNA-Valin | AY843597 | FAIVOVICH et al. (2005) |
| *Dendropsophus marmoratus* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AF308082 AF308114 | CHEK et al. (2001) |
| *Dendropsophus minutus* | Anura, Hylidae (30 Chr.), Dendrosophini | Partial 12S- and 16S rRNA | AF308081 AF308112 | CHEK et al. (2001) |
| *Dendropsophus bifurcus* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AF308073 AF308098 | CHEK et al. (2001) |
| *Dendropsophus bifurcus* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA + tRNA-Valin | AY362975 | This work |
| *Dendropsophus sarayacuensis* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AE308076 AF308104 | CHEK et al. (2001) |
| *Dendropsophus triangulum* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA + tRNA-Valin | AY326053 | DARST & CANNATELLA (2004) |
| *Dendropsophus leucophyllatus* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AF308072 AF308097 | CHEK et al. (2001) |
| *Dendropsophus ebraccatus* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AF308074 AF308101 | CHEK et al. (2001) |
| *Dendropsophus elegans* | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA | AF308075 AF308103 | CHEK et al. (2001) |
| *Dendropsophus salli* n. sp. | Anura, Hylidae (30 Chr.), Dendropsophini | Partial 12S- and 16S rRNA + tRNA-Valin | AY362976 | This work |

**S2.** DNA alignment of partial mitochondrial 12S and 16S rRNA genes (including the intermediate tRNA-Valin region) separated into loop and stem regions. Stem regions are boxed and numbered. Grey shaded areas were difficult to align among amphibians but were considered informative for the phylogenetic analyses of the *Dendropsophus leucophyllatus* group when underlined. The total number of sites for phylogenetic analyses of loop and stem regions was 1118 (stem regions 478, loop regions 640). The number of potentially informative sites within loop and stem regions was 209 (stem regions 39, loop regions 170). Dark grey shaded areas illustrate the sequence divergence between *Dendropsophus bifurcus* from Ecuador and *Dendropsophus salli* from Bolivia. Abbreviations and accession numbers (if not already given in S1): TNA: *Typhlonectes natans* (AF154051), AME: *Ambystoma mexicanum* (Y10948), XLA: *Xenopus laevis* (M10217), RCA: *Lithobates catesbeianus* (M57527), HMA: *Hypsiboas marianitae* (AY362977), DAN: *Dendropsophus anceps*, DSL: *Dendropsophus salli,* DBI: *Dendropsophus bifurcus,* DBIc: *Dendropsophus bifurcus* (CHEK et al. 2001), DSA: *Dendropsophus sarayacuensis,* DTR: *Dendropsophus triangulum,* DLE: *Dendropsophus leucophyllatus,* DEB: *Dendropsophus ebraccatus,* DEL: *Dendropsophus elegans,* DMI: *Dendropsophus minutus,* DMR: *Dendropsophus marmoratus.*