

# A new species of *Hyloxalus* Jiménez De La Espada, 1871 “1870” (Anura: Dendrobatidae: Hyloxalinae) from a cloud forest near Bogotá, Colombia, with comments on the *subpunctatus* clade

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## Abstract

A new species of frog of the genus *Hyloxalus* from a high Andean forest of the central parts of the Cordillera Oriental, Colombia is recognized and described using integrative taxonomy. Using phylogenetic analyses of 2590 base pairs (bp) of mitochondrial DNA, *Hyloxalus felixcoperari* sp. nov. was shown to belong to a lineage of the *subpunctatus* clade, part of a subgroup that comprises high altitude-occurring lineages (subgroup I) and sister taxa of *H. subpunctatus* sensu stricto from the Bogotá plateau region. The genetic distance between both taxa is 2.1% and 5.1% for the 16S and cytochrome b gene fragments respectively. *Hyloxalus felixcoperari* sp. nov. is a medium-sized frog, which can be distinguished morphologically from *H. subpunctatus* by having dark throat and chest and unwebbed toes, and bioacoustically by emitting an advertisement call consisting of trill-like calls repeated at a rate of 50–104 repetitions/min, with a dominant frequency ranging from 3028.9–3861.2 Hz. Molecular dating analysis revealed that the already known lineages of the *subpunctatus* clade subgroup I from the middle Cordillera Oriental of Colombia, recently diverged during the lower Pleistocene – Upper Pliocene, most probably due to environmental fluctuations. Threats and main aspects of the natural history of this new species including breeding and habitat are reported in this article.

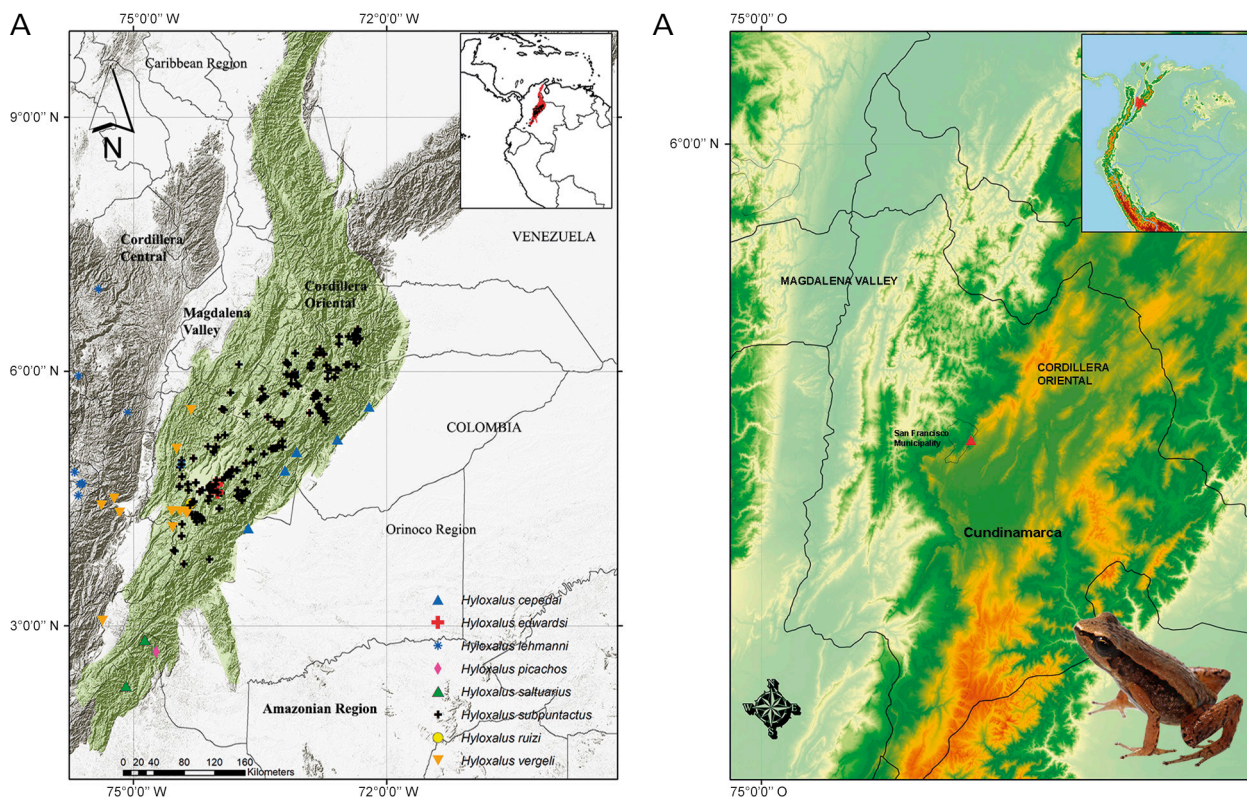
## Key words

Bioacoustics, Cordillera Oriental, high Andean, lower Pleistocene, mitochondrial DNA, recent radiation, upper Pliocene.

## Introduction

*Hyloxalus* Jiménez de la Espada, 1870 is a large and monophyletic genus of dendrobatid frogs with 60 nominal species (FROST, 2018; GRANT *et al.*, 2017), morphologically recognizable by having dorsal cryptic coloration, pale oblique lateral stripes, posteriorly granular dorsal skin texture, median lingual process absent, dextral larval vent tube and emarginated not umbelliform larval oral disc shape. Additionally, frogs of this genus do not have a swollen third finger in the adult males and the ventrolateral stripe and collar are usually absent (GRANT *et al.*,

2006; ANGANOY-CRIOLLO, 2013; FROST, 2018; GRANT *et al.*, 2017). Colombia harbors 22 nominal species from this genus (COPE, 1899; HELLMICH, 1940; SILVERSTONE, 1971; EDWARDS, 1974; SILVERSTONE, 1975; LYNCH, 1982; RIVERO & SERNA, 1986; RIVERO & GRANADOS-DIAZ, 1989; RIVERO, 1991; RIVERO & SERNA, 1991, 1995; GRANT & CASTRO, 1998; GRANT & ARDILA-ROBAYO, 2002). Among these species, 20 are typical of the Andean region and the following eight species are distributed or restricted to the Cordillera Oriental of Colombia (Fig. 1): *Hylo-*



**Fig. 1. A:** Species of the genus *Hyloxalus* distributed in the Cordillera Oriental of Colombia. **B:** Type locality (red triangle) of *Hyloxalus felixcooperari* sp. nov. from an Andean forest on the western slope of the Cordillera Oriental. Inset photo: *Hyloxalus felixcooperari* sp. nov. Adult male, holotype IAvH-Am-14540.

*xalus edwardsi* (LYNCH, 1982), endemic of the páramo in the central region of the Cordillera Oriental in Cundinamarca department, between 3070–3250 meters above sea level (COPE, 1899; LYNCH, 1982; RUIZ-CARRANZA, *et al.*, 1996; ACOSTA-GALVIS, 2000; ARDILA-ROBAYO & ACOSTA-GALVIS, 2000; LYNCH & RENJIFO, 2001; ACOSTA-GALVIS, 2004; BERNAL & LYNCH, 2008; ANGANOY-CRIOLLO, 2013; SÁNCHEZ, 2013). *Hyloxalus ruizi* (LYNCH, 1982), inhabits the Andean forests on the western slope of the Cordillera Oriental in Cundinamarca department, between 2410–2649 m above sea level (LYNCH, 1982; RIVERO & SERNA, 1988; RUIZ-CARRANZA *et al.*, 1996; ARDILA ROBAYO & ACOSTA-GALVIS, 2000; BERNAL & LYNCH, 2008). *Hyloxalus saltuarius* (GRANT & ARDILA, 2002) is distributed in the sub Andean forests located on the eastern slopes of Los Picachos National Natural Park in Caquetá department, between 1200–1600 m above sea level (GRANT & ARDILA-ROBAYO, 2002; BERNAL & LYNCH, 2008). *Hyloxalus vergeli* (HELLMICH, 1940) is distributed in the sub-Andean forests, dry forests and rain tropical forests of the upper and middle Magdalena river valley on the western slope of the Cordillera Oriental and eastern slope of the Cordillera Central in Cundinamarca, Huila and Tolima departments, between 520–2100 m above sea level (HELLMICH, 1940; COCHRAN & GOIN, 1970; RUIZ-CARRANZA, *et al.*, 1996; ACOSTA-GALVIS, 2000; BERNAL *et al.*, 2005; BERNAL & LYNCH, 2008; ACOSTA-GALVIS, 2012; SÁNCHEZ, 2013). *Hyloxalus lehmanni* (BERNAL & LYNCH, 2008) is from Albán

Municipality in Cundinamarca department at 2060 m above sea level. However, this record requires confirmation because the currently known distributional range of the species is restricted to the Central and Western cordilleras of Colombia (Fig. 1). *Hyloxalus subpunctatus* (COPE, 1899) was originally described from the Bogotá region and considered a complex of cryptic species inhabiting sub-Andean forests to páramos of both slopes of the Cordillera Oriental in Arauca, Boyacá, Casanare, Cundinamarca, Meta and Santander departments, between 1750–4020 m above sea level (STEBBINS & HENDRICKSON, 1959; COCHRAN & GOIN 1970; RUIZ-CARRANZA *et al.*, 1996; FANDIÑO *et al.*, 1997; ACOSTA-GALVIS, 2000; ARDILA-ROBAYO & ACOSTA-GALVIS, 2000; LYNCH & RENJIFO, 2001; GRANT, *et al.*, 2006; BERNAL & LYNCH, 2008; ANGANOY-CRIOLLO, 2013; SÁNCHEZ, 2013). Finally, two species were recently transferred from the genus *Allobates* by Grant *et al.* (2017): *Hyloxalus cepedai* (MORALES, 2000 '2002'), from the tropical wet forests of the foothills on the eastern slope of the Cordillera Oriental, in the Departments of Casanare and Meta at 560 m above sea level (MORALES, 2002; LYNCH, 2006; GRANT, *et al.*, 2017) and *Hyloxalus picachos* (ARDILA-ROBAYO *et al.*, 1999) from type locality (1450–1600 meters) at Los Picachos National Natural Park in Caquetá department (ARDILA-ROBAYO *et al.*, 1999; BERNAL & LYNCH, 2008).

Recent phylogenetic hypotheses of frogs of the *Hyloxalus* genus, based on molecular and morphological data (VENCES, *et al.*, 2003; GRANT *et al.*, 2006; PÁEZ-VA-

CAS *et al.*, 2010; PYRON & WIENS, 2011; MUÑOZ *et al.*, 2015; GRANT *et al.*, 2017), recognize the *Hyloxalus subpunctatus* group, including *H. cepedai* and *H. picachos* from Cordillera Oriental of Colombia, as the sister of the *H. bocagei* group from the eastern Andean slopes and the lowlands adjacent to the Amazon in southern Colombia, Ecuador and northern Peru.

Here, by analyzing information from morphology, bioacoustics and mitochondrial DNA, a new species of *Hyloxalus* from the central region of the Eastern Cordillera of Colombia near Bogotá area is described. Additionally, threats and main aspects of the natural history of this new species, including breeding and habitat are reported. Furthermore, by performing phylogenetic and molecular dating analyses, including the so far known lineages belonging to the *subpunctatus* and *bocagei* clades, evolutionary relations are assessed and aspects of the historical biogeography of the *subpunctatus* clade are discussed.

## Material and methods

### Fieldwork and reference collection

The new species was recorded during fieldwork carried out in Las Nubes Private Natural Reserve, San Francisco Municipality, Cundinamarca Department (4°56'22.1" N; 74°14'48.8" W; 2500–2574 m above sea level), located in Andean forests of the Cordillera Oriental, Colombia. This locality is composed of agrosystems of the Andes orobiome within the ecoregion of the Magdalena valley montane forests (DINERSTEIN *et al.*, 1995; OLSON & DINERSTEIN, 2002). The geographical coordinates and elevations were obtained at the collecting sites with a Garmin GPSMAP 60CSx (map datum WGS 84). The collected specimens were euthanized using Benzocaine (CHEN & COMBS, 1999), fixed in 10% formalin and preserved in 70% ethanol. A tissue sample from one individual was obtained immediately after euthanasia. The collected individuals are housed in the Biological Collections of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt Colombia, Villa de Leyva, Boyacá, Colombia (IAvH-Am) and comparative material of *Hyloxalus subpunctatus* was reviewed in the Museum of Natural History of the Pontificia Universidad Javeriana, Bogotá, Colombia (MUJ).

### Molecular data

#### Laboratory procedures and data assemblage

Molecular distinctiveness and phylogenetic relationships between the new species of *Hyloxalus* were assessed by integrating and analyzing molecular data of the present known lineages belonging to the *subpunctatus* and

*bocagei* clades (Table 1). *Hyloxalus leucophaeus* was used as an outgroup. The analyzed molecular data set comprised three mitochondrial (mtDNA) gene fragments; 962 bp of the 12S ribosomal RNA (12S rRNA) gene, 932 bp of the 16S ribosomal RNA (16S rRNA) gene and 696 bp of the cytochrome b (cytb) gene. All three gene fragments were obtained from a single individual of the new species of *Hyloxalus* and the 12S rRNA and cytb gene fragments from *H. subpunctatus* s. str. Total genomic DNA was extracted using a standard Phenol-Chloroform method. Gene fragments were amplified using the following primers pairs: 12S rRNA: t-Phe-frog/Val-frog (WIENS *et al.*, 2005), 16S rRNA: 16Sbr-H/16SC-16L (PALUMBI *et al.*, 1991; DARST & CANNATELLA, 2004 respectively) and cytb: CytbDen3-L/CytbDen1-H. (SANTOS & CANNATELLA, 2011). PCRs were carried out in a total volume of 30 µl containing one unit Taq polymerase (Bioline; Randolph, MA), 1 × of buffer (Bioline), a final concentration of 1.5 mM MgCl<sub>2</sub> (Bioline), 0.5 µM of each primer, 0.2 mM of each dNTP (Bioline), 0.2 µg of Bovine Serum Albumin (BSA) and approximately 50 ng of total DNA. PCR products were purified using the ammonium acetate protocol (BENSCH *et al.*, 2000) and they were sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Remaining DNA extractions are stored at -80°C in the tissue collection of the Instituto de Genética, Universidad Nacional de Colombia (for voucher numbers see Table 1). The thermocycling conditions were performed as indicated by the authors who reported the primers for each fragment. The GenBank accession numbers of the obtained sequences are MG637362–MG637366. The sequences were edited and aligned using Chromas 1.51 (<http://www.technelysium.com.au/chromas.html>) and BioEdit 7.0.5.2 (HALL, 1999).

### Phylogenetic analyses, genetic divergence and dating

The data set was analyzed using the following partition scheme: (i) unpartitioned, (ii) partitioned by gene (i.e., each gene fragment treated as a distinct partition) and (iii) maximum partitioning (i.e., each codon of the protein-coding gene cytb and each ribosomal gene fragment treated as distinct partitions). The optimal partitioning scheme and best-fit evolutionary models were assessed using PARTITIONFINDER v1.1.1 and the Bayesian Information Criterion (LANFPEAR *et al.*, 2012) resulting in the selection of the maximum partitioning scheme. The following resulting models were applied in a Bayesian analysis (BA) with MrBayes v 3.2.1 (RONQUIST *et al.*, 2012): 12S rRNA – GTR+I+G, 16S rRNA – GTR+I+G, cytb 1<sup>st</sup> codon – K80+I, cytb 2<sup>nd</sup> codon – F81+I, cytb 3<sup>rd</sup> codon – TrN+G. These models were incorporated into a single tree search (mixed model partition approach; NYLANDER *et al.*, 2004) and two parallel runs were carried out using four Markov chains, each starting from a random tree. The Markov chains were run for 10 mil-

**Table 1.** Species and GenBank accession numbers of DNA sequences used in the phylogenetic analyses.

Species	Locality/origin	Accession numbers			Voucher number	Source
		Cytochrome b	12S rDNA	16S rDNA		
<i>Hyloxalus cepedai</i>	Colombia: Meta, Villavicencio	MF614220	MF624226	MF624226	MAA574	GRANT <i>et al.</i> , 2017
<i>Hyloxalus felixcooperari</i> sp. nov.	Colombia: Cundinamarca, San Francisco	MG637362	MG637365	MG637364	IavH-Am-14540	This study
<i>Hyloxalus picachos</i>	Colombia: Guaviare, San José del Guaviare	MF614223	MF624230	MF624230	MCL813	GRANT <i>et al.</i> , 2017
<i>Hyloxalus subpunctatus</i>	Colombia: Cundinamarca, Bogotá (Topotypes)	MG637363	MG637366	DQ502273	MUJ 5212	This study, Grant <i>et al.</i> , 2006
<i>Hyloxalus subpunctatus</i> lineage 1	Colombia: Boyacá, Chiquinquirá	HQ290551	EU342693	EU342693	TNHCFS 4957	SANTOS <i>et al.</i> , 2009
<i>Hyloxalus</i> sp.	Colombia: Casanare, Agua Azul	HQ286374	EU342692	EU342692	TNHCFS 4940	Following PÁEZ-VACAS <i>et al.</i> , 2012; SANTOS <i>et al.</i> , 2009
<i>Hyloxalus vergeli</i>	Colombia: Huila, Neiva	MF614227	MF624234	MF624234	MUJ 5018	GRANT <i>et al.</i> , 2017
<i>Hyloxalus vergeli</i>	Colombia: Huila, Neiva	MF614226	MUJ5017	MUJ5017	MUJ 5017	GRANT <i>et al.</i> , 2017
<i>Hyloxalus vergeli</i>	Colombia: Huila, Neiva	MF614225	MF624232	MF624232	MUJ 4298	GRANT <i>et al.</i> , 2017
<i>Hyloxalus maculosus</i>	Ecuador: Napo, Puerto Misahualli	HQ286377	EU342694	EU342694	QCAZ 19971	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus bocagei</i>	Ecuador: Sucumbios, Reventador	HQ286378	HQ286386	HQ286386	QCAZ 37259	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus sauli</i>	Ecuador: Orellana, Yasuni Research Station	HQ286376	AY364555	AY364555	QCAZ 16541	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus yasuni</i> (lineage 1)	Ecuador: Orellana, Yasuni Research Station	HQ286381	EU342699	EU342699	QCAZ 16505	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus yasuni</i> (lineage 2)	Ecuador: Napo, Cotapino	HQ286382	AY364559	AY364559	QCAZ 16504	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus yasuni</i> (lineage 3)	Ecuador: Sucumbios, Bermejo	HQ286383	AY364560	AY364560	QCAZ 16503	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus italo</i> (lineage 1)	Ecuador: Morona Santiago, Santiago	HQ286379	AY364558	AY364558	QCAZ 16511	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus italo</i> (lineage 2)	Ecuador: Pastaza, Reserva HolaVida	HQ286380	AY364552	AY364552	QCAZ 16508	PÁEZ-VACAS <i>et al.</i> , 2012
<i>Hyloxalus leucophaeus</i>	Peru: Amazonas, Molinopampa	HQ286372	EU342689	EU342689	KU 211880	PÁEZ-VACAS <i>et al.</i> , 2012

lion generations. The burn-in was set to sample only the plateau of the most likely trees that were used for generating a 50% majority rule consensus. The posterior probabilities in this tree are a measure of clade frequency and, thus, credibility. The software TRACER 1.5.4 (RAMBAUT & DRUMMOND, 2007) was used to assess an acceptable level of the MCMC chain mixing and to estimate effective sample sizes for all parameters. Additionally, maximum likelihood (ML) analyses were run using RAxML 7.2.8 (STAMATAKIS, 2006) and the default GTR+G model across all partitions. Five independent Maximum Likelihood searches were performed with different starting conditions and the rapid bootstrap algorithm to explore the robustness of the branching patterns by comparing the best trees. Afterward, 1000 non-parametric thorough bootstrap values were computed and plotted against the best tree. In order to assess the genetic differences between the new species and the so far known lineages belonging to the *subpunctatus* and the *bocagei* clades, uncorrected *p* genetic distances for the 16S and cytb gene fragments were calculated using MEGA 7.0.21 (KUMAR *et al.*, 2016).

Based on the data set including all three gene fragments, split ages of lineages were estimated by an uncorrelated relaxed clock as implemented in BEAST 1.4.8 (DRUMMOND & RAMBAUT, 2007). For this, the node defining the *bocagei* + *subpunctatus* clades was calibrated with a normal prior of 23.63 million years ago (mya; with an interval of 16.61–30.0 mya), as estimated by PÁEZ-VACAS *et al.* (2010). Running settings were: (i) the random starting tree using the yule process as the tree prior to speciation. This pure-birth process does not allow for extinction (single rate for speciation  $\text{birth} = \lambda$  and the rate for extinction  $\mu = 0$ . HEATH, 2018); it was applied it here since all included sequences are from extant species. (ii) three independent analyses of 100 million generations with a sampling rate of every 1000 generations and (iii) the auto optimize option activated to automatically adjust the tuning parameters. Input sequence data was manually partitioned according to the different gene fragments in the XML file generated with BEAUTI. The substitution models estimated by PARTITIONFINDER were a priori assigned to each partition. The optimal burn-in (10000) and the convergence of the runs were defined using TRACER 1.5.4. The tree files from each run were combined using LOGCOMBINER (DRUMMOND & RAMBAUT, 2007). Linearized consensus trees including posterior probabilities and 95% highest posterior density (HPD) intervals for node

estimates were inferred from the tree output files using TREEANNOTATOR (DRUMMOND & RAMBAUT, 2007), and the resulting trees were visualized using FIGTREE v.1.3.1 (RAMBAUT, 2009).

## Morphology

### Adults

The criteria for the definition of morphological descriptions and diagnostic characters followed previous taxonomic proposals (MYERS *et al.*, 1991; COLOMA, 1995; GRANT *et al.*, 1997; KAPLAN, 1997; GRANT & CASTRO, 1998; GRANT, 2004; GRANT *et al.*, 2006; CISNEROS-HEREDIA & MCDIARMID, 2007; GRANT, 2007; GRANT *et al.*, 2007; DUELLMAN & LEHR, 2009). The morphological observations were made using a stereoscopic microscope Nikon optical device SMZ-1B, with High Intensity Illuminator NI-150 Nikon and App Scope 3xSRA41. Measurements and proportions were taken using a digital Mitutoyo precision calliper ( $\pm 0.1$  mm) and the following abbreviations are used: SVL (snout-vent length), HW (head width), HL (head length from tip of snout to the posterior border of skull; the posterior edge of prootic was noted through the skin), IND (internarial distance), IOD (interorbital distance), ED (eye diameter), END (eye-nostril distance), TD (tympanum diameter), UEW (upper eyelid width), ETS (distance between the anterior edge of the eye to the tip of snout), RW (rostral width, measured at the midpoint between eye-nostril distance), TL (tibia length from heel to convex surface of flexed knee), FL (femur length, measured from the centre of the cloaca to the distal end of the femur measured in the bent hind limb), FTL (foot length, distance from proximal margin of inner metatarsal tubercle to tip of Toe IV), HDL (hand length, measured from the proximal edge of the large medial palmar tubercle to the tip of the third finger), FA (forearm length, the distance from the tip of the elbow to the proximal edge of the base palmar tubercle), 3FD (third finger disc diameter) and 4TD (fourth toe disc diameter). Means are reported as  $\pm$  one standard error. Sexual maturity was determined by examination of the gonads and external or secondary sexual characteristics. Individuals with vocal slits and dark throats were scored as adult males. Photographs of the specimens in life were taken with a digital camera model Canon EOS 70D and the preserved specimens were photographed with a digital camera Canon EOS 5D Mark II.

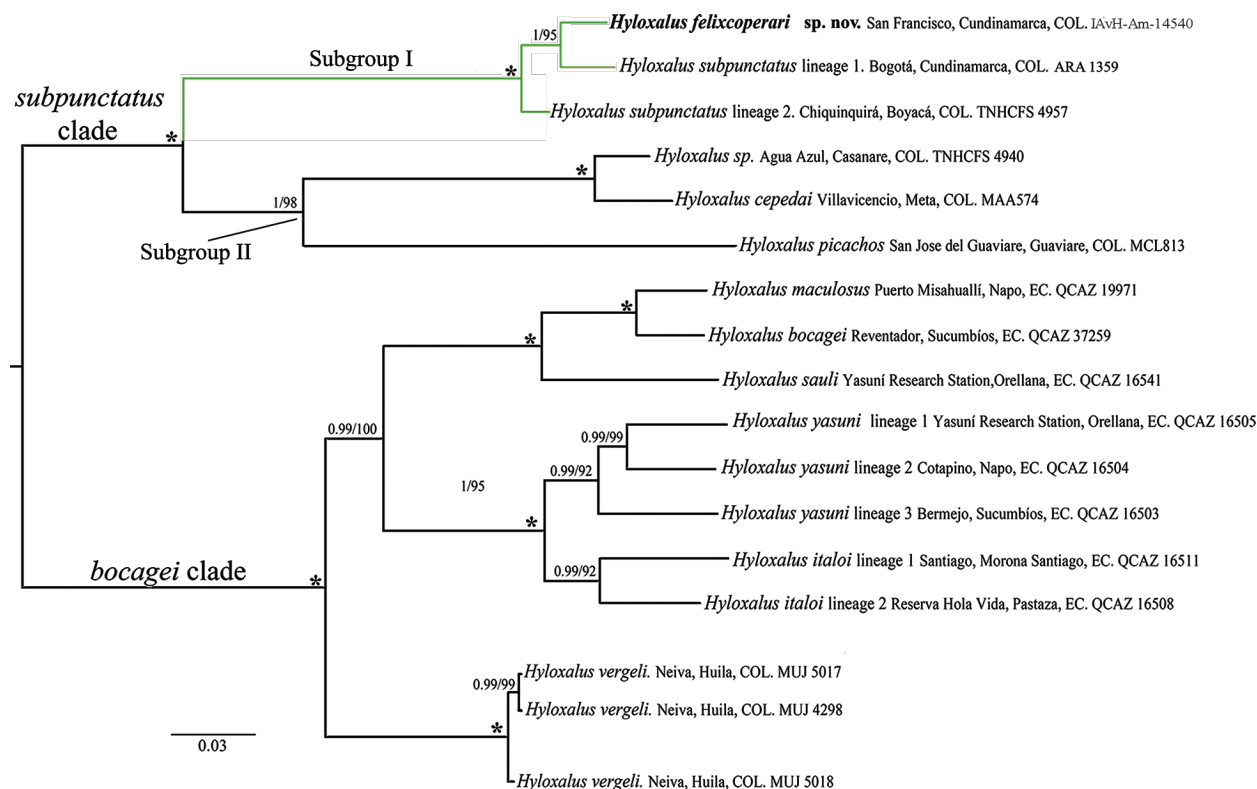
### Tadpoles

Specimens were fixed in 10% formalin for permanent storage. Three specimens of tadpoles were collected while back-riding on one female (Fig. 13). The description of the developmental stages of tadpoles, oral disc morphology, labial tooth row formula (LTRF), body morphology and the morphometric variables, followed previous proposals (GOSNER, 1960; ALTIG & MCDIARMID,

1999; MCDIARMID & ALTIG, 1999; ANGANOY-CRIOLLO, 2013). Color in life was transcribed from field notes. Abbreviations and standard measurements for tadpoles are as follow: TL (total length from tip of snout to tip of tail), BL (body length from tip of snout to junction of posterior body and tail musculature), TAL (tail length from junction of posterior body and tail musculature to tip of tail), BW (greatest body width), BH (highest body height), HW (head width at level of eyes), TMH (tail muscle height at base of tail), TMW (tail muscle width at base of tail), MTH (maximum height of tail), END (eye-naris distance), NSD (naris-snout distance), IND (internarial distance), IOD (interorbital distance) and ED (eye diameter). Measures were taken from preserved specimens to the nearest 0.1 mm under the stereoscope, using a digital calliper.

### Bioacoustics

Calling males were found on leaf litter associated with Chusque vegetation (*Chusquea* spp.) beside a small brook. Vocal recordings of unvouchered specimens were obtained positioning the microphone about 1 to 1.5 m from the calling males on the 15th of December 2016 at 16:10 hours and with a temperature of 16.7 °C and 60% relative humidity. Calls were recorded using a Marantz PMD 671 digital recorder with a unidirectional microphone Sennheiser MKH 60 P48. Temperature and humidity records were taken using an EXTECH thermo-hygrometer Model 445702. Acoustic analysis was conducted using the software RAVEN 1.3 for Windows (CHARIF *et al.*, 2008), with a sampling frequency of 44 kHz, 16-bit resolution, FFT (Fast Fourier Transformation) = 256 and Overlap = 50. The criteria for the definition and descriptions of acoustic parameters that include temporal and spectral variables followed previous proposals (DUELLMAN & PYLES, 1983; COCROFT & RYAN, 1995; COLOMA, 1995; KOK, 2010; KOCH *et al.*, 2011; KOK *et al.*, 2013; KÖHLER *et al.*, 2017). The temporal variables measured included the following: call (main acoustic unit in a frog vocalization), note (smaller subunits of calls separated from one another), number of notes per call (number of distinct acoustical signals recognizable in a call), call rate (number of calls per minute), note duration (duration individual note in seconds), oscillations (number of sound waves contained in each note), internote interval (from the beginning of one note to the beginning of the next note). Furthermore, spectral structure was evaluated with the dominant frequency (frequency of highest energy determined by the Fourier calculation), and the number of harmonics (number of harmonic frequencies present in the call). Advertisement calls were deposited in the sound collection of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt Colombia, Villa de Leyva, Boyacá, Colombia (BSA) and the URL's are available on the IAvH website (<http://coleccion.humboldt.org.co/rec/sonidos/BSA-15987/>)



**Fig. 2.** Bayesian inference tree showing the evolutionary relationships of *Hyloxalus felixcoperari* sp. nov. (bold) based on 2590 bp of mtDNA (partial 12S rRNA, 16S rRNA and cytb genes). Numbers before nodes: posterior probability values for the Bayesian analysis (BA) left and maximum likelihood (ML) analysis right; asterisks indicate maximum support.

## Results

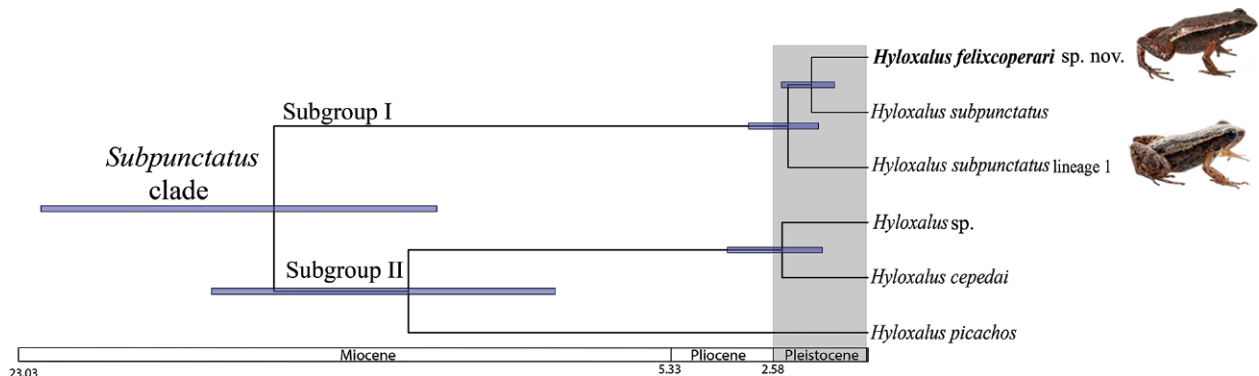
### Phylogenetic analyses, genetic divergence and dating

Both tree building methods revealed a congruent phylogenetic pattern consisting of two reciprocally monophyletic groups corresponding to the *subpunctatus* and *bocagei* clades (Fig. 2). Within the *subpunctatus* clade two subgroups were revealed: subgroup I formed by high altitude taxa (from 1750 to 4020 m above sea level) and subgroup II, formed by low to middle altitude taxa (from 560 to 1600 m above sea level. Fig. 2). Within subgroup I, *H. felixcoperari* sp. nov. was revealed as a sister taxon of *H. subpunctatus* with strong support (BA: 1, ML: 95%), and *H. subpunctatus* lineage 1 (from Boyacá, Chiquinquirá), appeared as sister of both taxa with maximum support. Within subgroup II, *Hyloxalus* sp. from Agua Azul Casanare was revealed as sister taxon of *H. cepedai* with maximum support and *H. picachos* appeared as sister of both taxa with robust support (BA: 1, ML: 98%). Within the *bocagei* clade, *H. vergeli* appeared as its basal sister taxa with maximum support. As expected, the phylogenetic relations among the other species of this clade matched the molecular phylogeny of PÁEZ-VACAS *et al.* (2010), with robust support (Fig. 2).

The uncorrected *p* distances revealed that within the *subpunctatus* clade subgroup I, sequence differentiation

values between *H. felixcoperari* sp. nov. and *H. subpunctatus* were 2.1% and 5.6% for the 16S and cytb gene fragments respectively (Tables S1 and S2). The differentiation between *H. felixcoperari* sp. nov. and *H. subpunctatus* lineage 1 were 3.1% and 7.2% for the 16S and cytb gene fragments respectively, and the differentiation between the later taxa and *H. subpunctatus* were 2.4% and 7.2% for the same gene fragments. The sequence divergence ranges of those taxa compared to the taxa comprising subgroup II was 14.1–14.8% and 19.0–36.7% for the 16S and cytb gene fragments respectively (Tables S1 and S2). Lastly the genetic distances between taxa from the *subpunctatus* clade and *bocagei* clade range from 13.9–18.7% for the 16S and 16.3%–31.8% for the cytb (Tables S1 and S2).

Our dating analyses (Fig. 3) revealed that the *subpunctatus* clade subgroup I (high altitude) and group II (middle altitude) diverged at 16.23 mya (highest posterior density interval [HPD] = 11.78–22.60 mya). Within the *subpunctatus* clade subgroup I the divergence time for the separation of *H. felixcoperari* sp. nov. and its closest relative *H. subpunctatus* was estimated at 1.53 mya (HPD=0.912–2.38 mya). The branch off of the *H. subpunctatus* lineage 1 was estimated at 2.17 mya (HPD=1.35–3.26 mya). Within the *subpunctatus* clade subgroup II the divergence time for the separation of *Hyloxalus* sp. from Agua Azul Casanare and *H. cepedai* was estimated at 2.34 mya (highest posterior density interval [HPD] = 1.24–3.84 mya) and the branch



**Fig. 3.** Divergence time estimates using BEAST. Purple bars at nodes represent 95% highest posterior density intervals. The light grey column on the right shows the Pleistocene epoch. Insets: *Hyloxalus felixcooperari* sp. nov. Adult male, paratype IAvH-Am-14540, *H. subpunctatus* lineage I. Adult male IAvH-Am -13953.

off of *H. picachos* was estimated at 12.55 mya (HPD = 8.54–17.92 mya).

***Hyloxalus felixcooperari* sp. nov.**

urn:lsid:zoobank.org:act:AE33C649-8D87-43C3-9D9F-50F7AB75AEA2

**Holotype.** Adult male, IAvH-Am-14538 (field number ARA 7497), collected by Andrés Acosta, John Cesar Neita and Luis Miguel López on the 14<sup>th</sup> of December 2016, deposited in the Amphibian Collection of the Biological Collections, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt Colombia, Villa de Leyva, Boyacá, Colombia.

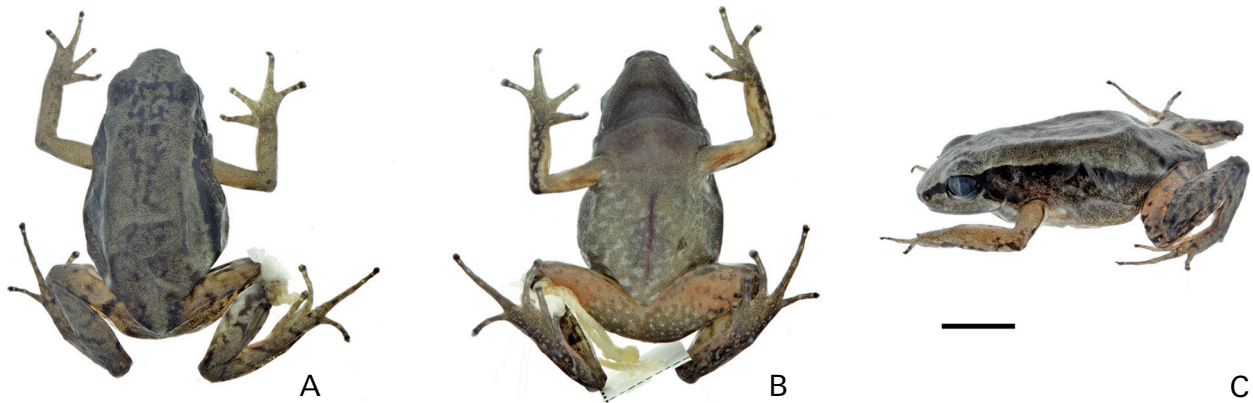
**Type Locality** (Fig. 1). Finca La Nube, Natural Private Reserve Paso de Nubes, Vereda La Laja, San Francisco Municipality, Cundinamarca Department, Colombia, 4° 56' 22.1" N / 74° 14' 48.8" W. Atitude: 2500–2574 m above sea level.

**Paratypes.** Adult males IAvH-Am-14539-40 and adult females IAvH-Am-14541-43, with same data as the holotype.

**Diagnosis** (Figs. 5–9). A species of *Hyloxalus* characterized by (1) SVL of adult males between 20.3–20.8 mm ( $20.6 \pm 0.2$ ,  $n=3$ ), and adult females between 21.4–23.8 mm ( $22.4 \pm 1.2$ ,  $n=3$ ); (2) disc on Finger III not expanded, the width of the adjacent phalange between 72.9–96.4% of disc; (3) when adpressed, Finger I equal or slightly shorter than Finger II; (4) fringe absent on the fingers; (5) disc on Toe IV slightly expanded, the width of the adjacent phalange between 63.2–75.4% of disc; (6) fringe absent on Toe IV and on other toes; (7) outer tarsal fold absent and inner tarsal fold, present and elongated tubercle-like; (8) toes unwebbed; (9) dorsolateral stripe absent; (10) oblique lateral stripe present, extending from groin to eye; (11) ventrolateral stripe absent; (12) throat, chest, and anterior part of the abdomen sexually dimorphic: in adult males, throat and chest dark and in adult

females, cream with irregular spots or dark brown reticulations; (13) abdomen cream with brown reticulations in females (Fig. 7), in adult males it turns light brown with diffuse cream spots; (14) Finger III not swollen in males; (15) testes white; (16) adult males without black, pale or dark grey arm gland; (17) cloacal tubercles absent; (18) incomplete pupil ring; (19) the call with dominant frequency between 3028.9–3861.2 Hz.

**Species comparisons** - Twenty species of *Hyloxalus* are so far recognized in Colombia, the majority of them restricted to the three Andean cordilleras (Fig. 1). Compared to the other eight species known to occur in the Cordillera Oriental, *Hyloxalus felixcooperari* sp. nov. is a medium sized frog with throat and chest dark but without black, pale or dark grey arm gland in the adult males; character which differentiate it from *H. lehmanni* and *H. saltuarius*. *Hyloxalus felixcooperari* sp. nov. differentiates from *H. edwardsi* and *H. ruizi* in neither having anal sheath nor basal toe webbing, and from *H. vergeli* by the absence of webbed toes. *Hyloxalus felixcooperari* sp. nov. differs from *H. cepedai* by the absence of dorso-lateral stripe (present in *H. cepedai*), and by the presence of complete oblique lateral stripe (absent in *H. cepedai*). *Hyloxalus felixcooperari* sp. nov. differs from *H. picachos* by Finger I slightly shorter than or sub equal to Finger II (having Finger I longer than II in *H. picachos*), and in having a larger body size (20.3 to 23.8 mm vs. 15.7 to 18.8 mm of SVL in *H. picachos*). *H. felixcooperari* sp. nov. can be confused with *H. subpunctatus* notably since adults of both species present complete oblique lateral stripe. However, they differ by having throat and chest dark in the adult males (immaculate in adult males of *H. subpunctatus*, Fig. 9), an incomplete pupil ring (complete in *H. subpunctatus*), fringe absent on toe IV and on the other toes (present in *H. subpunctatus*), the inner tarsal fold present and elongated, tubercle-like (barely visible and flattened in *H. subpunctatus*) and the call of *Hyloxalus felixcooperari* sp. nov. differs from the call of *H. subpunctatus* in the emission of a trill-like call (single notes emitted at a constant rate in *H. subpunctatus* sensu NAVAS, 1996; FANDIÑO *et al.*, 1997).



**Fig. 4.** *Hyloxalus felixcooperari* sp. nov. in alcohol 70%. Adult male IAvH-Am-14538 (SVL =20.3 mm, holotype). **A)** Dorsal view; **B)** Ventral view; **C)** Lateral view. Line scale = 5 mm.



**Fig. 5.** Lateral and frontal views of sexually dimorphic coloration in life of type series of *Hyloxalus felixcooperari* sp. nov. **A:** Holotype adult male IAvH-Am-14538; **B:** adult male IAvH-Am-14539; **C:** adult male IAvH-Am-14540; **D:** adult female IAvH-Am-14541; **E:** adult female IAvH-Am-14542; **F:** adult female IAvH-Am-14543.

**Description of the holotype.** (Fig. 5A) Adult male, 20.3 mm SVL; skin of dorsum and thighs slightly granular; cephalic region, eyelids and lateral surfaces smooth; head length diagonally from corner of mouth to tip of snout 7.4 mm; snout length (from anterior corner of eye to tip of snout) 3.0 mm; preaxillary area with bulged aspect (Fig. 5A); cloacal tubercles absent; head width between angle of jaws 7.0 mm; interorbital distance 3.0 mm; snout sloped, bluntly rounded in dorsal aspect, sharply rounded in lateral view (Fig. 5A); internarial distance (between center of naris) 2.8 mm; *Canthus rostralis* well-defined and rounded; loreal region weakly concave, vertical, not sloping outward to lip (Fig. 5A); eye length from its posterior to anterior corner 2.3 mm; eye-nostril distance (from anterior corner of eye to center of nostril) 1.8 mm; nares slightly protuberant, directed postero-dorsally; anterior three quarter of tympanum and *annulus tympanicus* partially visible, rest of tympanum concealed postero-dorsally by low supratympanic bulge, relatively large, its diameter 1.1 mm; teeth present on maxillary arch.

Forelimbs of moderate size, forearm length (from proximal edge of palmar tubercle to outer edge of flexed elbow) 4.4 mm; hand length (from proximal edge of palmar tubercle to tip of third finger) 5.5 mm; disc width moderately expanded from the adjacent phalange, 79.6% of Finger III. Finger III not swollen. Fringes absent on

fingers. Finger I slightly shorter than Finger II when appressed (Fig. 6). Finger II reaches distal subarticular tubercle of finger III; Finger IV beyond or reaches the distal subarticular tubercle of finger III. Relative lengths of appressed fingers III>IV>II>I. Subarticular tubercles 1-1-2-2. Supernumerary tubercles absent, subarticular tubercles subovoid; thenar tubercle elliptical; palmar tubercle weakly subelliptical; metacarpal fold or ridge absent.

Hindlimbs moderate; foot length (from proximal edge of outer metatarsal tubercle to tip of fourth toe) 9.7 mm, foot length 47.9% of SVL. Toe webbing and toe fringes absent. Relative lengths of adpressed toes IV>III>V>II>I. Disc of toe III barely extends beyond the penultimate subarticular tubercle of the toe IV; toe V reaching the level of penultimate subarticular tubercle of toe IV. Discs of the toes slightly expanded, width of the adjacent phalange 68.9% of disc of toe IV. Tibia length (from outer edges of flexed knee to heel) 9.2 mm, its length is equivalent to 45.7% of SVL; subarticular tubercles 1-1-2-3-2; supernumerary tubercles absent; inner metatarsal tubercle elliptical; outer metatarsal tubercle sub-circular, smaller than inner metatarsal tubercle, diameter of outer metatarsal tubercle 44.4% of inner metatarsal tubercle; outer tarsal fold absent; inner tarsal tubercle prominent elongate tubercle-like, oblique to the tarsus but with ridge neither folded nor extended to pre-axial side of toe I; median metatarsal tubercle not present.





**Fig. 6.** Ventral view of **A:** hand and **B:** foot of *Hyloxalus felixcooperari* sp. nov. Holotype IAvH-Am-14538 adult male. Line scale = 2 mm.

**Description and variation of the type series** - Variations in meristic variables are provided in Table 2. Adult males with SVL between 20.3 and 20.8 mm ( $n=3$ ), unpigmented (white) testes and throat and chest dark; adult females with SVL between 21.4 and 23.8 mm ( $n=3$ ); skin of dorsum and thighs slightly granular; cephalic region, eyelids and lateral surfaces smooth; preaxillary area with bulged aspect (a tubercle-like in adult male IAvH-Am-14540); cloacal tubercles absent; head width (between angles of jaws) 32.9–36.4% of SVL, and 90.1–104.8% of head length; interorbital distance 26.4–44.5% of head width; snout sloped, bluntly rounded in dorsal aspect, sharply rounded in lateral view; *Canthus rostralis* well defined and rounded; loreal region weakly concave, vertical, not sloping outward to lip; eye diameter 28.8–39.4% of the head length; eye-nostril distance 46.9–60.8% of snout length and 60.6–104.4% of the eye diameter; nares slightly protuberant, directed postero-dorsally; anterior three quarter of tympanum and *annulus tympanicus* partially visible, rest of tympanum concealed postero-dorsally by low supratympanic bulge, relatively large: in its greatest diameter 40.8–50.0% of the eye diameter; teeth present on maxillary arch.

Forelimbs of moderate size (Fig. 6), forearm length 20.0–24.0% of SVL and 70.0–91.0% of hand length; hand length 25.5–28.5% of SVL; disc width moderately expanded from the adjacent phalange, 72.9–96.4% of Finger III. Finger III not swollen in either adult males or in adult females. Fringes absent on fingers. Finger I slightly shorter than or sub-equal to Finger II when appressed. Finger II reaches distal subarticular tubercle of finger III (in IAvH-Am-14543); Finger IV extends beyond or extends to the distal subarticular tubercle of finger III. Relative lengths of appressed fingers III>IV>II>I. Subarticular tubercles 1-1-2-2. Supernumerary tubercles absent, subarticular tubercles sub-ovoid; thenar tubercle elliptical; palmar tubercle weakly subelliptical; thenar tubercle from one-half to one third than palmar tubercle; metacarpal fold or ridge absent.

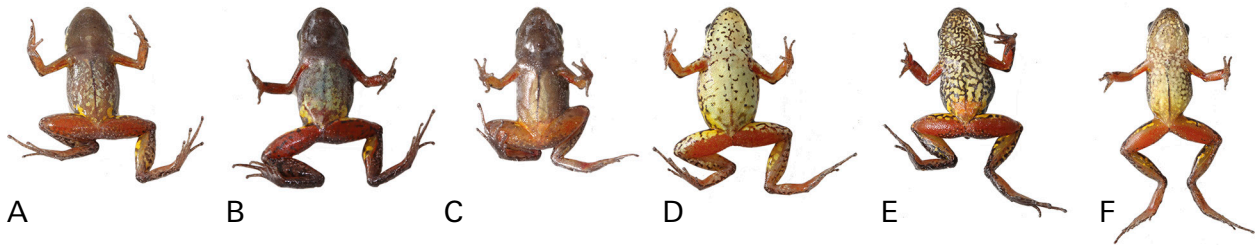
Hindlimbs moderate (Fig. 6), shank 43.9–47.9% of SVL and foot length 41.7–47.9% of SVL. Toe webbing

**Table 2.** Meristic variation of *Hyloxalus felixcooperari* sp. nov. Mean,  $\pm$  one SD and range are given for adults. SVL = Snout vent length, HW = Head width, HL = Head length, IOD = Interorbital distance, ED = Eye diameter, END = Eye-naris distance, SL = Snout length, TD = Tympanum diameter, HDL = Hand length, FL = Forearm length, TL = Tibia length, FTL = Foot length. All measurements in mm.

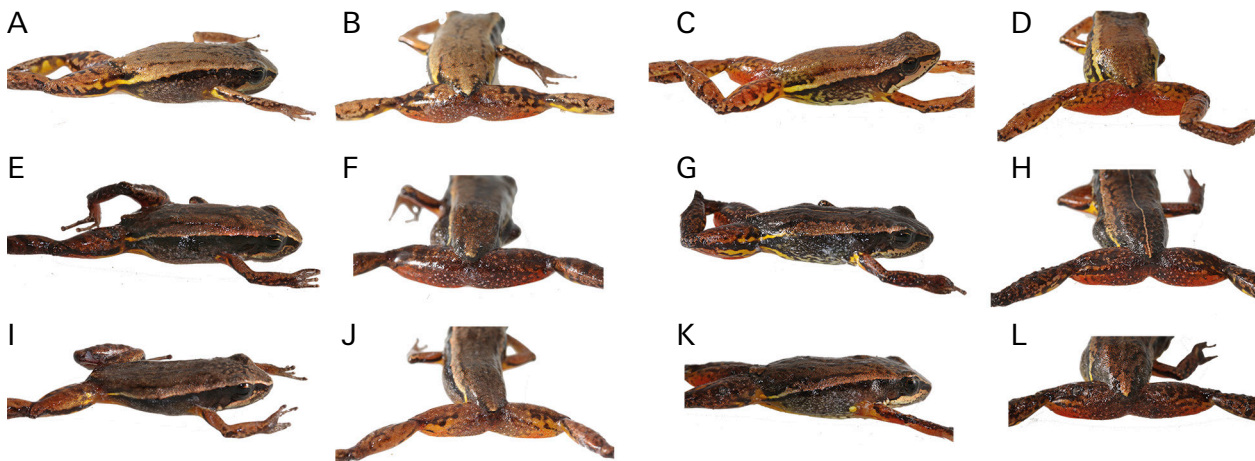
	Males ( $n=3$ )	Females ( $n=3$ )
SVL	20.6 $\pm$ 0.24 (20.3–20.8)	22.4 $\pm$ 1.27 (21.4–23.8)
HW	7.0 $\pm$ 0.20 (6.8–7.2)	7.7 $\pm$ 0.49 (7.2–8.1)
HL	7.0 $\pm$ 0.48 (6.5–7.4)	7.8 $\pm$ 0.16 (7.7–8.0)
IOD	2.8 $\pm$ 0.17 (2.6–3.0)	2.9 $\pm$ 0.89 (1.9–3.6)
ED	2.2 $\pm$ 0.16 (2.0–2.3)	2.6 $\pm$ 0.41 (2.2–3.0)
END	1.7 $\pm$ 0.36 (1.4–2.1)	2.1 $\pm$ 0.01 (2.13–2.16)
SL	3.2 $\pm$ 0.37 (2.9–3.6)	3.7 $\pm$ 0.17 (3.5–3.8)
TD	1.0 $\pm$ 0.09 (0.9–1.1)	1.2 $\pm$ 0.08 (1.1–1.2)
HDL	5.5 $\pm$ 0.11 (5.3–5.6)	6.1 $\pm$ 0.62 (5.6–6.8)
FL	8.9 $\pm$ 0.48 (8.6–9.5)	9.7 $\pm$ 0.12 (9.6–9.8)
TL	9.21 $\pm$ 0.07 (9.1–9.2)	10.4 $\pm$ 0.22 (10.2–10.6)
FTL	9.3 $\pm$ 0.61 (8.6–9.7)	10.5 $\pm$ 0.34 (10.1–10.8)

and toe fringes completely absent. Relative lengths of adpressed toes IV>III >V>II>I. Disc of toe III barely beyond than the penultimate subarticular tubercle of the toe IV; toe V reaching the level of penultimate subarticular tubercle of toe IV. Discs of the toes slightly expanded, width of the adjacent phalange 63.2–75.4% of disc of toe IV. Tibia length 43.9–47.9% of SVL; subarticular tubercles 1-1-2-3-2; supernumerary tubercles absent; inner metatarsal tubercle elliptical; outer metatarsal tubercle sub-circular, smaller than inner metatarsal tubercle, diameter outer metatarsal tubercle 44.4–70.7% of inner metatarsal tubercle; outer tarsal fold absent; inner tarsal tubercle prominent elongate tubercle-like, oblique to the tarsus but with ridge neither fold nor extended to pre-axial side of toe I; median metatarsal tubercle not present.

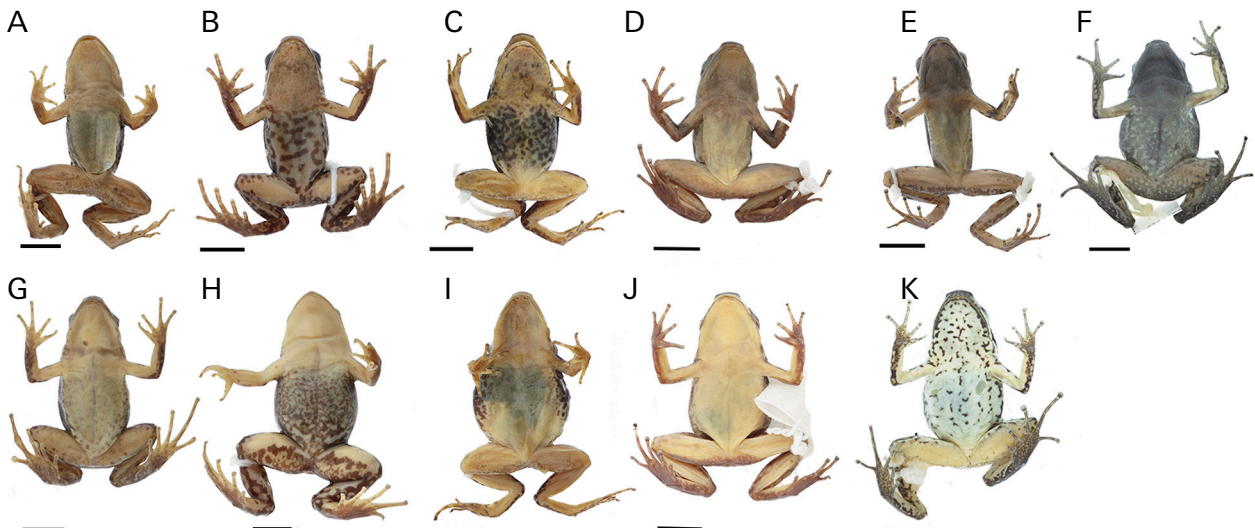
**Color in life** (Figs. 5, 7 and 8). Based on field notes and photographs of the type series: in dorsal view: light brown with irregular spots and dark brown reticulations; the mid-dorsal line is conspicuous in IAvH-Am-14542. Forelimbs are reddish brown with irregular spots and dark brown reticulations. Dorsal surfaces of thighs colored reddish brown, with dark brown incomplete transverse bands; anterior thigh is reddish brown with a dark brown longitudinal band; axillary and inguinal region usually with yellow spots and longitudinal band; (in IvH-Am-14539-41) pale orange axillary region. Sides of head have a black band that extends from the anterior tip of the snout to the posterior region of groin; lateral region of the body dark brown with small irregular and diffuse white spots. Male IAvH-Am-14541 with irregular pale yellow spots; upper lip light brown or cream with irregular thin dark brown spots on the edge; iris copper with black reticulations and pupil ring incomplete; posterior thigh in the distal region yellow with longitudinal band or dark



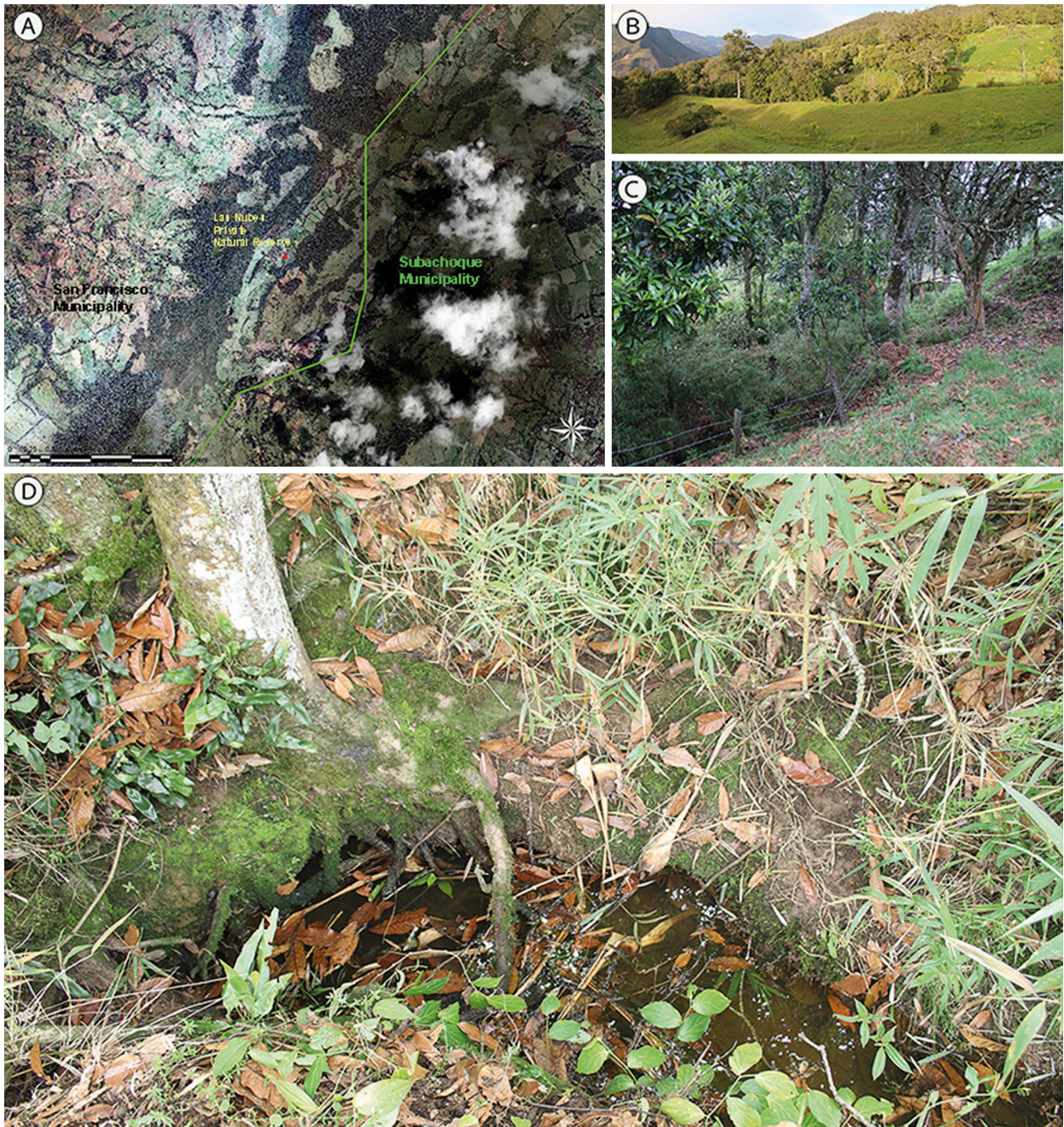
**Fig. 7.** Ventral views of sexually dimorphic coloration in life of type series of *Hyloxalus felixcoperari* sp. nov. **A:** Holotype adult male IAvH-Am-14538; **B:** adult male IAvH-Am-14539; **C:** adult male IAvH-Am-14540; **D:** adult female IAvH-Am-14541; **E:** adult female IAvH-Am-14542; **F:** adult female IAvH-Am-14543.



**Fig. 8.** Color pattern variation of flanks and posterior thigh in life of type series of *Hyloxalus felixcoperari* sp. nov. **A, B:** Holotype adult male IAvH-Am-14538; **C, D:** adult male IAvH-Am-14539; **E, F:** adult male IAvH-Am-14540; **G, H:** adult female IAvH-Am-14541; **I, J:** adult female IAvH-Am-14542; **K, L:** adult female IAvH-Am-14543.



**Fig. 9.** ventral view in preservative of some *Hyloxalus* species from the Cordillera Oriental of Colombia: adult males: **A:** *Hyloxalus subpunctatus* from Bogotá plateau region (Topotype), IAvH-Am 6584 (SVL = 20.0 mm.); **B:** *Hyloxalus subpunctatus* from Chingaza National Natural Park, IAvH-Am-9074 (SVL = 19.22 mm.); **C:** *Hyloxalus pulchellus* from Silvia Municipality, Cauca Department, IAvH-Am-3494 (SVL = 19.5 mm.). Species with dark throat and chest from *Hyloxalus ramosi* group: **D:** *Hyloxalus lehmanni*, adult male IAvH-Am 1793 (SVL = 18.10 mm.) obscure variation (sensu GRANT & CASTRO, 1998); **E:** *Hyloxalus saltuarius* from Acevedo municipality, Huila Department, adult male IAvH-Am 7645 (SVL = 18.87 mm.); **F:** *Hyloxalus felixcoperari* sp. nov. adult male IAvH-Am-14538 (SVL = 20.3 mm, holotype). Adult females: **G:** *Hyloxalus subpunctatus* from Bogotá plateau region, IAvH-Am 2170 (SVL = 23.4 mm.); **H:** *Hyloxalus subpunctatus* from Pesca Boyacá, IAvH-Am-8837 (SVL = 23.4 mm.); **I:** *Hyloxalus pulchellus* from Silvia Municipality, Cauca Department adult female IAvH-Am-3487 (SVL = 25.1 mm.); **J:** *Hyloxalus lehmanni*, adult female IAvH-Am 1554 (SVL = 20.83 mm.); **K:** *Hyloxalus felixcoperari* sp. nov. adult female IAvH-Am-14541 (SVL = 22.0 mm., Paratype). Line scale = 5 mm.



**Fig. 10.** A: ESRI satellite image from the type locality (red triangle) of *Hyloxalus felixcoperari* sp. nov. at Las Nubes Private Natural Reserve, San Francisco Municipality (border in green line). Coordinates: 4°56'22.1" N/74°14'48.8" W, Altitude range: 2500–2574 m above sea level, Cundinamarca Department. B: General view of the landscape associated with the protective cover of the creek that corresponds to the species habitat. C and D: Microhabitat associated to small ponds formed along the creek in which the specimens were collected. Photos: Andrés Acosta-Galvis.

brown reticulations; dark brown cloacal region with small white melanophores; light brown oblique lateral stripe extending from groin to eye. Species is sexually dimorphic. The ventral surfaces as follow: in adult males a dark brown throat and chest; light brown belly (in IAvH-Am-14538 with irregular cream spots). In adult females, a cream throat, chest and belly with irregular spots or dark brown reticules. Orange ventral thigh at the cloacal region with scattered and small white melanophores; reddish or light brown palmar and plantar surfaces.

**Color in preservative** (ethanol 70%). Brown to blackish brown dorsal surface (Fig. 4) with dark brown and white irregular marks in adult females. Light brown forelimbs with irregular spots and dark brown reticulations. Light brown dorsal surfaces of thighs with dark brown and incomplete transverse bands; light brown anterior thigh with a dark brown longitudinal band; cream axillary and inguinal region. Flanks of the head with a black band that extends from the anterior tip of the snout to the posterior region of the groin; lateral region of the body dark brown

**Table 3.** Call parameters of three species from the highlands of the Cordillera Oriental and Cordillera Central of Colombia. Length and time in seconds. Frequencies in Hz.

Parameter	<i>H. subpunctatus</i>	<i>H. pulchellus</i>	<i>H. felixcoperari</i> sp. nov.
Call duration	0.15–0.80 s	0.4–0.6 s	1.23–1.90 s
Call rate	13–64	18	50–104
Notes per call	—	4–6	13–19
Note length	—	0.05	0.030–0.061 s
Internote duration	0.010–0.049	0.06–0.12	0.053–0.095 s
Harmonics	—	2	2
Frequency ranging	2800–3600	—	2701.9–5971.7
Dominant Frequency	—	2700–3000	3028.9–3861.2
References	FANDIÑO <i>et al.</i> (1997), NAVAS & BEVIER (2001)	COLOMA (1995), KOCH <i>et al.</i> (2011)	This work

with small irregular and diffuse white spots; posterior thigh dark brown with longitudinal band or dark brown and cream reticulations; dark brown cloacal region with small white melanophores; light brown oblique lateral stripe; pale orange ventral thigh at the cloacal region with scattered small white melanophores; light brown palmar and plantar surfaces with irregular spots and dark brown melanophores.

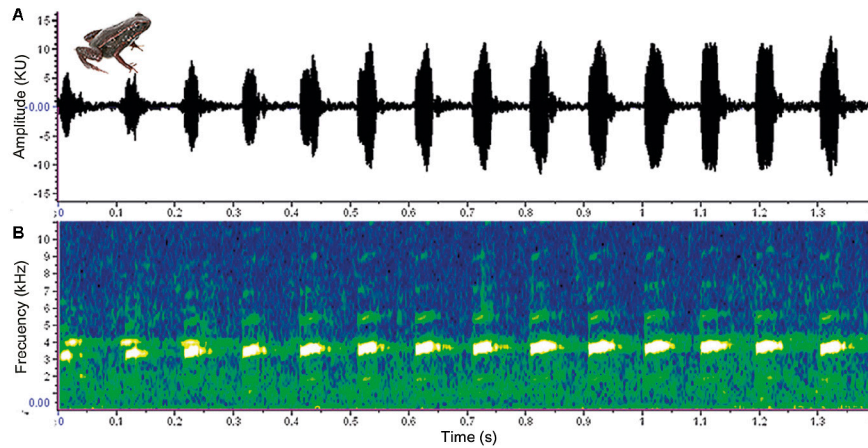
**Etymology.** The specific epithet of this dendrobatid frog is dedicated to Felix Acosta-Gualtero (1935–2015), who dedicated his life to the consolidation of the solidarity economy (Cooperativism) in Colombia, based on principles of participation, reciprocity and cooperation.

**Advertisement call** (Fig. 11 and 12). Five minutes of advertisement calls were recorded (sounds collection code BSA-15987, <http://coleccion.humboldt.org.co/rec/sonidos/BSA-15987/>). The adult males vocalized hidden under the leaf litter, near a slow flowing stream up to 1 m distance from the water, at the end of the rainy season. On December 15<sup>th</sup>, 2016 two unvouchered specimens were recorded between 11:17–15:56 hours at a temperature of 16.7° C and at 60% relative humidity. Temporal structure: the advertisement call of *Hyloxalus felixcoperari* sp. nov. consists of 13–19 notes per call, (mean  $14.1 \pm 1.24$ ,  $n=21$ ); advertisement call duration varied between 1.23–1.90 s (mean  $1.37 \pm 0.13$ ,  $n=21$ ), starting through the first four notes to gradually increasing its amplitude. The call rate varied between 50–104 calls/min. The note duration was between 0.030–0.061 s (mean  $0.046 \pm 0.064$ ,  $n=93$ ) with 104–166 (mean  $135.2 \pm 19.1$ ,  $n=49$ ) number of oscillations contained in each note. The internote interval varied between 0.053–0.095 s (mean  $0.062 \pm 0.007$ ,  $n=100$ ). Spectral structure: two harmonics were developed, with the dominant frequency 3028.9–3861.2 Hz. The distribution of sound energy increases progressively through the higher harmonics.

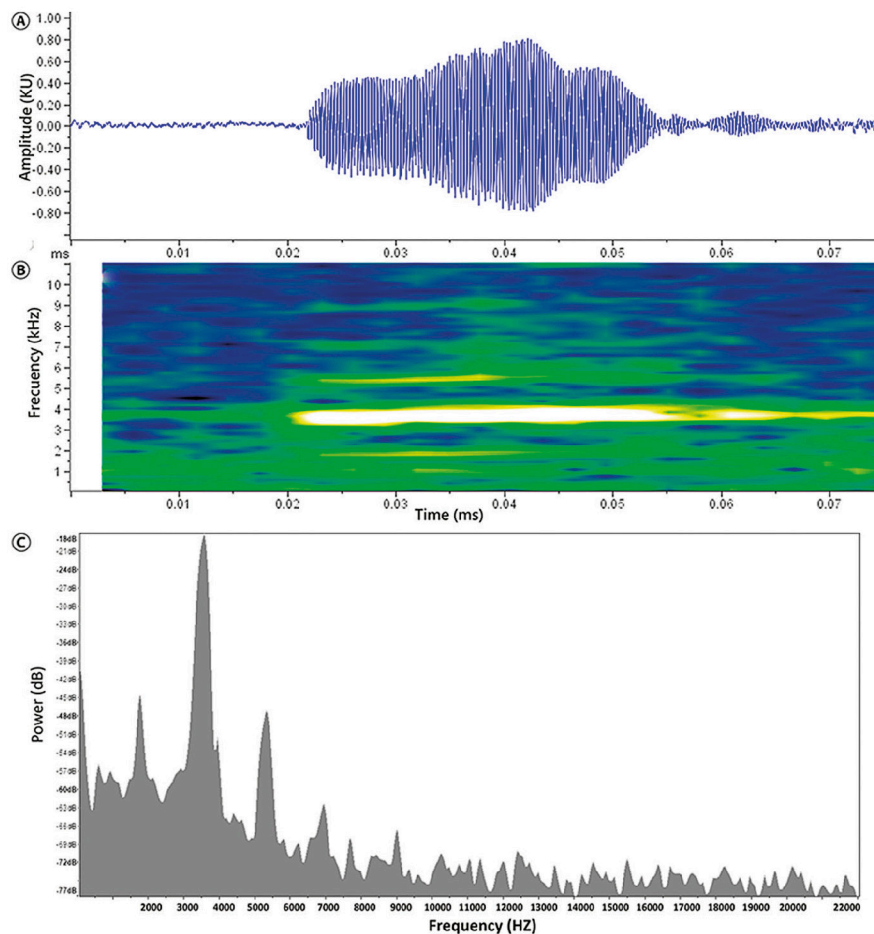
Furthermore, *H. felixcoperari* sp. nov. has distinctive vocalizations compared to *H. subpunctatus* and *H. pulchellus* (Table 3). The calls of *H. felixcoperari* sp. nov. emits a trill-like call, while in *H. subpunctatus* are composed of single notes (“peep”) emitted at a constant rate (NAVAS, 1996; FANDIÑO *et al.*, 1997) and *H. pulchellus* consists of a buzz call (COLOMA, 1995).

**Larval transportation and tadpole description** (based on back-riding tadpoles; Fig. 13). Within the type series one of the females was carrying three tadpoles (IAvH-Am-14543). The following description is based on three tadpoles in stage 26 (IAvH-Am-14544; Fig. 13). The tadpoles of *Hyloxalus felixcoperari* sp. nov. are characterized by being exotrophic and lotic (McDIARMID & ALTIG, 1999); total length 8.6–9.7 mm; body length 3.8–4.4 mm (39.8–48.1% of total length); body ovoid and depressed; highest body width 2.5–2.9 mm, highest body height 1.6–2.0 mm; snout bluntly rounded in dorsal and lateral views. Very small subcircular naris, directed anterodorsally, opening 0.2–0.3 mm from tip of snout; distance from naris to anterior margin of eye 0.3–0.6 mm; internarial distance 0.4–0.5 mm (20.0–24.7% head width at level of eyes). Eyes dorsal and directed laterally; eye diameter 0.4–0.5 mm, interorbital distance 0.7–0.9 mm (38.1–44.6% head width at level of eyes). Coiling of intestines from central to sinistral; sinistral and conical spiracle, vent tube free, opening directed posterodorsally, barely visible. Vent tube dextrally attached to ventral fin, opening directed dextrally. Developing hind limb bud of 0.6 mm in length and 0.3 mm width. Tail length 4.8–5.8 mm (51.8–60.1% of total length); caudal musculature robust that extends to a major degree in the dorsolateral region at the junction of the body; tail gradually reduced until it reaches its tip; tail muscle width at base of tail 0.8–1.1 mm; tail muscle height at base of tail 0.9–1.1 mm. The height of the dorsal fin gradually increases until reaching the same height of the ventral fin; maximum tail height almost equal to the body height, from 1.1 to 1.8 mm. Lateral-line system not detectable. The oral disc (in back-riding tadpoles) weakly emarginated in stage 26, with one row to one biserial row of marginal papillae; labial tooth row formula from 0/0,3 to 5 marginal papillae on anterior lip; jaw sheaths not fully pigmented; denticles not covering all ridges of the labial tooth rows; a barely or not visible notch at the middle of the free-edge of the upper jaw sheath.

When trying to establish comparisons in the early stages of the development of the tadpoles (back-riding tadpoles), there are limitations that do not allow the identification of relevant taxonomic differences, mainly in the oral disc (ANGANROY-CRIOLLO, 2013). In stage 26 (*sensu* GOSNER, 1960), the tadpoles of *H. felixcoperari* share



**Fig. 11.** Advertisement call of an unvouchered adult male of *Hyloxalus felixcooperari* sp. nov. BSA-15987; gradual change in amplitude is evident from the fourth note. **A:** Oscillogram, **B:** Spectrogram.



**Fig. 12.** Vocalisation of *Hyloxalus felixcooperari* sp. nov. **A:** Expanded oscillogram; **B:** Spectrogram of the fifth note in Fig. 11 and **C:** Power spectrum.

the following features with tadpoles of *H. subpunctatus*: notch on upper jaw sheath absent, oral disc anteroventral on body, caudal musculature slender at body-tail junction, and spiracle located slightly posterior to the mid-body.

**Color of tadpole in life** (Fig. 13). Dorsal surfaces: dark brown body and interorbital region; the region surrounding the eyes and tail is light brown with irregular to dif-

fuse dark spots. Eyes black. Lateral surfaces: light brown cephalic region with irregular dark spots and cream ventrolateral region; cream tail muscle (cream myotomes) with very thin coffee brown reticles and a dark brown longitudinal line in the anterior and dorsolateral portion; translucent dorsal fin with tiny irregular dark spots; translucent ventral fin. Ventral surfaces: translucent belly with intestines and heart barely visible; cream color



**Fig. 13.** Left: Lateral view of a female *Hyloxalus felixcooperari* sp. nov. carrying tadpoles in stage 26 (sensu GOSNER, 1960) IAVH 14543 (SVL=21.4). Right: Lateral, dorsal and ventral views of a tadpole IAVH 14544 in stage 27 in preservative. Scale bars = 5 mm.

on remaining surfaces and yellowish cream intestines (Fig. 13).

**Color of tadpole in preservative.** Brown to dark brown dorsal surfaces and interorbital region and body. Translucent venter with some scattered and diffuse melanophores. Cream caudal musculature with scattered dark brown flecks; translucent upper fin with scattered dark brown; translucent lower fin.

**Distribution.** *Hyloxalus felixcooperari* sp. nov. is currently known only from the type locality at Natural Private Reserve Paso de Nubes, between 2500–2577 m above sea level. The locality corresponds to a cloud Andean forest fragment located on the western slope of the Cordillera Oriental of Colombia (Fig. 1).

**Natural History.** *Hyloxalus felixcooperari* sp. nov. is a diurnal species inhabiting Andean cloud forests associated with open areas on the western slope of the Cordillera Oriental. The species' biological activity between 9:00–16:00 hours is related to the hours of the highest temperature in the area. The adult males call for mating while hidden inside the litter in the surrounding vegetation along slow-course creeks. They establish small mating territories separated from one another by areas ranging from 1 to 4 meters. The species seems to be more active at the end of the rainy season and can be considered rare due to their relative low abundances (only six specimens were obtained in four hours of sampling performed by three collectors). The microhabitat consists of vegetation remnants at the drainage edge in a pasture matrix. Grasses and herbaceous species dominate the grazing stratum; above them grow dense tangles composed by *Chusquea* spp. and a tree species *Weinmannia* sp. (Encenillo) (Fig. 10).

*Hyloxalus felixcooperari* sp. nov. is sympatric but not syntopic with other anuran species such as: (i) *Rheobates palmatus* occurring in nearby streams and associated with fast flowing streams with small ponds, (ii) *Dendropsophus padreluna* and *D. molitor*; inhabiting permanent and seasonal ponds and (iii) an undescribed species of the genus *Pristimantis* from the forests and protective vegetation cover of streams.

**Conservation status.** *Hyloxalus felixcooperari* sp. nov. seems to be a rare species and it was present only in one of the three streams evaluated, occupying areas severely fragmented and seriously affected by anthropogenic disturbance and where agrochemicals used for potato crops are common. In fact, one of the collected individuals, an adult female IAVH-Am-14542 showed malformations of the manus, possibly indicating some degree of chemical impact, but this aspect needs to be further assessed. The original type locality habitat has been subject to extensive conversion by livestock for more than 40 years, producing degradation and disappearance of the original landscape. Currently, the type locality is part of a protected area from the "Reservas Naturales de la Sociedad Civil", a system of private natural reserves recognized under Article 109 of Law 99 of 1993 of the Republic of Colombia.

## Discussion

In this study, integrative taxonomy allowed us to confirm that *H. felixcooperari* sp. nov. is distinct from other *Hyloxalus* species and lineages with cryptic coloration from high altitudes of the Cordillera Oriental. In agreement with previous studies (i.e. GRANT *et al.*, 2006; SANTOS *et al.*, 2009; PAÉZ-VACAS *et al.*, 2010; PYRON & WIENS, 2011; VENCES *et al.*, 2003; GRANT *et al.*, 2017) our phylogenetic analyses corroborated the validity of the *Hyloxalus subpunctatus* clade and revealed it as sister to the *H. bocagei* clade. Furthermore, our analyses revealed two subgroups within the *subpunctatus* clade: subgroup I formed by high altitude occurring taxa and subgroup II, formed by low to middle altitude occurring taxa. Our RMC analyses suggested that those subgroups separated during the Miocene epoch (Fig. 3). In the ancestral reconstruction of the distribution of members of the *Hyloxalus bocagei* clade performed by PAÉZ-VACAS *et al.*, (2010), it was suggested that the ancestors of both, the *subpunctatus* + *bocagei* clade and the *subpunctatus* subgroups (represented in the analysis by one sequence of each subgroup), were Andean. This suggests that for the *subpunctatus* clade there was also a trend for the dispersal of species from Andean ancestors to lower areas during the Miocene. In agreement with the historical status of "cryptic species" within *H. subpunctatus* sensu lato our phylogeny showed that *H. subpunctatus* sensu stricto and an individual from a geographically distant population previously studied; *H. subpunctatus* TNHCFS 4957 from Chiquinquirá, Boyaca (SANTOS *et al.*, 2009), designated here as the *H. subpunctatus* lineage 1, clearly differentiated genetically. Acosta-Galvis (in prep), has also shown morphologic and bio-acoustic differences between them, confirming that both correspond to different, separately evolving evolutionary lineages currently under description. In consequence, together with *H. felixcooperari* sp. nov this situation is related to fragmentary knowledge and morphological similarity rather than strict "cryptic

speciation". The small genetic distances and relatively recent divergence dates suggest that these three lineages from high altitudes of the middle Cordillera Oriental of Colombia correspond to a recently radiated species group. Their mean ages of estimated separation (including their HPD), fall within the Lower Pleistocene – Upper Pliocene period (0.912–3.26 mya), a time when the Cordillera Oriental had most probably reached its current elevation (Gregory-Wodzicki, 2000). We hypothesize that the dispersal barriers formed by the fragmentation of the montane vegetation produced by the cold temperatures during that period (HOOGHIEMSTRA & VAN DER HAMMEN, 2004; HOOGHIEMSTRA *et al.*, 2006) triggered and have maintained the independent recent evolution of these lineages. As the temperatures became warmer, populations expanded together with the montane vegetation, allowing proximity and possibly secondary contact of the already differentiated lineages. The same pattern of recent diversification has been observed in a high altitude group of Andean anoles (VARGAS-RAMÍREZ & MORENO-ARIAS, 2014). SANTOS *et al.*, (2009) hypothesised that the extant northern Andean highlands dendrobatid fauna most likely originated after prolonged *in situ* diversification after the inception of the poison frog clade, but the pace of species formation has slowed down. In contrast for the *subpunctatus* clade, our results suggest a pattern of rapid *in situ* diversification accompanied by an increase of species formation. The recent diversification of these frogs contrasts with the old diversification during the Late Miocene and Early Pliocene of the partially sympatric hyloid frog *Dendropsophus molitor*, associated with the uplift of the Cordillera Oriental (GUARNIZO *et al.*, 2009). Our observations support the hypotheses that the climatic fluctuations during the Pleistocene may have triggered speciation and radiation events in the northern Andes, increasing its diversity and producing high levels of endemism (LYNCH *et al.*, 1997; DUELLMAN, 1999).

GRANT *et al.* (2006) argue that transport of tadpoles on the dorsum of parent nurse frogs is a dendrobatid synapomorphy as the most primitive condition and dependent exclusively on the males carrying the tadpoles. This condition is reported for *Hyloxalus subpunctatus* (STEBBINS & HENDRICKSON, 1959; FANDIÑO *et al.*, 1997). In contrast it seems that females of *Hyloxalus felixcooperari* may transport the larvae. However, because this latter observation is limited to a single specimen, it is still unknown whether this derived condition corresponds to nurse frogs of both sexes, or it is an exclusive female condition.

## Comparative material

*Hyloxalus cepedai* (MORALES, 2000): COLOMBIA: Arauca Department, Tame municipality, Vereda Sabana de La Vega, Casiriba farm, 6°22'52.1"N/71°55'1.3"W, 750 m above sea level, IAvH-Am-10156, IAvH-Am-10162; Casanare Department, Nunchía municipality, Vereda Vega Piedecuesta, Las Canarias farm, 450 m above sea level, IAvH-Am-6713; Yopal municipality, Corregimiento El Charte, Vereda Rincón del Soldado, headwaters the Secreto forest, 5°23'48.8"N/72°29'14.6"W, 1018 m above sea level, IAvH-Am-10713.

*Hyloxalus lehmanni* (SILVERSTONE, 1971): COLOMBIA: Nariño Department, La Planada Nature Reserve, 7 Km S Chucunes, 1780 m above sea level: IAvH-Am-1527, IAvH-Am-1540-1, IAvH-Am-1553-4, IAvH-Am-1794-6, IAvH-Am-1793, IAvH-Am-1797.

*Hyloxalus picachos* (ARDILA-ROBAYO *et al.*, 1999): COLOMBIA: Caquetá Department, San Vicente de Cagúan municipality, Guayabal Police Inspection, Andalucía Farm, 2°44'41"N/74°53'22"W, 1550 m above sea level, IAvH-Am-6520-35.

*Hyloxalus pulchellus* (Jimenez De la Espada, 1875): COLOMBIA: Cauca Department, Silvia Municipality, 2400 m above sea level, IAvH-Am-0020, 2320 m above sea level, IAvH-Am-3448-9, IAvH-Am-3485-9, and IAvH-Am-3490-5. Nariño Department, La Cocha Lake, 2790 m above sea level, IAvH-Am-3446-7.

*Hyloxalus subpunctatus* (COPE, 1899): COLOMBIA: Boyacá Department, Cocuy Municipality, Sierra Nevada del Cocuy National Park, Frailejon valley, southern flank of the Sierra Nevada of Cocuy, 3800 m above sea level, 6°43'N/ 72°45'W, MUJ 406; Garagoa Municipality, Ciénaga-Valvanera Vereda, Private Natural Reserve El Secreto, 2100 m above sea level, 5°07'N/ 73°15'W, MUJ 1582; Cundinamarca Department, Chingaza National Park: MUJ 598-600, MUJ 1091, MUJ 1097-8; near of La Siberia creek junction La Playa river, 2600 m above sea level, 4°35'N/73°36'W: MUJ 447-8; near site La Playa, 3140 m above sea level, 4°34'N/73°46'W, MUJ 545, MUJ 548-551; road Chingaza National Park to Cáqueza Municipality, site La Playa, 3000 m above sea level, MUJ 1120; 3140 m above sea level, 4°34' N/73°57'30" W, MUJ 111-2; Buitrago Lakes, 3360 m above sea level, 4°45'N/73°50'W, MUJ 1099-1101, MUJ 1103-04, MUJ 1107-08; nearby Buitrago Lakes, 3200 m above sea level, 4°45'N/73°50'W, MUJ 1115-18, 3600 m., 4°46' N.-73°50' W, MUJ 435-37; Vereda California, site Piedras Gordas, 3250 m above sea level, 4°44' N.-73°51'W, MUJ 555, near Piedras Gordas creek, 2850 m above sea level, 4°44'N.-73°52'W, MUJ 567-68; 3210 m above sea level, 4°45'N/73°51'W: MUJ 1094, MUJ 1102, MUJ 1109; site Piedras Gordas creek, 3210 m above sea level, 4°45'N/73°51'W: MUJ 1106; 3250 m above sea level, MUJ 1095-6; 3200 m above sea level, MUJ 1113; 3240 m above sea level, MUJ 1122-23; road to Lake "El Medio", 3210 m above sea level, 4°32'N/73°45'W, MUJ 1114; site Frailejon valley, 3150 m above sea level, 4°36'N/73°47'W, MUJ 1119, MUJ 1124-5; 3000 m above sea level, MUJ 1129; 3500m. 4°36' N/73°46' W., MUJ 446, MUJ 671, MUJ 1126; Km. 5 road to "La Paila" way to Fomeque Municipality, 3300 m above sea level, 4°32'N/73°46'W, MUJ 1121; road to site La Paila, 3150 m above sea level, 4°28' N/73°47' W, MUJ 445; Chuza dam, near Casino, 2967 m above sea level, 4°38'N/73°44', MUJ 1127-28; site Chuza, forest near road to Chuza dam, 2850 m above sea level, 4°39' N.-73°43' W, MUJ 1131, 3300 m above sea level, 4°38' N/73°44'W., 9 Kilometers north of Camp Monteredondo, 3140 m above sea level, 4°40' N/73°44' W, MUJ 1130; 2 Km. before Frío River, 3400 m above sea level, 4°30'N/73°45' W, MUJ 574; road Chingaza National park to Fomeque Municipality, site El Arnical, 3300 m above sea level, 4°30' N/73°46' W, MUJ 556; Chingaza National park to Guasca Municipality, Siecha Lake, 3600 m above sea level, 4°47' N/73°51' W, MUJ 693-4.

*Hyloxalus saltuarius* (GRANT & ARDILA-ROBAYO, 2002): COLOMBIA: Huila Department, Acevedo Municipality, western slope of Cabaña Las Brisas, 1 km from the Bocatoma, Los Guácharos Cave Natural National Park, 1700-2000 m above sea level, IAvH-Am-7644-5.

*Hyloxalus vergeli* (HELLMICH, 1940): COLOMBIA: Huila Department, Neiva Municipality, Vereda Tamarindo, Alto La Tribuna, Private Reserve Hocol, La Tribuna Research and Education Center farm, 780 m above sea level, 3° 4'N/75°22.3'W, MUJ 4297-4328, MUJ 4344-4345, MUJ 4370, MUJ 5017-5018; Cundinamarca Department, Sylvania Municipality: IAvH-Am-521, IAvH-Am-523 IAvH-Am-527.

**Table S1.** Uncorrected *p* distances for the fragment of 16S gene (932 bp) of the *Hyloxalus* species, expressed as percentages (averages). Below the diagonal are between lineages divergences, along the diagonal and in bold are within lineage divergence.

	<i>N</i>	<i>feli</i>	<i>sub</i>	<i>sub1</i>	<i>sp.</i>	<i>cep</i>	<i>pic</i>	<i>mac</i>	<i>boc</i>	<i>sau</i>	<i>yas</i>	<i>ita</i>	<i>ver</i>
<i>H. felixcooperari</i> sp. nov.		—											
<i>H. subpunctatus</i>	1	2.1	—										
<i>H. subpunctatus</i> Lineage 1	1	3.1	2.4	—									
<i>H. sp.</i> TNHCFS 4940	1	14.7	14.8	14.1	—								
<i>H. cepedai</i>	1	14.6	14.4	14.4	3.2	—							
<i>H. picachos</i>	1	14.5	14.3	13.4	12.8	14.3	—						
<i>H. maculosus</i>	1	14.7	13.9	14.7	15.6	14.7	18.3	—					
<i>H. bocagei</i>	1	14.5	14.5	15.0	15.6	14.6	18.7	3.0	—				
<i>H. sauli</i>	1	16.0	16.1	15.4	16.5	16.4	17.8	5.6	5.7	—			
<i>H. yasuni</i>	3	14.6	14.7	14.7	16.4	16.3	17.3	10.1	10.3	10.9	<b>5.0</b>		
<i>H. italoï</i>	2	14.4	14.8	15.0	16.4	15.8	18.0	9.6	10.1	10.8	5.7	<b>4.8</b>	
<i>H. vergeli</i>	1	14.7	15.1	14.9	15.5	15.1	16.3	9.2	8.2	9.2	9.4	10.1	<b>0.1</b>

**Table S2.** Uncorrected *p* distances for the fragment of *cytb* gene (696 bp) of the *Hyloxalus* species, expressed as percentages (averages). Below the diagonal are between lineages divergences, along the diagonal and in bold are within lineage divergence.

	<i>N</i>	<i>feli</i>	<i>sub</i>	<i>sub1</i>	<i>sp.</i>	<i>cep</i>	<i>pic</i>	<i>mac</i>	<i>boc</i>	<i>sau</i>	<i>yas</i>	<i>ita</i>	<i>ver</i>
<i>H. felixcooperari</i> sp. nov.		—											
<i>H. subpunctatus</i>	1	5.6	—										
<i>H. subpunctatus</i> Lineage 1	1	7.2	7.2	—									
<i>H. sp.</i> TNHCFS 4940	1	36.7	29.2	29.2	—								
<i>H. cepedai</i>	1	34.1	31.6	26.9	1.4	—							
<i>H. picachos</i>	1	25.1	20.9	19.0	22.4	24.4	—						
<i>H. maculosus</i>	1	19.8	19.8	16.3	23.9	23.9	23.6	—					
<i>H. bocagei</i>	1	19.9	18.1	18.1	29.9	29.9	18.2	7.1	—				
<i>H. sauli</i>	1	31.8	31.8	34.2	27.5	27.5	25.6	22.7	20.4	—			
<i>H. yasuni</i>	3	29.6	29.6	26.1	28.8	28.8	21.8	18.1	16.4	21.0	<b>11.4</b>		
<i>H. italoï</i>	2	28.4	28.4	25.4	28.6	28.6	22.5	15.3	12.4	25.8	12.9	<b>13.4</b>	
<i>H. vergeli</i>	1	28.0	28.0	25.9	20.0	21.9	18.1	18.3	14.9	23.6	16.6	16.4	0

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