PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

EVOLUÇÃO DAS RÃS ANDINAS DE RIO *HYLOSCIRTUS* (ANURA: HYLIDAE): RELAÇÕES FILOGENÉTICAS, REVISÃO TAXONÔMICA E DELIMITAÇÃO DE ESPÉCIES

TESE DE DOUTORADO

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SUMARIO

Cápitulo III. A new red-eyed of stream treefrog of *Hyloscirtus* (Anura: Hylidae) from Peru, with comments on the taxonomy of the genus.

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RESUMO

A compreensão das relações evolutivas tem aumentado significativamente nos últimos anos para diferentes grupos de anfíbios neotropicais, principalmente baseada em hipóteses filogenéticas bem suportadas com evidência molecular e morfológica. Recentemente, novas classificações taxonômicas, padrões e processos de especiação, reinterpretação da evolução de caracteres fenotípicos e evidência da existência de vários táxons tradicionalmente consideradas como uma única espécie foram inferidos pelos avances filogenéticos. No entanto, só recentemente as rãs andinas associadas a riachos foram reconhecidas como uma unidade monofilética e agrupadas no gênero *Hyloscirtus*. Apesar de estudos anteriores sobre a sistemática de *Hyloscirtus,* a relações filogenéticas entre muitas de suas espécies ainda permanecem não resolvidas. Por isso aqui eu apresento cinco capítulos que fornecem a revisão mais importante na sistemática e um análise filogenética abrangente para 29 das 34 espécies usando um máximo de 6.859 pares de bases para quatro genes mitocondriais e sete genes nucleares provenientes de 157 espécimes, considerando toda a distribuição geográfica do gênero nos Andes neotropicais e América central. Usando critério da máxima parcimônia e implementação de reconstrução de homologia dinâmica, análise filogenética corroboram a monofilia de *Hyloscirtus* e recuperado como intimamente relacionado com *Aplastodiscus, Bokermannohyla* e *Hypsiboas*. Se discute a evolução de alguns caracteres fenotípicos mediante a optimização no contexto da topologia mais parcimoniosa obtidas aqui, revelando que a historia de origem independente mais complexa do previamente valorado. A partir da revisão de 1420 espécimes de 136 localidades, os resultados revelam uma diversidade de espécies antes desconhecida para *Hyloscirtus* dos quais pelo menos 15 linhagens são candidatas para novas espécies, e nove são formalmente delimitadas e descritas a partir da evidência morfológica, bioacústica e molecular. Adicionalmente um novo rearranjo taxonômico do gênero é proposto que inclui a definição de quatro grupos de espécies. As relações filogenéticas inferidas aqui para o gênero *Hyloscirtus* abrem uma janela para a compreensão da história evolutiva e diversidade dos hilídeos neotropicais.

ABSTRACT

Understanding the evolutionary relationships has increased significantly in recent years for different groups of neotropical amphibians, mainly based on well-supported phylogenetic hypotheses with molecular and morphological evidence. Recently, new taxonomic classifications, patterns and processes of speciation, reinterpretation of the evolution of phenotypic traits and evidence of various taxa traditionally regarded as a single species, were inferred by phylogenetic advances. However, only recently the Andean frogs associated with streams have been recognized as a monophyletic unit and grouped in the genus *Hyloscirtus*. Although there some previous studies on the systematics of *Hyloscirtus*, the phylogenetic relationships among many of its species still remain unresolved. Therefore here I present five chapters that provide the most important and systematic revision and comprehensive phylogenetic analysis for 29 of the 34 species using a maximum of 6,859 base pairs for four mitochondrial genes and seven nuclear genes from 157 specimens, considering the entire geographical distribution of the genus in the Neotropical Andes and Central America. Using maximum parsimony criterion and implementation of reconstruction of dynamic homology, phylogenetic analysis corroborate the monophyly of *Hyloscirtus* and recovered as closely related to *Aplastodiscus, Bokermannohyla* and *Hypsiboas*. The evolution of some phenotypic characters by in the context of the most parsimonious topology obtained here, show that the evolutionary history is more complex than previously suspected. From revision of 1420 specimens of 136 localities, the results suggest a diversity of species previously unknown to *Hyloscirtus* of which at least 15 lines are candidates for new species, and nine are formally defined and described from morphology, bioacoustics and molecular evidence. Additionally a new taxonomic rearrangement of the genus is proposed to include a definition of four species groups. The phylogenetic relationships inferred and knowledge generated here for *Hyloscirtus* open a window for understanding the evolutionary history and diversity of Neotropical tree frog.

APRESENTAÇÃO

As rãs arborícolas associadas a riachos do gênero *Hyloscirtus* constituem um elemento significativo da fauna de anuros da região andina neotropical. A distribuição geográfica se estende desde as florestas úmidas do centro da Costa Rica até os flancos do oeste e leste dos Andes na Colômbia e no Equador, os vales inter-andinos colombianos e as drenagens amazônicas de Venezuela, Colômbia, Equador, Peru e Bolívia (Frost, 2014). A distribuição altitudinal se estende desde os 300 até 3.500 m, aproximadamente; não obstante, a maior parte das espécies é restrita a regiões elevadas (Duellman et al., 1997; Coloma et al., 2012; Frost, 2014). Grande parte dos representantes habita principalmente florestas em galeria e habitats ribeirinhos, mas as espécies distribuídas em páramo podem ser encontradas esporadicamente fora das poças de água próximas aos córregos.

O gênero *Hyloscirtus* inclui 34 espécies de rãs arborícolas que se submergem em córregos (Frost, 2014; Rivera-Correa e Faivovich, 2013; Almendáriz et al., 2014), das quais o monofiletismo é suportado tanto por dados morfológicos quanto por dados moleculares (Faivovich et al., 2005; Sánchez, 2010; Wiens et al., 2010; Pyron e Wiens, 2011). No momento, estas espécies são divididas em três grupos taxonômicos: *H. armatus* (Duellman et al., 1997), *H. bogotensis* (Duellman, 1972) e *H. larinopygion* (Duellman and Hillis, 1990). O monofiletismo de cada um desses grupos está suportado principalmente por dados moleculares (Faivovich et al., 2005; Wiens et al., 2010). Sinapomorfias da morfologia das larvas suportam o monofiletismo do grupo *H. armatus* (Duellman et al., 1997; Faivovich et al., 2005; Lötters et al., 2005) e da morfologia dos adultos suportam o monofiletismo do grupo *H. bogotensis* (Duellman et al., 1972; Faivovich et al., 2005). Apesar de que uma combinação de caracteres distingue o grupo *H. larinopygion* dos outros dois grupos, não existem caracteres fenotípicos ainda reportados como sinapomorfias.

A espécie tipo do gênero é *Hyloscirtus bogotensis* (Peters, 1882). Peters (1882) descreveu esta espécie no gênero *Hylonomus*, mas posteriormente a transferiu para o gênero *Hyloscirtus* devido a homonimia primaria de *Hylonomus* Dawson, 1860. A presença de diapófises sacrais arredondadas foi o diagnostico taxonônomico para *H. bogotensis*. Lutz (1968) definiu *Hyloscirtus* (monotípico nessa época) por possuir discos pequenos, não maiores que os dígitos, e o tímpano não visível, e sugeriu que estava relacionada aos gêneros *Hyla* Laurenti, 1768 e *Aplastodiscus* Lutz, 1950. Posteriormente, *Hyloscirtus* foi transferida à sinomimia de *Hyla* por Duellman (1970) sem maiores comentários relacionados com nesta mudança.

Duellman et al. (1997) apresentou uma análise filogenética restrita aos grupos de espécies de *Hyla* distribuídos parcial ou totalmente nos Andes. Segundo os resultados de Duellman et al. (1997), os grupos de espécies *H. armata, H. bogotensis* e *H. larinopygion* formam um grupo monofilético suportado por três transformações na morfologia das larvas: disco oral grande, orientado ventralmente; papilas marginais completas; fórmula de fileiras de dentes labiais 4/6 ou mais. Esses autores sugeriram também o relacionamento dos grupos de espécies *H. armata* e *H. larinopygion* pela presença do prepollex grande sem apresentar espinho projetado em adultos machos. Não obstante, Faivovich et al. (2005) mencionaram que machos adultos do grupo *H. bogotensis* também apresentam uma morfologia do prepollex similar à dos grupos *H. armata* e *H. larinopygion*.

Faivovich et al. (2005), apresentaram uma proposta filogenética que no momento constituiu a hipótese filogenética mais completa da família Hylidae. Estes autores ressuscitaram o gênero *Hyloscirtus* para alocar as espécies tradicionalmente incluídas nos grupos *Hyla armata, Hyla bogotensis* e *Hyla larinopygion* e o diagnosticaram com 56 caracteres de genes mitocondriais e nucleares. Além disso, consideraram a presença de franjas dermais largas nos dígitos como sinapomorfias morfológicas putativas. A classificação taxonômica proposta por estes autores apresenta o gênero *Hyloscirtus* na tribo Cophomantini, subfamília Hylinae, com os seguintes relacionamentos: (*Myersiohyla* (*Hyloscirtus* (*Bokermannohyla* (*Aplastodiscus* + *Hypsiboas*)))). Wiens et al. (2005) publicaram uma análise filogenética da família Hylidae incluindo seis espécies de *Hyloscirtus* onde este gênero foi recuperado como monofilético e irmão do gênero *Boana* Wiens et al. (2005).

Sánchez (2010) estudou o desenvolvimento das larvas de *Hyloscirtus* e avaliou algumas características do disco oral, narinas, pele e tubo cloacal dos girinos. Este autor propôs uma estrutura associada ao tubo ventral que cobre os membros como sinapomorfia morfológica do gênero. Além disso, propôs dois grupos morfológicos não congruentes com os três grupos de espécies propostos por Duellman (1997) e Faivovich et al. (2005). Os grupos propostos por Sánchez foram estabelecidos com base na fórmula de fileira de dentes, a morfologia da cobertura da mandíbula superior e a forma das narinas dos girinos.

Wiens et al. (2010) e Pyron e Wiens (2011) apresentaram uma proposta das relações filogenéticas de Hylidae e Amphibia, respectivamente. Não obstante, estes autores utilizaram os mesmos dados de *Hyloscirtus* fornecidos por Faivovich et al. (2005) e Wiens et al. (2005), ambos trabalhos recuperaram a estrutura filogenética dos três grupos taxonômicos, mas com fraco suporte para o grupo *H. larinopygion* em Pyron e Wiens (2011). Coloma et al. (2012) aumentaram a amostra taxonômica para incluir 17 espécies do gênero com ênfase no grupo *H. larinopygion*, descreveram duas novas espécies, proveram descrições dos girinos e da osteologia, e discutiram tempos de divergência e distância genética entre as espécies. Recentemente, Faivovich et al. (2013) testaram o monofiletismo de *Myersiohyla,* gênero relacionado a *Hyloscirtus,* e descreveram duas novas espécies. Também, encontraram as relações de *Hyloscirtus* de acordo à inferência de máxima parcimônia proposta por Coloma et al. (2012) e discutiram alguns estados de caráter compartilhados na tribo Cophomantini.

Apesar dos esforços mencionados acima, que incluem as mais recentes contribuições recentes na sistemática do grupo *Hyloscirtus larinopygion*, a diversidade, taxonomia e filogenia de todo o gênero *Hyloscirtus* não tem sido avaliada. Sendo assim, os objetivos desta pesquisa foram:

(i) Testar o monofiletismo do gênero *Hyloscirtus*
(ii) Explorar as relações filogenéticas entre as espécies
(iii) Testar o monofiletismo dos grupos de espécies propostos por outros autores
(iv) Elucidar o número potencial de espécies novas no gênero com base em dados moleculares
(v) Descrever as espécies novas integrando distintas linhas de evidência disponível
(vi) Interpretar a evolução dos alguns caracteres fenotípicos no gênero no contexto do resultado da análise filogenética aqui apresentada

O estudo é apresentado nos seguintes cinco capítulos:

Cápitulo I. A New Species of *Hyloscirtus* (Anura: Hylidae) From Colombia, with a Rediagnosis of *Hyloscirtus larinopygion* (Duellman, 1973)

Artigo publicado na revista *Herpetologica*

Hyloscirtus larinopygion é rediagnosticada, e uma nova espécie anteriormente confundida com esta é descrita da Cordilheira Central dos Andes da Colômbia. A nova espécie se diferencia das demais do grupo de *H. larinopygion* por seu padrão de colorido, coloração da íris, e na presença de uma "pad" nupcial com projeções epidérmicas castanhas escuras. Discutimos variação e diversidade de pads nupciais, o tubérculo calcar, e alguns aspectos relacionados com a região cloacal inchada no grupo de *H. larinopygion*. Finalmente, discutimos a biologia reprodutiva e os problemas que envolvem a determinação de sinapomorfias putativas de *Hyloscirtus*.

Cápitulo II. Acoustic Signals in Andean Stream Treefrogs: Comparative Analysis of the Advertisement Calls of *Hyloscirtus antioquia* and *H. larinopygion*

Artigo com submissão prevista para a revista *Salamandra*

Descrevemos e comparamos o canto de anúncio de *Hyloscirtus antioquia* e *H. larinopygion*, duas espécies de riachos andinos e estreitamente relacionadas, pertencentes ao grupo de *H. larinopygion*. Gravamos indivíduos de sete localidades no norte da Colômbia, incluindo a localidade tipo de *H. antioquia*. O canto de anúncio consiste em sinais estereotipados de uma única nota multipulsada com 5–8 pulsos, duração de 0,122–0,276 s, e pico de frequência relativamente baixo (1642,7–1894,9 Hz). Foram encontradas diferenças significativas em as variáveis espectrais e temporais entre ambas as espécies; no entanto, nenhuma característica acústica pode ser considerada como diagnóstica. A hipótese filogenética mais completa do grupo de *H. larinopygion* sugere que os cantos múlti-pulsados são homoplásticos e evoluíram de forma independente, em pelo menos, dois eventos.

Cápitulo III. A new species of stream treefrog of the genus *Hyloscirtus* (Anura: Hylidae) from Peru, with comments on *Hyloscirtus larinopygion* species group

Artigo com submissão prevista para a revista *South American Journal of Herpetology*

Descrevemos uma nova espécie pertencente ao gênero *Hyloscirtus*, do nordeste do Peru. Esta é diagnosticada pela combinação de: presença de um espinho prepólico alargado, curvo e saliente; membros anteriores hipertrofiados; uma prega supracloacal e uma supratimpânica engrossada presente; tamanho grande; ausência de "pad" nupcial; padrão de cor e coloração da íris. Consideramos que a nova espécie pode estar estreitamente relacionada com *H. condor* e *H. tapichalaca*, do sul do Equador, por compartilharem alguns dos estados de caracteres apresentados. Além disso, discutimos alguns aspectos relacionados à sistemática do grupo de *H. larinopygion*.

Cápitulo IV. A new species of *Hyloscirtus* Peters, 1882 (Anura: Hylidae) with hypertrophied forelimbs from southeastern Ecuador

Artigo com submissão prevista para a revista *Journal of Herpetology*

Descrevemos uma nova espécie pertencente ao gênero *Hyloscirtus*, do sudeste do Equador, historicamente confundida com *Hyloscirtus lindae.* A nova espécie difere de seus congêneres pela combinação de membros anteriores hipertrofiados, presença de um alargado, curvado e saliente espinho prepólico; prega supra-cloacal grande e grossa; discos adesivos de coloração laranja-avermelhado ou vermelho-vivo, superfícies ocultas dos membros cinza-azuladas, e com espículas dorsalmente. O novo táxon é espécie irmã de *H. tapichalaca* e parte do clado sul do grupo de *H. larinopygion*, um grupo de quatro espécies restritas à região sudeste do Equador e norte do Peru. Discutimos brevemente sobre a morfologia dos braços no grupo de *H. larinopygion*

Cápitulo V. Evolution of the Stream Treefrogs *Hyloscirtus* (Anura: Hylidae): Phylogenetic Relationships, Taxonomic Revision and Species Limits

Artigo com submissão prevista para a revista *Zoological Scripta*

As rãs arborícolas associadas a riachos do gênero *Hyloscirtus*, distribuídas desde a Costa Rica até a Bolívia, constituem um elemento significativo da fauna de anuros da região dos Andes. Apesar dos estudos prévios sobre a sistemática de *Hyloscirtus* nestes últimos anos, um estudo das relações filogenéticas e uma revisão taxonômica extensiva das espécies deste gênero ainda não foram realizadas. Tendo isto em consideração, nesta tese é apresentada uma análise filogenética e uma revisão taxonômica de *Hyloscirtus* com base numa densa amostragem de espécies terminais. Os principais objetivos da tese são testar o monofiletismo do gênero, explorar as relações filogenéticas entre as espécies, testar o monofiletismo dos grupos de espécies propostos, elucidar o número potencial de espécies novas para o gênero com base em dados moleculares, descrever as possíveis novas espécies integrando diferentes linhas de evidencia disponíveis, e interpretar a evolução de caracteres fenotípicos entre as espécies no contexto dos resultados da análise filogenética. Considerando estes tópicos, eu apresento uma análise filogenética molecular que inclui 29 das 34 espécies nominais atualmente reconhecidas, usando até 6.859 pares de bases de sequencias de DNA que provém de quatro genes mitocondriais e sete genes nucleares extraídos de 157 espécimes distribuídos em sete países. Utilizando máxima parcimônia e a implementação do critério de reconstrução de homologia dinâmica, a análise filogenética revela que o gênero *Hyloscirtus* é

monofiletico no que diz respeito a outros gêneros da tribo Cophomantini e que está diagnosticado por 42 series de transformação em genes nucleares, mitocondriais e ribosomais. Além disso, o grupo de espécies *Hyloscirtus bogotensis* e várias das espécies atualmente reconhecidas para *Hyloscirtus* foram corroboradas como não monofileticas. Adicionalmente, o estado taxonômico de *Hyloscirtus denticulentus* (Duellman, 1972) foi reavaliado. Os dados moleculares revelam uma extraordinária diversidade de espécies não reportada previamente para *Hyloscirtus* dos quais pelo menos 13 linhagens são candidatas para novas espécies, destas linhagens sete são formalmente delimitadas e descritas com base em evidência fenotípica e dados de DNA. Eu proponho um novo rearranjo taxonômico do gênero que inclui a definição de quatro grupos de espécies, um deles é proposto como novo neste trabalho e todos são suportados também com dados fenotípicos. Discuto além a inesperada posição filogenética de *H. jahni.* A análise resultou em uma hipótese filogenética fortemente suportada para a maior parte dos clados propostos que provém uma base histórica para a discussão da evolução de alguns caracteres fenotípicos para o gênero *Hyloscirtus*. Caracteres morfológicos como a morfologia dos apêndices anteriores, excrescências nupciais e glândulas mentais são discutidos.

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Cápitulo I

A New Species of *Hyloscirtus* (Anura: Hylidae) From Colombia, with a Rediagnosis of *Hyloscirtus larinopygion* (Duellman, 1973)

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A NEW SPECIES OF *HYLOSCIRTUS* (ANURA: HYLIDAE) FROM COLOMBIA, WITH A REDIAGNOSIS OF *HYLOSCIRTUS LARINOPYGION* (DUELLMAN, 1973)

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ABSTRACT: *Hyloscirtus larinopygion* is rediagnosed, and a new species formerly confused with that species is described from the highlands of the Central Cordillera in the Andes Mountains of Colombia. The new species differs from the other species of the *H. larinopygion* group by its color pattern, iris coloration, and presence of a nuptial pad with dark brown epidermal projections. We discuss variation and diversity of nuptial pads, the calcar tubercle, and some aspects related to the swollen cloacal region in the *H. larinopygion* group. Finally, we discuss reproductive biology and problems involving the definition of a putative synapomorphy of *Hyloscirtus*.

KEY WORDS: Hylidae; Cophomantini; *Hyloscirtus larinopygion* species group; Systematics; Taxonomy; Andes Mountains.

THE HYLID genus *Hyloscirtus* includes 32 species (Frost, 2013) of stream-dwelling treefrogs whose monophyly is supported both by molecular and morphological data (Faivovich et al., 2005; Sánchez, 2010; Wiens et al., 2010; Pyron and Wiens, 2011). Its species are divided in three species groups, the *H. armatus, H. bogotensis*, and *H. larinopygion* groups. The monophyly of each of these groups is mostly supported by molecular data (Faivovich et al., 2005; Wiens et al., 2010), and larval morphological synapomorphies further support the monophyly of the *H. armatus* group (Duellman et al., 1997; Faivovich et al., 2005; Lötters et al., 2005).

The *Hyloscirtus larinopygion* group currently comprises 13 colorful species distributed in the Andes Mountains of Colombia and Ecuador: *H. caucanus* (Ardila-Robayo et al., 1993), *H. criptico* Coloma et al., 2012, *H. larinopygion* (Duellman, 1973), *H. lindae* (Duellman and Altig, 1978), *H. pacha* (Duellman and Hillis, 1990), *H. pantostictus* (Duellman and Berger, 1982), *H. princecharlesi* Coloma et al., 2012, *H. psarolaimus* (Duellman and Hillis, 1990), *H. ptychodactylus* (Duellman and Hillis, 1990), *H. sarampiona* (Ruiz-Carranza and Lynch, 1982), *H. staufferorum* (Duellman and Coloma, 1993), *H. tapichalaca* (Kizirian et al., 2003), and *H. tigrinus* Mueses-Cisneros and Anganoy-Criollo, 2008.

Species included in the *H. larinopygion* group have relatively restricted ranges, although *H. larinopygion* is widely distributed in the Cordillera Central and Occidental in Colombia and is present in Provincia de Carchi in northern Ecuador. While studying material identified in collections as *H. larinopygion* from the northern Cordillera Central, in the Departamento de Antioquia, Colombia, it became evident that the specimens actually correspond to a different and new species. The goals of this paper are to rediagnose *Hyloscirtus larinopygion*, describe the new species, and discuss some taxonomic characters of the *H. larinopygion* group.

MATERIALS AND METHODS

Specimens were fixed in 10% formaldehyde and stored in 70% ethanol. Measurements are those established by Duellman (1970) and Heyer et al. (1990). All measurements were taken with digital calipers and rounded to the nearest 0.1 mm. Abbreviations are SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), END (nostril–eye distance), NSD (nostril–tip-of-snout distance), IND (inter–nostril distance), AMD (distance between the anterior margins of eyes), TD (tympanum diameter), TL (tibia length), FL (foot length), TFD (third-finger diameter), and FTD (fourth-toe diameter). Webbing formulae follow the terminology of Savage and Heyer (1967) as modified by Myers and Duellman (1982). The description of nuptial pads follows the terminology of Luna et al. (2012). Sex was determined by examination of secondary sexual characters (nuptial pads, vocal slits, and expansion of the vocal sac) or, when in doubt, by examination of gonads. Color pattern in life was described from field notes and color photographs. Drawings were made using a Zeiss stereomicroscope with a drawing tube. Information on other species was taken from preserved specimens (Appendix), notes and photographs of living specimens, and literature. Institutional abbreviations used throughout this paper are MHUA-A (Museo de Herpetología, Universidad de Antioquia, Medellín, Colombia), ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia), QCAZ (Museo de Zoología, Pontificia Universidad Católica del Ecuador) and DHMECN (División de Herpetología, Museo Ecuatoriano de Ciencias Naturales).

RESULTS

Hyloscirtus **larinopygion** *(Duellman, 1973)*

(Figs. 1–3, 6)

Diagnosis.—The following characters distinguish *Hyloscirtus larinopygion* from the other species of the *H. larinopygion* group: dorsal coloration that varies from light brown to dark brown, sometimes with a dark brown reticulated pattern; white-grayish or blue-grayish flanks with black stripes, sometimes anastomosed or reticulated; iris gold or silver (see below), with black reticulation; nuptial pad large, creamy white, covering medial margin of prepollex and matacarpal II and with a distal component that partially covers the dorsal surface of the finger; broad elliptical prepollex, not modified as a projecting spine. *Hyloscirtus larinopygion* has been formerly confused with *H. psarolaimus* (Duellman and Hillis 1990), *H. criptico* Coloma et al. 2012, and the new species described below. It differs from *H. psarolaimus* in that the latter has a dull bronze iris with black reticulations (golden or silver in *H. larinopygion*), has a middorsal line in most individuals (absent in *H. larinopygion*), and lacks nuptial pads in adult males (present in *H. larinopygion*). *Hyloscirtus criptico* differs from

H. larinopygion in having small orange flecks and stippling on the dorsum and venter (absent in *H. larinopygion*), having a dark gray iris (golden or silver with black reticulation in *H. larinopygion*), and lacking nuptial pads in adult males (present in *H. larinopygion*). The new species described below differs from *H. larinopygion* in having a reddish brown dorsum with orange markings that turn to yellow toward the black flanks and are delimited with blue or pale gray outline (dorsum light brown to dark brown, sometimes with a dark brown reticulated pattern; white-grayish or blue-grayish flanks with black stripes, sometimes anastomosed or reticulated in *H. larinopygion*), iris gray with burgundy reticulation (golden or silver, with black reticulation in *H. larinopygion*), nuptial pad with dark brown epidermal projections and with a larger distal component (creamy white in *H. larinopygion*, with the distal component covering a smaller area). See Table 1 for a summary of diagnostic characters of all species of the *Hyloscirtus larinopygion* group.

Description.—The description provided by Duellman (1973) is adequate; however, it was based only on a female individual, and more specimens, including several males, have been collected in the last 20 yr. These new specimens indicate that males of this species have a large creamy white nuptial pad. It covers the medial margin of prepollex and Metacarpal II, with a distal component that partially covers the dorsal surface of the finger. Male SVL 53.6–60.5 (*n* = 10), female SVL 62.0–68.4 (*n* = 4). See below for further comments.

Remarks. – *Hyloscirtus larinopygion* was described from near the city of Popayan in the Departmento del Cauca, Cordillera Central of Colombia (see Duellman 1973). Duellman and Altig (1978) identified as *H. larinopygion* specimens from San Francisco (Departmento del Putumayo, Colombia) and Papallacta (Provincia de Napo, Ecuador). Subsequently, Duellman and Hillis (1990) described these populations as *Hyla psarolaima* (now *Hyloscirtus psarolaimus*). Ardila-Robayo et al. (1993) considered some populations from Departmento de Antioquia (i.e., localities in the municipalities of Bello, Belmira, and Sonsón), as the most-northern records of *H. larinopygion* along the Central Cordillera in Colombia; however, these populations belong to the new species described below. In addition, some populations in the provinces of Carchi and Imbabura, northern Ecuador, that were traditionally assigned to *H. larinopygion* (Duellman and Coloma, 1993; Toral et al., 2002) have recently been described as *H. criptico* by Coloma et al. (2012).

Duellman (1973) described a fold above the cloaca in the holotype (KU 144127). We find that this fold is very likely an artifact of the position of preservation (legs extended in a posterior direction; Fig. 1A); in 26 examined specimens of *H. larinopygion*, we did not observe this structure. Duellman (1973) described the iris color as being silver with fine black reticulations. However, several specimens collected along in the range of *H. larinopygion* and photographs of many living specimens (i.e., ICN, MHUA, and QCAZ specimens), did not show this color pattern, and no color slides of the type specimen are available (W. Duellman, personal observation). Nevertheless, differences in iris coloration had already been described by Mueses-Cisneros and Perdomo-Castillo (2011) in *H. tigrinus*, another species of the *H. larinopygion* group. Until more observations on iris coloration become available, we consider the iris of *H. larinopygion* to be golden or silver with black reticulation.

Coloma et al., (2012) recently presented a phylogenetic hypothesis based on mitochondrial DNA evidence (12S and 16S sequences) for most of the currently known species. Their results indicated that *Hyloscirtus larinopygion* is the sister taxon of *H. lindae* plus *H. pantostictus*. They also described the tadpole and ontogenetic changes for a population from Ecuador. Bernal et al. (2004) briefly described the advertisement call of *H. larinopygion* from Municipio de Ibagué, Departamento del Tolima, Colombia, without providing information on the number of individuals or voucher specimens.

Distribution.—*Hyloscirtus larinopygion* is the most widely distributed species of the group, and is currently known from the Cordillera Central and Occidental of Colombia to northern Ecuador, approximately between 2000 and 2800 m elevation (Fig. 2).

Hyloscirtus **antioquia** *sp. nov.*

(Figs. 2–6, Tables 1, 2)

Hyla larinopygion Ardila-Robayo et al. (1993:564; part)

Hyloscirtus larinopygion Palacio et al. (2006:72; part)

Hyloscirtus larinopygion Mueses-Cisneros and Anganoy-Criollo (2008:135, fig. 6A; part)

Holotype.—MHUA-A 7227, adult male, Colombia, Antioquia, Municipio de Bello, Serranía Las Baldías, Corregimiento de San Félix, Vereda Las Huertas, Quebrada El Hato (6°19′53.93″N, 75°38′10.10″W, 2660 m, datum = WGS84), km 5 along San Félix road—Truchera San Félix. Collected on 5 June 2010 by Mauricio Rivera, Alejandro Montoya, and Felipe Duarte (Fig. 4).

Paratypes. – All adults. MHUA-A 7228–9 (males): collected with the holotype. MHUA-A 5707 (female) and MHUA-A 5708 (male): collected at the type locality on 11 May 2008 by Laura Bravo. MHUA-A 7230–32 (males): collected at the type locality on 5 June 2012 by Mauricio Rivera, Esteban Alzate, Felipe Duarte, Diego Rivera, and Carlos Botéro. ICN 9384–86 (three adult males) and ICN 9387 (one adult female): Colombia, Antioquia, Municipio de Bello, Corregimiento de San Félix, Serranía Las Baldías, 6.6–8.1 km along San Felix road—Antenas, 2820–3100 m, collected 9 July 1981 by John Lynch and Pedro Ruiz-Carranza. ICN 9388–93, 9401 (males): Colombia, Antioquia, Municipio de Belmira, Vereda Los Patos, Quebrada Los Patos, 3.7 km N of Belmira, 2620 m, collected on 10 July 1981 by John Lynch and José V. Rueda. MHUA-A 6139 (female): Colombia, Antioquia, Municipio de Belmira, Vereda Río Arriba, Alto el Morro, finca Quebradona (6°38′17.0″N, 75°39′49.0″W, 2917 m, datum = WGS84), collected on 28 November 2008 by Alejandro Montoya. MHUA-A 7449–50, 7453, 7568 (males) and MHUA-A 7451–52 (females): Colombia, Antioquia, Municipio de Belmira, Vereda Montañitas, Reserva de Corantioquia (6°36′49.0″N, 75°39′13.10″W, 2820–2875 m, datum = WGS84), collected during 9–18 June 2012 by Esteban Alzate, Felipe Duarte and Laura Pinto. MHUA-A 2645, 2647 (males) and MHUA–A 2646 (female): Colombia, Antioquia, Municipio de Envigado, Loma El Escobero, Parque Ecólogico San Sebastian – La Castellana (6°08′2.32″N, 75°30′39.90″W, 2500 m, datum = WGS84), collected on 21 June 2003 by Lucas Barrientos. MHUA-A 4140 (male): same locality, collected on 3 December 2005 by Mauricio Arias. MHUA-A 1707—08 (males) and MHUA-A 1709, 1716 (females): Colombia, Antioquia, Municipio de Santa Rosa de Osos, Llanos de Cuivá (6°36′7.75″N, 75°18′13.83″W; 2700 m, datum = WGS84), collected 13–16 October 2001 by Paul Gutiérrez. ICN 9399 (male): Colombia, Antioquia, Municipio de Sonsón, 12 km E Sonsón, 2560 m, collected on 10 July 1981 by John Lynch and José V. Rueda. MHUA-A 6138 (male): Colombia, Antioquia, Municipio de Sonsón, Vereda San Francisco, Páramo las Palomas trail, 2920 m, collected on 22 November 2008 by Juan Pablo Hurtado. MHUA-A 6012, 6014 (males): Colombia, Antioquia, Municipio de Yarumal, Corregimiento Llanos de Cuivá, Vereda San Antionio del Rio, finca Los Lagos (6°51′7.0″N, 75°29′28″W, 2660 m, datum = WGS84), collected on 19 July 2008 by Felipe Duarte.

Diagnosis.— *Hyloscirtus antioquia* can be distinguished from the other 13 species of the *H. larinopygion* group by the following characters: reddish brown dorsum with a rough reticular pattern of orange markings that turn to yellow towards the black flanks and are delimited with blue or pale gray outlines; thighs with yellow and black stripes; discs grayish black dorsally and bluish gray ventrally, and iris gray with burgundy reticulation; large nuptial pad with dark brown epidermal projections; and broad elliptical prepollex that is not modified as a projecting spine.

Hyloscirtus antioquia has been previously confused with *H. larinopygion*, but differs from the latter in color pattern (dorsum light brown to dark brown, white-grayish or blue-grayish flanks with black stripes in *H. larinopygion*, see above for details), iris coloration (iris golden or silver, with black reticulations in *H. larinopygion*), and morphology and color of the nuptial pad (creamy white nuptial pad, and a smaller distal component in *H. larinopygion*; see Fig. 6). *Hyloscirtus lindae* and *H. pantostictus* also have a gray iris but without reticulation (dull bluish gray with minute black flecks in *H. lindae* and gray without flecks *H. pantostictus*) and both species have orange digital discs; *H. pantostictus* also has orange spots all over the body (absent in *H. antioquia*). The broad elliptical prepollex, not modified as a projecting spine, distinguishes *H. antioquia* from *H. tapichalaca* (prepollex modified as a projecting spine). See Table 1 for a summary of some diagnostic characters of all species of the *Hyloscirtus larinopygion* group.

Description of holotype.—Adult male of 56.6 mm SVL (Fig. 4). Body robust. Head barely wider than long; head as wide as body; head width 37% of SVL; head length 35% of SVL. Snout rounded in dorsal view and truncate in profile (Fig. 5A–B); canthus rostralis rounded, indistinct; loreal region concave; lips rounded, not flared; nostrils slightly protuberant, directed anterolaterally, slightly posterior to anterior margin of lower jaw. Internarial region and top of head flat. Interorbital distance slightly shorter than upper eyelid. Eye prominent; its diameter larger than eye–nostril distance. Tympanum and tympanic annulus visible, rounded and inclined medially towards the longitudinal body axis, such that almost the complete tympanum is visible from above; its diameter equals 51% eye diameter. Supratympanic fold prominent, starting behind the eye and extending to the anterior margin of the insertion of the arm, slightly covering the dorsal margin of tympanum.

Region between head and suprascapulae quite depressed. Vomerine teeth in two large, transverse series abutting medially, posterior to the choanae; each series is slightly S-shaped, and bears 15 teeth. Choanae small, ovoid, separated by a minimum distance larger than 3.5 times their maximum diameter. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin). Vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac not evident externally, single, median and subgular. Mental gland absent.

Forearm robust; axillary membrane absent; low ulnar tubercles present. Fingers short, thick and bearing large, ovoid discs, with circumferential groove of each disc clearly defined by the size difference between the disc and the smaller pad; width of disc on Finger III is 13% greater than the tympanum diameter. Relative lengths of fingers: 1 < 2 < 4 < 3. Fingers with wide dermal fringes, webbed basally; webbing formula: I–II 2–3 III $2^{3/4}$–$2^{1/2}$ IV (Fig. 5C). Subarticular distal tubercles large, single, rounded, and conic; distal tubercles on Finger IV globular. Large supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, thick, elliptical. Broad elliptical prepollex, not modified as a spine. Nuptial pad large, covering medial margin of prepollex and Metacarpal II; with a distal component that partially covers the dorsal surface of the finger; with dark brown epidermal projections. Hind limbs robust; tibia length 53% SVL; foot length 46% SVL. Small pyramidal calcar tubercle present; tarsal fold absent; small tubercles along the outer margin of the tarsus, starting in the tibio-tarsal articulation and reaching the base of outer metatarsal tubercle. Inner metatarsal tubercle large, ovoid; outer metatarsal tubercle small, round. Toes short, with lateral fringes, bearing discs smaller than those on fingers. Relative length of toes: 1 < 2 < 3 = 5 < 4; toe webbing formula: I 2–2^{+} II $1^{1/3}$–$2^{1/3}$ III $1^{2/3}$–$2^{2/3}$ IV $2^{2/3}$–$1^{1/2}$ V (Fig. 5D). Subarticular tubercles large, round, and conical; single rows of smaller supernumerary tubercles along the axis of every toe. Cloacal opening directed posteroventrally, at midlevel of thighs; no supracloacal flap; margins of vent with numerous small folds; two large, swollen glandular areas at proximal posterior thighs; cloacal sheath short. Dorsal skin, gular region, and pectoral region smooth; flanks, belly, and proximal ventral region of thighs granular. Pectoral fold absent.

Color in life of the holotype.—Dorsal surfaces dull reddish brown with a conspicuous reticular pattern of orange markings that become yellow toward the black flanks. Anterior and posterior surfaces of thighs and shanks black with yellow bars thinly outlined in blue. Dorsal surfaces of fingers and toes dirty gray with some black horizontal bars; discs dark gray dorsally and bluish gray ventrally. Gular region brown. Ventral surfaces dirty gray in medial area; ventral surfaces of hands, feet, and webbing dirty gray. Pericloacal region grayish brown. Iris gray with burgundy reticulation.

Color in preservative of the holotype.—Dorsal surfaces brown with gray reticulated markings; flanks black with gray blotches. Anterior and posterior surfaces of thighs and shanks black with light gray bars. Fingers and toes dirty gray with some black horizontal bars; discs dark gray dorsally. Gular region, belly, and ventral surfaces of hands, feet, webbing, and pericloacal region dirty gray.

Measurements of the holotype (in mm).—SVL 56.6; HL 19.8; HW 21.3; ED 6.7; END 4.5; NSD 2.6; IND 4.7; AMD 11.2; TD 2.8; TL 29.8; FL 26.3; TFD 3.7; FTD 3.2.

Variation and sexual dimorphism.—Measurements are given in Table 2. The number, size, and shape of lateral markings varies among individuals; hue varies as well, with some individuals almost orange. One specimen has irregular lichen-like orange markings on the dorsum (ICN 18597), whereas some have orange vertical bars on the thighs (ICN 9384, 9390, 9392; MHUA-A 7228, 7230). Dark gray or black markings occasionally occur on the belly (ICN 9390). Calcar tubercle more developed in some individuals (ICN 9387, 18597; MHUA-A 2647) than others. Vomerine teeth series in contact medially (ICN 9389, 9392) or with an obvious gap (ICN 9384, 9387, 9390). Number of vomerine teeth 14–18/12–20. In some individuals, the series are slightly S-shaped. The toe-webbing formula varies as follows: I (2–2^{+})–(2–2^{+}) II ($1^{1/2}$–$1^{1/3}$)–($2^{1/2}$–$2^{1/3}$) III ($1^{1/2}$–$1^{2/3}$)–($2^{2/3}$ – 3^{-}) IV ($2^{2/3}$ –3)–($1^{1/2}$ –$1^{2/3}$) V. Females are larger than males (male SVL 53.4–58.0, *n* = 20; female SVL 58.6–63.4, *n* = 6), the forearm is more slender, and secondary sexual characters (nuptial pad, vocal slits, vocal sac) are absent. The ovarian oocytes in a female (MHUA-A 5707) are unpigmented and have a diameter of 2.5–3.0 mm (*n* = 30).

Distribution and natural history.—The new species is known from eight localities north of the Cordillera Central of Departmento de Antioquia, Colombia (Fig. 2). *Hyloscirtus antioquia* inhabits cloud forests of high mountains and subpáramo, 2500–3200 m a.s.l. It occurs in streams or small puddles formed by flowing water and seems to prefer small trees and Common Bamboo, *Chusquea* sp. Individuals have been observed perched less than 3 m high. Male acoustic activity begins at the beginning of the night and decreases toward early morning. The call consists of low-pitched notes that resemble the stridulation of crickets, and along the noise of the stream it is sometimes difficult to hear vocalizing males. Some individuals have been observed resting among bracts of *Espeletia* sp. and different species of terrestrial bromeliads in the forest, distant from streams. Amplexus and egg clutches remain unknown. Adults often release a bitter white secretion and strong smell; there is a change of color from reddish brown to a gray during handling.

Remarks.—We assigned the new species to the genus *Hyloscirtus* on the basis of the wide dermal fringes on fingers and toes (Faivovich et al., 2005), the sole adult morphological synapomorphy so far identified for the genus. There are no known morphological synapomorphies for the *Hyloscirtus larinopygion* species group, and we assign the new species tentatively to this group on the basis of its similarity with *H. larinopygion*.

Etymology.—The specific epithet is in allusion to the Departmento de Antioquia, Colombia, where this new species is distributed. Although the origin of the word *Antioquia* is unclear, it is considered by some historians to be an Amerindian word meaning "mountain of gold". This species is named after one of the most diverse regions of amphibians in the neotropics and in recognition of the Grupo Herpetológico de Antioquia for their contribution to the knowledge of the herpetofauna of the region. The name is used here as a noun in apposition.

DISCUSSION

The description of *H. antioquia* raises to 14 the number of known species in the *H. larinopygion* group. The colorful patterns of most of these frogs have historically biased the diagnoses of species toward coloration characters, and there has been little or no discussion about other morphological characters, such as nuptial pads, calcar tubercles, and swollen cloacal region.

Nuptial pads in the *H. larinopygion* group are known to occur in *H. sarampiona* (Ruiz-Carranza and Lynch, 1982)*, H. caucanus, H. larinopygion, H. lindae* (Ardila-Robayo et al., 1993), *H. staufferorum* (Duellman and Coloma, 1993), *H. tigrinus* (Mueses-Cisneros and Anganoy-Criollo, 2008; Mueses-Cisneros and Perdomo-Castillo 2011), *H. princescharlesi* (Coloma et al., 2012), *H. pantostictus* (M. Rivera-Correa, personal observation), and *H. antioquia* sp. nov. (this paper). Nuptial pads have been reported to be absent in *H. pacha, H., psarolaimus*, *H. ptychodactylus* (Duellman and Hillis, 1990), *H. criptico* (Coloma et al., 2012), and *H. tapichalaca* (Kizirian et al., 2003).

A notable difference between the nuptial pads of *H. larinopygion* and *H. antioquia* is that in the latter the nuptial pad is covered with dark brown epidermal projections, whereas it is creamy white in *H. larinopygion* (Fig. 6). In both cases, the shape of the pad is similar: a medial component that expands distally and covers dorsally a portion of the digit, although the latter is notably broader in *H. antioquia* than in *H. larinopygion*. These differences are constant in 28 males of *H. antioquia* and 20 of *H. larinopygion*. Our study of five male specimens of *H. tigrinus* (including two paratypes) failed to find the nuptial pads reported by Mueses-Cisneros and Anganoy-Criollo (2008) and Mueses-Cisneros and Perdomo-Castillo (2011).

Hyloscirtus tigrinus was defined mainly based on the coloration and the presence of a well-developed fleshy calcar tubercle (Mueses-Cisneros and Anganoy-Criollo, 2008). This character state is present only in the holotype (adult female). The males of the type series and two additional specimens studied (including one living individual from Balsa de los Sapos, PUCE, Ecuador) have a fleshy calcar tubercle that is poorly developed (Fig. 7A). The calcar tubercle has been used in diagnoses of species in the *H. larinopygion* group; however, we found that this structure is polymorphic (Fig. 7B–E) and that its prominence is easily exacerbated in dehydrated individuals. Therefore, descriptions of size and shape of the calcar tubercle in species of the *H. larinopygion* group must be considered carefully.

The specific name *larinopygion* comes from the swollen cloacal region, unusual in other known neotropical hylids (Duellman, 1973). This swelling occurs because of a series of large flat tubercles or bumps lateral and ventral to the cloacal opening. In spite of the putative uniqueness of the cloacal region in *H. larinopygion* (Duellman, 1973), and subsequent descriptions noticing the presence of glandular swellings of the cloaca, these swellings were not included in early characterizations of the *H. larinopygion* group (Duellman and Hillis, 1990; Duellman et al., 1997). This character should be properly defined, and its taxonomic distribution studied in the other species groups of *Hyloscirtus*. Interestingly, in *H. tapichalaca*, these tubercles are associated with a chevron-shaped white blotch that is conspicuous in a presumed antipredator posture (Kizirian et al., 2003), which so far is unknown in other species of the group.

Mental Glands and Reproductive Biology of Hyloscirtus

Mental glands are cutaneous secretory structures apparently involved in reproductive activities such as courtship and mating, and have been previously reported in the *Hyloscirtus bogotensis* and *H. armatus* species groups (Duellman, 1972; Faivovich and De la Riva, 2006). Additionally, mental glands have been observed in other species of the tribe Cophomantini, such as the *Hypsiboas benitezi* species group (Faivovich et al., 2006), *H. cinerascens* and *H. punctatus* of the *H. punctatus* species group (Hoogmoed, 1979; Brunetti et al., 2012), *H. heilprini,* a species of the *H. albopunctatus* group (Trueb and Tyler, 1974), and more recently in *Bokermannohyla* (Faivovich et al., 2009). However, mental glands are not present in species of the *Hyloscirtus larinopygion* group, suggesting that this character state might have reversed in this clade. The reproductive biology of *Hyloscirtus* is largely unknown, and only a few observations are available for *H. platydactylus* (La Marca, 1985), which is part of the *H. bogotensis* group. As more information becomes available, it may become possible to assess whether the absence of mental glands in the *H. larinopygion* group is related to changes in reproductive behavior in comparison with the other species groups of *Hyloscirtus*.

Wide Dermal Fringes or Thick Digits?

Faivovich et al. (2005) suggested that wide dermal fringes are a putative synapomorphy of *Hyloscirtus*. Coloma et al. (2012) stated that lateral fringes are absent on the fingers in *H. criptico* and *H. princecharlesi*. However, their figures 7B,C and 12B,C show the typical "thick" fingers and toes of species of *Hyloscirtus*, where the discs are barely wider than the digits. There seems to be little variation in *Hyloscirtus* in this regard (the two species of the *H. armatus* group being the exception; see Faivovich et al., 2005). However, for what we consider to be similar morphologies, different authors have referred to as dermal fringes (e.g., Duellman and Altig, 1978; Ruiz-Carranza and Lynch 1982; Ardila-Robayo et al., 1993; Mueses-Cisneros and Angonoy-Criollo, 2008), explicitly mentioned their absence (Duellman and Coloma, 1993; Coloma et al., 2012), or omitted any mention of them (Duellman, 1972; Duellman, 1973; Duellman and Berger, 1982; Duellman and Hillis, 1990). Kizirian et al. (2003), for example, described the fingers of *H. tapichalaca* as lacking lateral fringes but being broad. We believe that these differences stem more from different perceptions on how fringes should be defined (see Grant et al., 2006, for extensive discussion), and different mechanical styles of descriptions, than from actual morphological differences.

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APPENDIX

Specimens Examined

Hyloscirtus caucanus*:* COLOMBIA, Cauca, Páez, Hacienda Montenegro, Km. 34.5 Belalcazar–Tacueyo, 2400 m., ICN 7071 (holotype), 7002, 7056, 7072–76, 7238, 7241–48, 7250–52, 7055, 7239, 7253.

Hyloscirtus criptico*:* ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2760–2885 m., QCAZ 43516–18, 43528 (paratypes).

Hyloscirtus larinopygion*:* COLOMBIA, Caldas, Villa Maria, Km. 7 Villa Maria–Mariquita, ICN 34433; Caldas, Pensilvania, Km. 24 Pensilvania–Arboleda, 2000 m., ICN 36518–19; Cauca, Popayan, Santa Teresa stream, 2200 m., KU 144127 (holotype); Huila, Belalcazar, Parque Nacional Natural Nevado del Huila, 2900 m., ICN 41880; Quindío, Salento, Hacienda La Caleria, 2300 m., ICN 15626–27; Risaralda, Pereira, Parque Regional Ucumarí, Las Delicias stream, 2340 m., ICN 36133–38, 34970–72; Tolima, Ibague, Juntas, El Silencio, 2600–2820 m., ICN 9380–82, 9670; Valle del Cauca, El Cairo, Las Amarillas, 2140–2200 m., ICN 28926, 28828–29, 42779; Valle del Cauca, Farallones de Cali, Campamento Corea, 2600 m., ICN 13595. ECUADOR, Carchi, Espejo, Morán, 2452 m., QCAZ 41826.

Hyloscirtus lindae: ECUADOR, Napo, Papallacta, 2.660 m., KU 164402 (holotype), 155476 (paratype); Pacto Sumaco, Parque Nacional Sumaco, Refugio La Laguna, 2476–2775 m., QCAZ 41232, 41294–98, 45345–47.

Hyloscirtus pacha: ECUADOR, Morona Santiago, Plan de Milagro, 2350 m., KU 202762 (holotype); Plan de Milagro, Km 8 Plan de Milagro–Cuenca, 2150–2300 m., QCAZ 48237–41.

Hyloscirtus pantostictus: ECUADOR, Sucumbios, km 5 Santa Bárbara–La Bonita, 2650 m., KU 190000 (holotype); Santa Barbara, 2590–2800 m., QCAZ 10488,11660–67, 12171, 12174, 14084, 30529–31, 38421, 40331, 41393, 41412, 42350, 45434–38, 45443–44, 45446, 45450–53.

Hyloscirtus princecharlesi: ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2720–2794 m., QCAZ 43654, 44893 (paratypes).

Hyloscirtus psarolaimus: ECUADOR, Napo Papallacta, Km 11 Papallacta–Baeza, 2660 m., KU 164313 (holotype), QCAZ 13252, 23070; Carchi, Tulcán–Santa Bárbara road, QCAZ 15366; Morona Santiago, San Vicente, Parque Nacional Sangay, 15 Km to road Lagunas de Atillo, 2815 m., QCAZ 31671; Sucumbios, La Sofía, Campamento Río Verde, 2726 m., DHMECN 6493–94.

Hyloscirtus ptychodactylus: ECUADOR, Cotopaxi, Pilalo, 2320 m., KU 209780 (holotype).

Hyloscirtus sarampiona: COLOMBIA, Cauca, Parque Nacional Natural Munchique, Sopladero stream, 33 Km., 2190 m., ICN 7440 (holotype), 7441 (paratype).

Hyloscirtus staufferorum: ECUADOR, Napo, 27 Km N Jondachi, 2040 m., KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m., QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m., QCAZ 45962–63, 45965–67.

Hyloscirtus tapichalaca: ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m., QCAZ 15083–85, 16704–06, 17776–77 (paratypes).

Hyloscirtus tigrinus: COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m., ICN 53804 (holotype), ICN 53805–06 (paratypes).

TABLE 1.—Comparison of some diagnostic characters in species of the *Hyloscirtus larinopygion* group. Besides the characters shown, *H. tapichalaca* differs from all other species in the group for having the prepollex modified as a spine, and absence of the omosternum (Kizirian et al., 2003). Sources: (1) Ardila-Robayo et al. (1993); (2) Coloma et al. (2012); (3) Duellman (1973); (4) Duellman and Altig (1978); (5) Duellman and Berger (1982); (6) Duellman and Hillis (1990); (7) Duellman and Coloma (1993); (8) Kizirian et al. (2003); (9) Mueses-Cisneros and Anganoy-Criollo (2008); (10) Mueses-Cisneros and Perdomo-Castillo (2011); (11) Ruiz-Carranza and Lynch (1982); (*) this study.

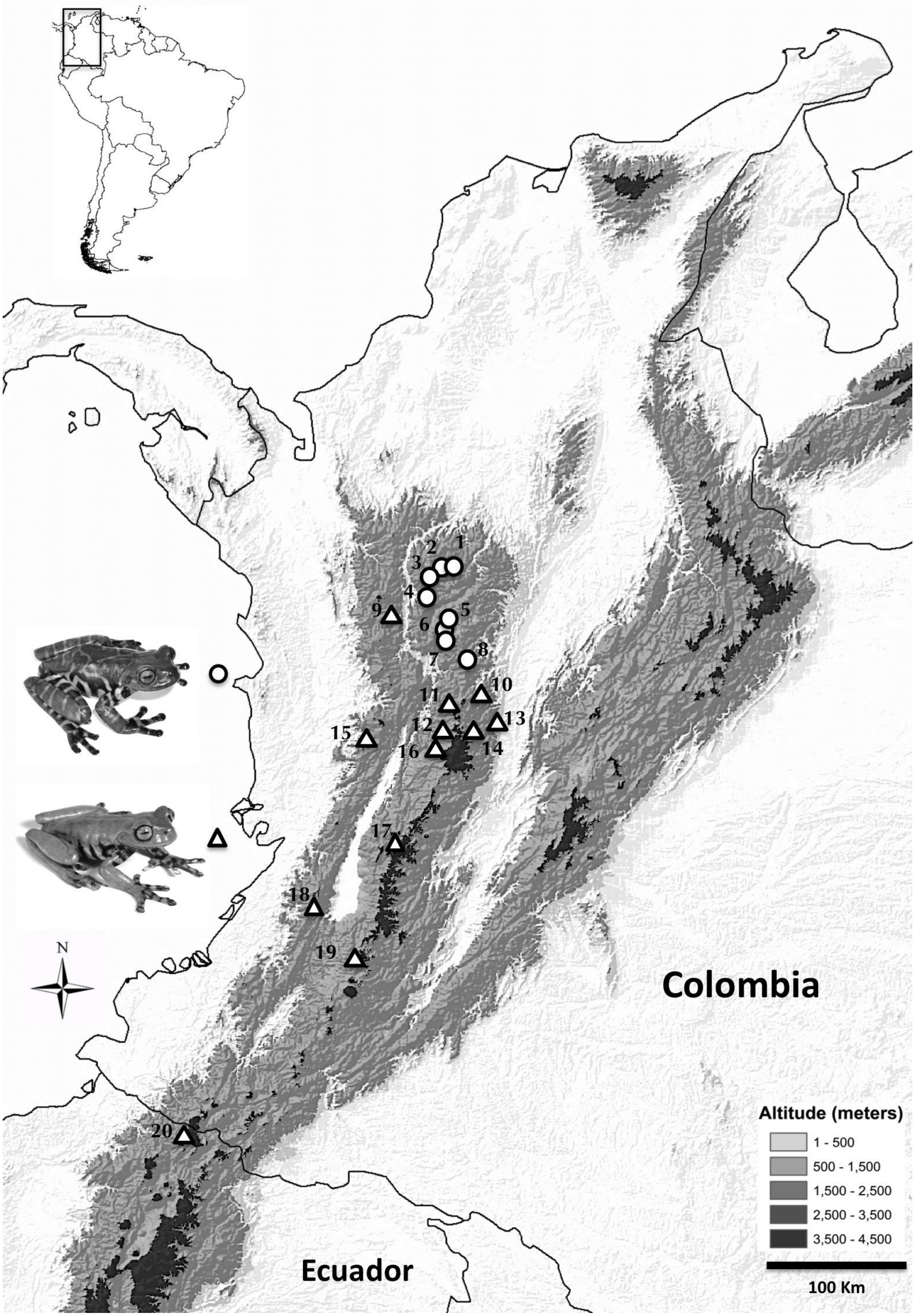
Species	Dorsal pattern	Flanks	Iris color	Vomerine teeth series	Nuptial pad	Calcar tubercle	Ulnar tubercles	Source
H. antioquia	Reddish brown; with or without scattered orange blotches	Gray or black with yellow markings delimited with blue or pale gray outline	Gray with burgundy reticulations	In contact or not, 12–20 teeth/each	Medial and distal component; dark brown epidermal projections	Present	Present rounded	* (1)
H. caucanus	Brown or reddish brown with dark brown scattered blotches; with or without middorsal stripe	Black or brown with orange spots and thick black stripes with tiny white dots	Pale yellow with brown reticulations	In contact, 10–14 teeth/each	Distal component only; creamy white	Present or Absent	Present, acute, white pigmented	* (1)
H. criptico	Grayish brown, densely stippled with minute cream flecks and bright orange blotches	Black with white and brown markings with tiny orange blotches	Dark gray	In contact or not, 8–15 teeth/each	Absent	Present	Absent	* (2)
H. larinopygion	Light brown to dark brown; with or without dark brown reticulation	White or light bluish gray with black vertical bars or blotches	Golden or silver with black reticulations	In contact or not, 8–15 teeth/each	Medial and distal component; creamy white	Present or Absent	Present rounded or acute	* (3)
H. lindae	Dark metallic brown	Dark metallic brown and dirty gray	Dull bluish gray with minute black flecks	In contact, 11–13 teeth/each	Distal component; creamy white	Present or Absent	Present rounded or absent	* (2, 4)
H. pacha	Dark brown with metallic orange flecks	Brown with creamy white blotches	Olive brown	In contact, 11–15 teeth/each	Absent	Present	Present rounded or absent	* (2, 6)
H. pantostictus	Olive-brown with small orange spots	Olive-brown with small orange spots	Dark gray	In contact or not, 10–14 teeth/each	Medial and distal component, light brown epidermal projections	Present or Absent	Present rounded	* (2, 4)
H. princecharlesi	Black with many round-oval orange blotches	Black with large round orange blotches	Dark gray	In contact or not, 11–15 teeth/each	Medial and distal component; creamy white	Absent	Absent	* (2)
H. psarolaimus	Grayish brown with dark brown and minute cream flecks; with or without middorsal stripe	Cream with dark brown spots	Dull bronze with black reticulation	In contact, 10–18 teeth/each	Absent	Present	Present rounded or absent	* (2, 6)
H. ptychodactylus	Orange-tan to reddish brown with minute orange-tan flecks enclosed in black markings; with or without middorsal stripe	Uniform black or with reddish brown extensions of the ground dorsal color	Pale blue	In contact, 11–15 teeth/each	Absent	Present	Present	* (2, 6)

H. sarampiona	Pale olive with orange spots	Black	Gold with thin black reticulation	In contact or not, 8–16 teeth/each	Medial and distal component; dark brown epidermal projections	Present or Absent	Present, rounded or acute	* (11)
H. staufferorum	Uniform dark brown	Uniform dark with white bars	Metallic brown	In contact, 16–25 teeth/each	Creamy white	Present	Present acute or absent	* (2, 7)
H. tapichalaca	Gray or dark brown	Gray brown with diffuse pink orange marks more conspicuous in axillary and inguinal regions	Yellow-gold with black reticulation	No contact, 4–6 teeth/each	Absent	Present	Absent	* (8)
H. tigrinus	Yellow-green or yellowish brown with transverse black stripes, sometimes reticulated; with or without middorsal stripe	Yellow-green with black reticulation	Light gray or yellow with black reticulation	In contact, 10–15 teeth/each	Absent	Present	Absent	* (9, 10)

TABLE 2.—Measurements (in mm) of the type series of *Hyloscirtus antioquia* sp. nov. See text for abbreviations.

	Males (*n* = 20)		Females (*n* = 6)	
Measurement	Mean ± SD	Range	Mean ± SD	Range
SVL	55.7 ± 1.64	53.4–58.0	61.0 ± 2.30	58.6–63.4
HL	18.1 ± 0.66	17.0–19.8	19.2 ± 0.97	18.1–20.8
HW	19.4 ± 1.01	18.0–21.3	20.5 ± 0.97	18.9–22.3
ED	6.3 ± 0.56	5.3–7.4	6.2 ± 0.54	5.3–6.7
END	4.9 ± 0.52	4.2–6.3	5.1 ± 0.58	4.6–5.9
NSD	2.6 ± 0.17	2.2–2.9	2.7 ± 0.42	2.3–3.4
IND	4.7 ± 0.43	3.9–5.3	4.8 ± 0.43	4.2–5.2
AMD	10.7 ± 0.6	9.5–12.0	11.6 ± 0.40	10.5–12.3
TD	2.9 ± 0.3	2.2–3.5	3.2 ± 0.35	2.9–3.7
TL	28.2 ± 1.2	25.4–30.5	30.9 ± 1.17	29.5–33.0
FL	24.6 ± 1.10	22.4–26.3	27.6 ± 0.97	25.9–29.3
TFD	3.0 ± 0.36	2.5–3.7	3.5 ± 0.30	3.1–4.0
FTD	2.7 ± 0.32	2.3–3.2	2.9 ± 0.29	2.6–3.4

FIG 1. – Holotype of *Hyloscirtus larinopygion,* KU 144127, SVL 53.6 mm; (A) dorsal view; (B) ventral view; (C) lateral view. The arrow indicates an artifactual fold above the cloaca. Photo: M. Bustamante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19
20
N
Colombia
Ecuador
Altitude (meters)
1 - 500
500 - 1,500
1,500 - 2,500
2,500 - 3,500
3,500 - 4,500
100 Km

FIG 2. – Geographic distribution of *Hyloscirtus antioquia* sp. nov. (circles), and *H. larinopygion* (triangles). *Hyloscirtus antioquia*: (1) San Antonio del Río, Yarumal; (2) Llanos de Cuivá, Santa Rosa de Osos; (3) Páramo de Belmira, Belmira; (4) Serranía Las Baldías, Bello (holotype locality); (5) Santa Elena, Medellín; (6) Loma El Escobero, Envigado; (7) Alto de San Miguel, Caldas; (8) Páramo de Sonsón, Sonsón. *Hyloscirtus larinopygion*: (9) Páramo de Urrao, Antioquia; (10) Pensilvania, Caldas; (11) Villamaría, Caldas; (12) Ucumari, Risaralda; (13) Murillo, Tolima; (14) Juntas, Tolima; (15) Serranía de los Paraguas, Chocó-Valle del Cauca; (16) Salento, Quindío (17) Parque Natural Las Hermosas, Tolima-Valle del Cauca; (18) Parque Natural Farallones de Cali, Valle del Cauca; (19) Popayan, Cauca (type locality); (20) Carchi, Ecuador.

FIG 3. – (A) *Hyloscirtus antioquia* sp. nov. in life (MHUA-A 7230, paratype, adult male, SVL 53.4 mm); (B) *H. larinopygion* in life (QCAZ 41826, adult male, SVL 55.2 mm). Photos: M Rivera-Correa and S. Ron.

FIG 4. – Dorsal and ventral view of the holotype of *Hyloscirtus antioquia* sp. nov., MHUA-A 7227; SVL 56.6 mm.

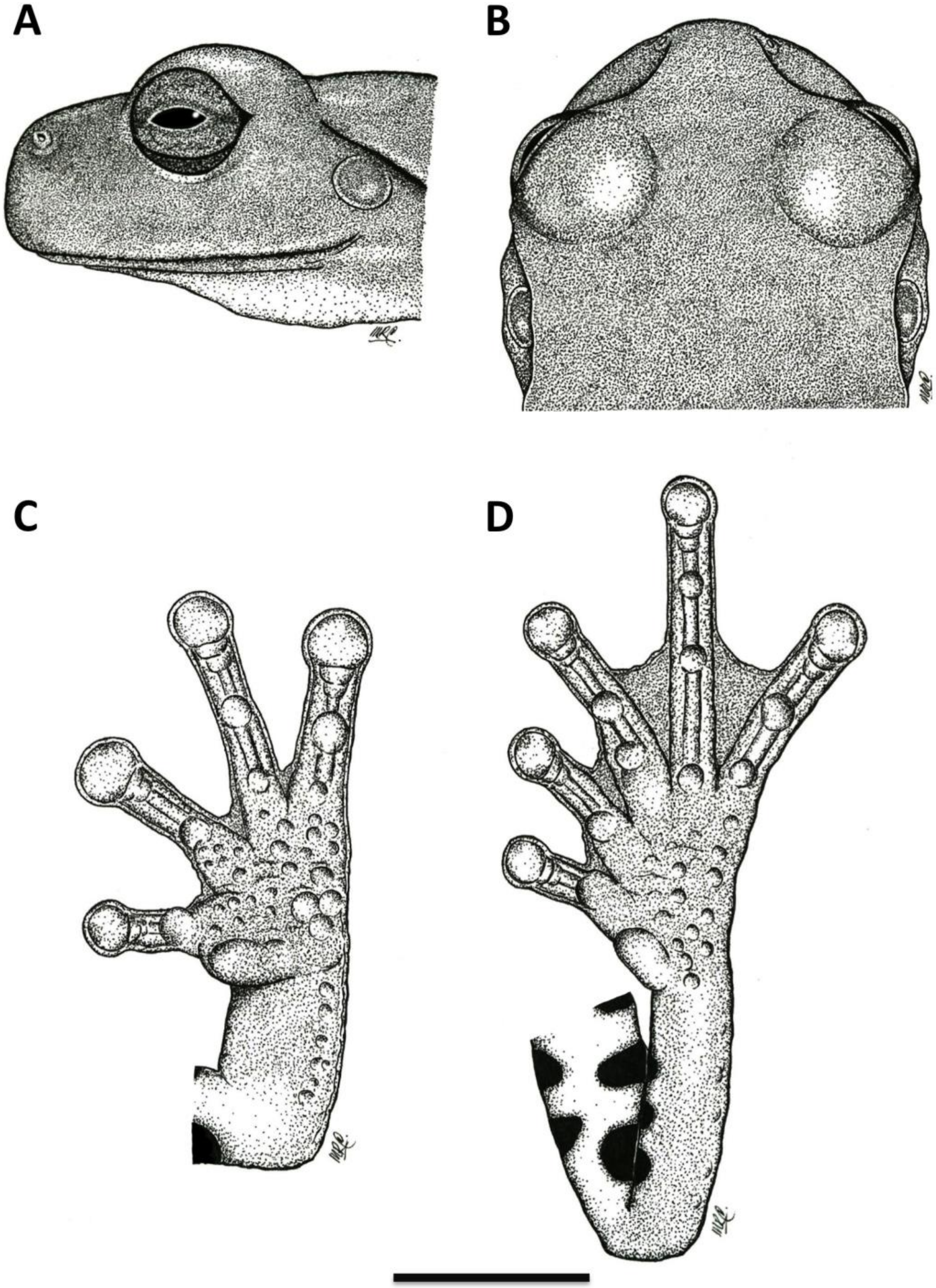

FIG 5. – Holotype of *Hyloscirtus antioquia* sp. nov., MHUA-A 7227. (A) Head in lateral view (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bar = 10 mm.

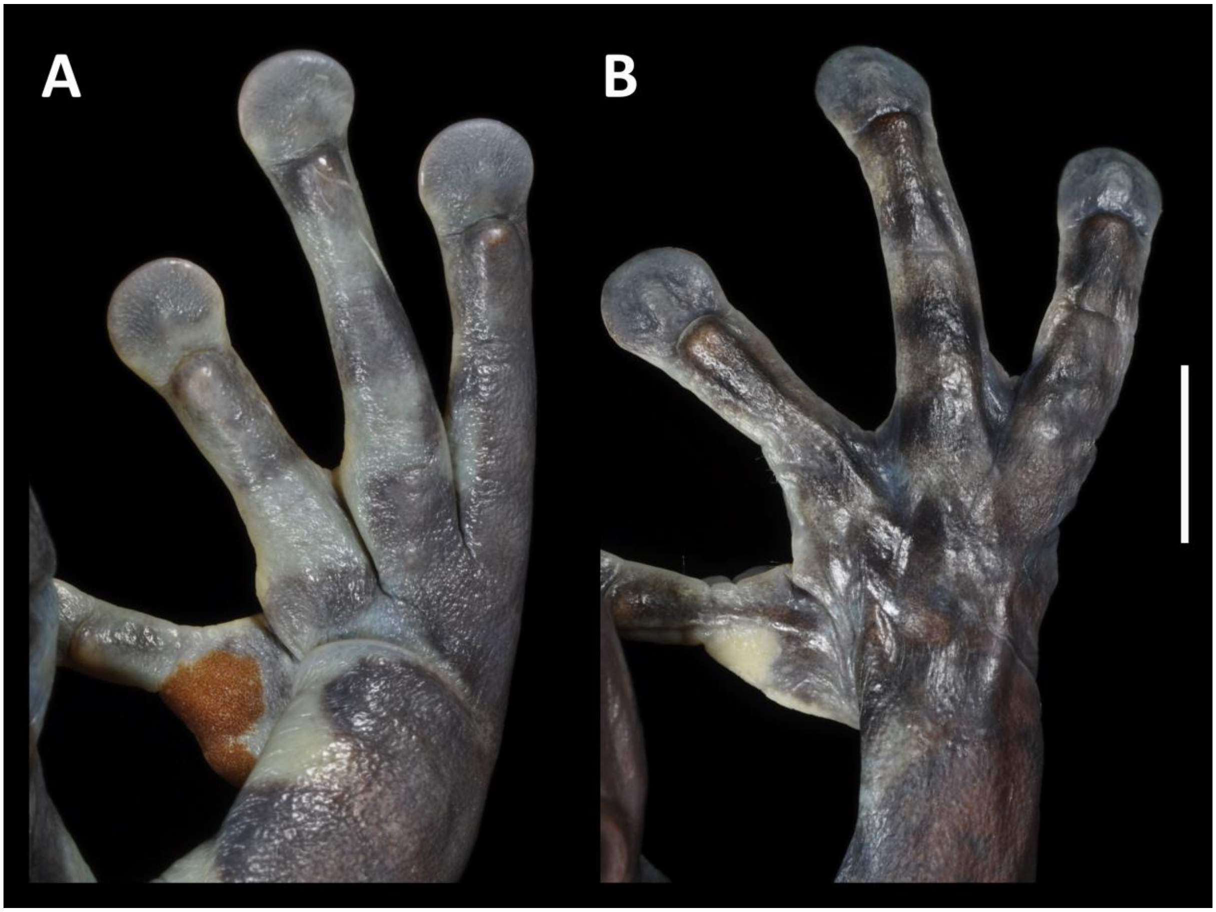

FIG 6. – Dorsal view of right nuptial pads in males of (A) *Hyloscirtus antioquia* (ICN 9390); (B) *H. larinopygion* (TG 2150). Scale bar = 5 mm.

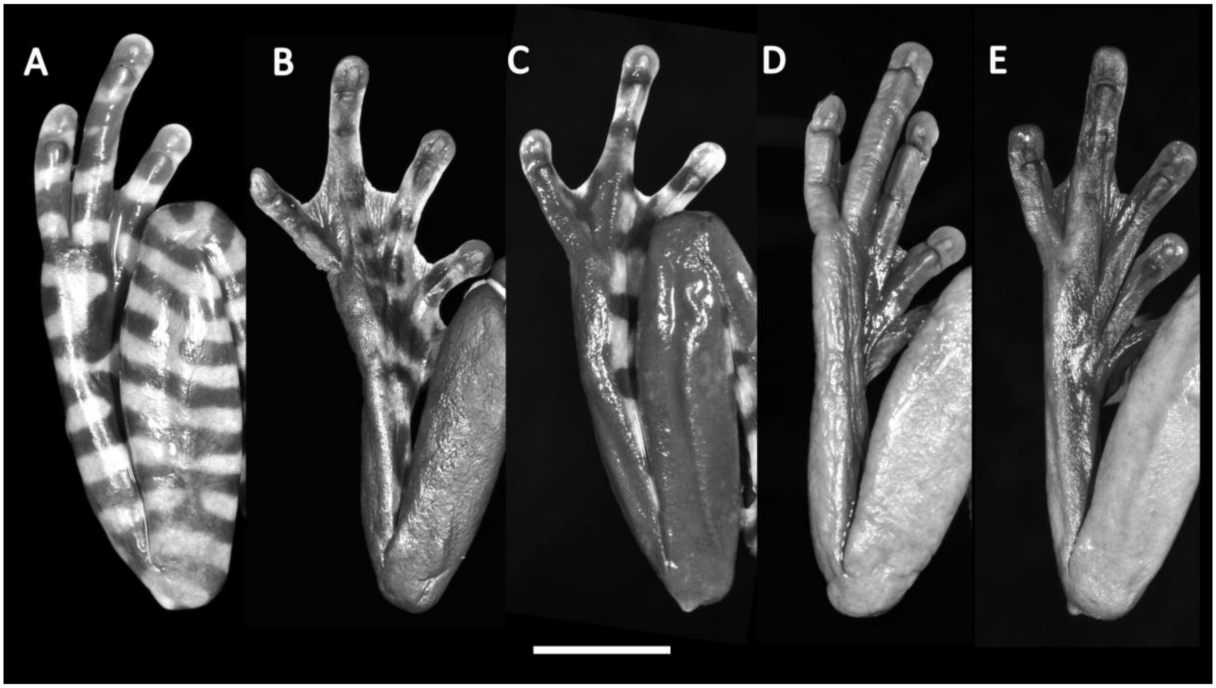

FIG 7. – Dorsal view of left hindlimb of (A) *Hyloscirtus tigrinus* (ICN 53806); (B, C) *H. larinopygion* (TG 2150 and MRC 575); (D, E) *H. sarampiona* (JMR 2431 and JMR 2434). Note the poor development of calcar tubercle in *H. tigrinus* and variation in different individuals of *H. larinopygion* and *H. sarampiona.* Scale bar = 10 mm.

Cápitulo II

Statistical differences and biological implications: A comparative analysis of the advertisement calls of two Andean stream treefrogs (Hylidae: *Hyloscirtus*) and the evolution of acoustic characters

Accepted in *Salamandra*

Statistical differences and biological implications:
A comparative analysis of the advertisement calls of two Andean stream treefrogs (Hylidae: *Hyloscirtus*) and the evolution of acoustic characters

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Suggested running head: Advertisement calls in *Hyloscirtus larinopygion* grou

Abstract

We describe and compare the advertisement call of *Hyloscirtus antioquia* and *H. larinopygion*, two sibling species of the Andean stream treefrogs of the *Hyloscirtus larinopygion* group. We recorded individuals from seven localities in Colombia, including the type locality of *H. antioquia.* The advertisement call of both species consists of a single, low-pitched mulitipulsed note, with some overlap of frequency and duration of pulses (dominant frequency 1642.7–1756.5 Hz and 5–6 periodic pulses in *H. antioquia* and 1722.7–1894.90 Hz and 5–6 periodic pulses in *H. larinopygion*). We found significant statistical differences in spectral and temporal variables between both species; however, no acoustic trait can be considered diagnostic. Our results highlight an important topic in evolutionary biology that deserves a thorough examination in Andean anurans, not all statistically significant differences in auditory signals do matter in male recognition and speciation. Furthermore, in the context of the most comprehensive phylogenetic hypothesis available for *Hyloscirtus*, the optimization of acoustical characters suggests that call structure has a complex history involving multiple transformations.

Keywords

Behaviour, bioacoustics, calls parameters, Colombia, *Hyloscirtus larinopygion* group.

Introduction

Most anurans employ acoustic communication for territorial dominium and reproduction.

Advertisement calls are acoustic signals usually emitted by male anurans to attract females and fend off other males (RYAN 1980, DUELLMAN & TRUEB 1994), often they vary interspecifically and sometimes in same species, making them important sources of characters for species diagnosis (COCROFT & RYAN 1995, GERHARDT & HUBER 2002, PADIAL et al. 2008) and phylogenetic inference (DUELLMAN 2007, SCHNEIDER & SINSCH 2007). The vocalisations also play an important role in comparative behavioural studies with anurans as model system (BOSCH & BOYERO 2003, ROBILLARD et al. 2006, WELLS 2007, WOLLENBERG et al. 2007, BOTH & GRANT 2012).

Hyloscirtus antioquia RIVERA-CORREA & FAIVOVICH 2013 is a colourful, stream treefrog (Fig. 1a) allied to *H. larinopygion* (DUELLMAN 1973) (Fig. 1b), with which it was confused previously (DUELLMAN & HILLIS 1990, ARDILA-ROBAYO et al. 1993, RIVERA-CORREA & FAIVOVICH 2013). *Hyloscirtus antioquia* and *H. larinopygion* are externally distinguishable by their colour patterns and the shape and keratin colour of the nuptial pads of adult males (RIVERA-CORREA & FAIVOVICH 2013). Both species are referred to the *H. larinopygion* group, and a recent phylogenetic analysis suggests they are sister species (BRUNETTI et al. 2015). *Hyloscirtus antioquia* is restricted to the northern parts of the Cordillera Central of Colombia, whereas *H. larinopygion* is widely distributed in the Cordillera Central and Occidental of Colombia and northern Ecuador (COLOMA et al. 2012, RIVERA-CORREA & FAIVOVICH 2013). Sympatry of *H. antioquia* and *H. larinopygion* is currently unknown, and the highlands of the Andes in southern of Departamento de Antioquia (up to 3300 m in Páramo de Sonsón) seem to act as a geographic barrier between them.

The advertisement call of *H. antioquia* has not been described, and although the advertisement call of *H. larinopygion* was described briefly by BERNAL et al. (2004), few quantitative data were provided. Thus, the objectives of this study are to 1) describe for the first time the advertisement call of *H. antioquia*, 2) provide a more detailed description of the advertisement call of *H. larinopygion*, and 3) compare the advertisement calls of these two closely related species and discuss patterns of the evolution of advertisement call structure among species of the *H. larinopygion* species group.

Materials and methods

We recorded advertisement calls during eight field trips between February 2010 and December 2012 (Table 1; Fig. 1c). Recordings were made with a digital recorder (Sony PCM-D50) connected to a directional microphone (Sennheiser K6/ME66). Recordings were made at a distance of 1.0–2.2 m. from the calling male. In total, we analysed 234 calls obtained from six males of *H. antioquia* (106 calls) and six males of *H. larinopygion* (128 calls), recorded at several localities (Fig. 1c; Table 1). After each recording, we measured the male's body size (snout–vent length, SVL) to 0.1 mm with digital callipers (Mitutoyo) and air temperature with a digital thermometer (Casio Pathfinder Triple Sensor) (Table 1). Specimens collected and voucher recordings are deposited in Museo de Herpetología Universidad de Antioquia (MHUA), Medellín, Colombia. Additional calls were obtained from the audio files recorded by the late P. M. Ruiz-Carranza to *H. antioquia* and deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (See ICN Voucher in Table 1). All recordings were analysed using Raven Pro 1.5 software for Mac OS X (Bioacoustics Research Program 2013), with Hann's sampling window, FFT window size of 256 points, sampling rate of 44.1 kHz, 16-bit precision, and overlap of 90%. Call parameter definitions follow MCLISTER et al. (1995), COCROFT & RYAN (1995) and GERHARDT & HUBER (2002). Following these authors, in this study we consider the dominant call frequency to be the frequency with the greatest amount of acoustic energy and a note as the sound unit produced by a single expiratory event of the frog.

We report numerical parameters as mean ± SD and the respective range (Table 2). The following temporal parameters were measured from oscillograms: call duration (= note duration), number of pulses per call, and pulse duration. We used power spectra to calculate the dominant call frequency (= fundamental frequency), and frequency bandwidth (the difference between the 5% and 95% frequencies, hereafter low frequency and high frequency). We compared body size and each temporal and spectral acoustic trait between *H. antioquia* and *H. larinopygion* using resampling statistical tests (BLANK 2011). In addition, we reduced redundancy of acoustic traits measured by conducting a principal component analysis (PCA) with Varimax-rotation. We tested possible relationships between air temperature and acoustic trait values by conducting linear regression on PCs. Finally, we used a PCA plot to visualize advertisement call divergence between species. Graphs of spectrogram, waveform and power spectrum were obtained with Seewave 1.6, R 2.15.1 package. Seewave settings, window name (Fourier transform window) = Hann; window length = 256 samples; overlap = 90%.

Results

Hyloscirtus antioquia and *H. larinopygion* were found inside gallery forests alongside streams. Acoustic activity started at dusk and decreased toward early morning (18:00–03:00, approximately). Calling males of both species were observed vocalizing from shrubs, low vegetation (< 2 m directly over slow-flowing streams) and common bamboo (*Chusquea* sp). The advertisement call of both species is a low-pitched and pulsed single note that resembles the sound produced by crickets through stridulation; this pulsed note call is audible to the human ear as melodious “brrrrrr”. The signal is degraded and the pulses are not always distinguishable at a distance of ca. 20 m. The amplitude modulation of each note consists of ascending intensity in the first half of the call and decreasing in the second half (Fig. 2). Pulses are a rhombus-shaped (see oscillogram), with the third and fourth pulses containing the greatest energy. The fundamental frequency coincides with the dominant frequency.

The advertisement call of *H. antioquia* consists of a single, multipulsed note of 5–6 periodic pulses (Figs 2a–c), with a slow and gradual rise of frequency throughout the call. Call duration was 0.155 ± 0.022 s (0.122–0.181 s) with call interval duration of 1.673 ± 0.357 s (1.055–2.687 s). The advertisement call of *H. larinopygion* consists of a single, multipulsed note of 6–8 periodic pulses (Figs 2d–f), with a slow gradual rise of the frequency from through the call. Call duration was 0.197 ± 0.016 s (0.137–0.276 s) with interval duration of 1.079 ± 0.294 s (0.763–2.596 s). Advertisement calls in *H. antioquia* were emitted at a rate of 23–35 calls/minute (mean = 28 ± 4.12), while those of *H. larinopygion* were emitted at a rate 20–48 calls/minute (mean = 34 ± 13.23). A summary of quantitative call traits of both species is provided in Table 2.

Male SVL was significantly greater (resampling test P= 0.006) in *H. larinopygion* than *H. antioquia*, although the mean difference between species was only approximately 1.68 mm SVL. All temporal and spectral characteristics of calls were statistically significantly different (Table 2; Figs 3a–b). The variation in the measured call traits was successfully summarized by two principal components (Table 3); PC1 was mainly correlated with call frequency while the PC2 with call duration and pulse number/call. Temperature and spectral call traits presented a marginal positive relationship (β= 1.616, P= 0.056). We did not remove this possible effect of temperature from our comparative analysis because (1) the predictive power on spectral trait variation by temperature was relatively low (R^2= 0.32), (2) our results (Fig. 3c) did not change when we used residuals of the temperature-PC1 linear regression analysis instead of the original

PC1 values (data not shown), and (3) the air temperatures of recordings for the two species did not differ (resampling test P = 0.422).

Discussion

The spectral and temporal call traits were significantly different between *H. antioquia* and *H. larinopygion* (Table 2). However, despite statistical differences in recorded calls, these differences are too small to clearly diagnose the two species; similar results have been found in other Neotropical anurans (e.g., TSUJI-NISHIKIDO et al. 2012). Consequently, at present *H. antioquia* and *H. larinopygion* can only by diagnosed using morphological (RIVERA-CORREA & FAIVOVICH 2013), and molecular traits (RIVERA-CORREA unpublished data). Call trait overlap among closely related species of *Hyloscirtus* has been found previously (COLOMA et al. 2012) and in other anuran species, it is associated with allopatric distributions (DUELLMAN & PYLES 1983, COCROFT et al. 1990, KWET et al. 2005, PEREYRA et al. 2012, WEN et al. 2012) such that pre-mating isolation mechanisms are not required to prevent hybridization. From this perspective, our results highlight an important topic in evolutionary biology that deserve more study in Andean anurans: not all statistically significant differences in auditory signals do matter in male recognition and speciation.

The higher call frequency in *H. larinopygion* than in *H. antioquia* is not attributable to differences in body size or ambient temperature. In anurans an inverse relationship between body size and dominant call frequency is common (GERHARDT & HUBER 2002, VARGAS-SALINAS & AMÉZQUITA 2014); however, males of *H. larinopygion* recorded in our study were larger (albeit slightly) than males of *H. antioquia*, yet the frequency of their calls was higher. Similarly, in some species it has been found that dominant call frequency is positively related with ambient temperature, but temperatures did not differ between our recordings of the two species; moreover, accumulated evidence suggests the relationship between spectral call traits and temperature is weak or non-existent in many anuran species (GERHARDT 1978, RYAN 1987, GERHARDT & HUBERT 2002).

In addition to *H. antioquia* and *H. larinopygion* (BERNAL et al. 2004, this study), the advertisement calls of eight species of the *H. larinopygion* group have been described: *H. condor, H. criptico, H. lindae, H. pacha, H. pantostictus, H. psarolaimus, H. staufferorum* and *H. tapichalaca* (KIZIRIAN et al. 2003, COLOMA et al. 2012, ALMENDÁRIZ et al. 2014). These calls all consist of a single note of short duration (133.0–542.0 ms; COLOMA et al. 2012, ALMENDÁRIZ et al. 2014, this study) and a broad band-pulse or multiple short pulses. Although ALMENDÁRIZ et al. (2014) described the call of *H. condor* as pulsed, our re-analysis of their audio-recordings shows that the structure of calls is not pulsed (Fig. 4; see also their figure 9).

Vocalizations can be informative for phylogenetic inferences (e.g., PÄCKERT et al. 2003, ROBILLARD et al. 2006, WOLLENBERG et al. 2007), although the reduced number of discrete characters in acoustic signals usually hinders their utility. Nevertheless, characterization of the advertisement call structure, and studies on the acoustical behaviour, can provide useful insights on the evolution of phenotypic characters of the group and it can contribute to the establishment of the phylogenetic relationships among species (see GOICOECHEA et al. 2009). Based on current knowledge of advertisement call structure and the most comprehensive phylogenetic hypothesis available for *Hyloscirtus* (see ALMENDÁRIZ et al. 2014, BRUNETTI et al. 2015), the optimization of acoustical characters indicates that call structure has a complex history involving multiple transformations. The evidence indicates that the unpulsed call in *Hyloscirtus* is plesiomorphic and was replaced by multiple short pulses at least three times in the genus (Fig.

4), including at least two independent origins in the *H. larinopygion* species group: one in *H. psarolaimus* and another in the clade containing *H. antioquia, H. larinopygion, H. lindae* and *H. panstostictus* (Fig. 4). Information on the advertisement calls of the remaining five species of the group (*H. caucanus, H. princecharlesi, H. ptychodactylus, H. sarampiona* and *H. tigrinus*) and *H. charazani* in the *H. armatus* group are necessary to better understand the evolution of acoustic signals in these riparian species.

In contrast to *H. staufferorum*, whose advertisement calls can be heard from up to 500 m distance (COLOMA et al., 2012), *H. antioquia* and *H. larinopygion* calls are audible only at short distances (less than 50 m; M. Rivera-Correa and T. Grant pers. obs.). Moreover, locating vocalizing individuals of these species, even at close proximity (i.e. < 2 m), is extremely difficult. This could be due to signal masking by the stream background noise and/or scattering of signals by vegetation (GERHARDT & HUBER 2002, WELLS 2007).

Finally, environmental noise and habitat structural complexity can be important selective forces modulating signal evolution in species with acoustic communication (WILEY & RICHARDS 1982, PATRICELLI & BLICKLEY 2006, FENG et al. 2006, VARGAS-SALINAS & AMÉZQUITA 2013); however, the evolutionary pressures acting on the advertisement calls in *Hyloscirtus* are unstudied. The advertisement call in this clade is a candidate model for future studies addressing the environmental factors that affect call structure and we hope the information provided herein will help inform and motivate further research on this topic.

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Table 1. Call recording localities of the specimens of *Hyloscirtus antioquia* and *H. larinopygion*. Abbreviations used: Ind (Nº of individuals); T. Air (Air temperature); NV (unvouchered specimen); Ant (Departamento de Antioquia); Cal (Departamento de Caldas); Ris (Departamento de Risaralda); Tol (Departamento de Tolima); * Type locality

Species	**Locality**	**Ind**	**Calls**	**T. Air (°C)**	**Acronym**
H. antioquia	Serranía las Baldías*, Bello (Ant); 2600 m	4	76	15.6–16.2	MHUA-A 7227–9
	S.Sebastian-La Castellana, Envigado (Ant); 2500 m	1	14	15.7	MHUA-A 5707
	Páramo de Sonsón, Sonsón, (Ant); 2800 m	1	16	16.0	ICN 18597
H. larinopygion	Cuchilla Jardín-Támesis, Jardín, (Ant); 2600 m	1	50	16.0	NV
	Villamaría, (Cal); 2620 m	1	18	16.2	GGD022
	Ucumarí, Pereira, (Ris); 2500 m	2	40	16.4	MHUA-A 9423 MRC 575
	Cajamarca, (Tol); 2600 m	2	20	15.8	NV

Table 2. Summary and results of statistical comparisons of call traits (mean ± standard deviation, range) between *Hyloscirtus antioquia* and *H. larinopygion*. N = Sample size.

Call variables	***H. antioquia***	**N**	***H. larinopygion***	**N**	**Resampling test**
Body size (mm)	54.8±0.392 (53.8–56.6)	6	56.5±0.286 (56.4–57.2)	6	P= 0.006
Call duration (s)	0.155±0.022 (0.15–0.16)	106	0.197±0.016 (0.16–0.25)	128	P= 0.011
No. pulses/call	5.515±0.069 (5.29–5.75)	654/106	7.130±0.394 (6.0–8.0)	816/128	P= 0.001
Pulse duration (s)	0.016±0.0002 (0.0158–0.0176)	492	0.018±0.0002 (0.0172–0.0188)	612	P= 0.002
Low frequency (Hz)	1497.6±22.387 (1464.2–1593.8)	106	1659.4±34.915 (1550.4–1722.7)	128	P= 0.003
Dominant frequency (Hz)	1680.7±18.592 (1642.7–1756.5)	106	1834.2±35.394 (1722.7–1894.90)	128	P= 0.003
High frequency (Hz)	1783.3±34.541 (1722.7–1919.4)	106	2008.9±36.084 (1894.9–2067.2)	128	P= 0.004

Table 3. Results of a principal component analysis summarizing variation in the advertisement calls of *Hyloscirtus antioquia* and *H. larinopygion*. The highest loadings for each principal component are > 0.9.

	Principal component	
Call variables	**PC1**	**PC2**
Dominant frequency (Hz)	0.982	0.130
Low frequency (Hz)	0.986	0.128
High frequency (Hz)	0.961	0.249
Call duration (s)	0.090	0.969
Pulse duration (s)	0.618	0.586
Pulse number/call	0.208	0.963
Eigenvalue	4.044	1.555
% of variance explained	54.893	38.434

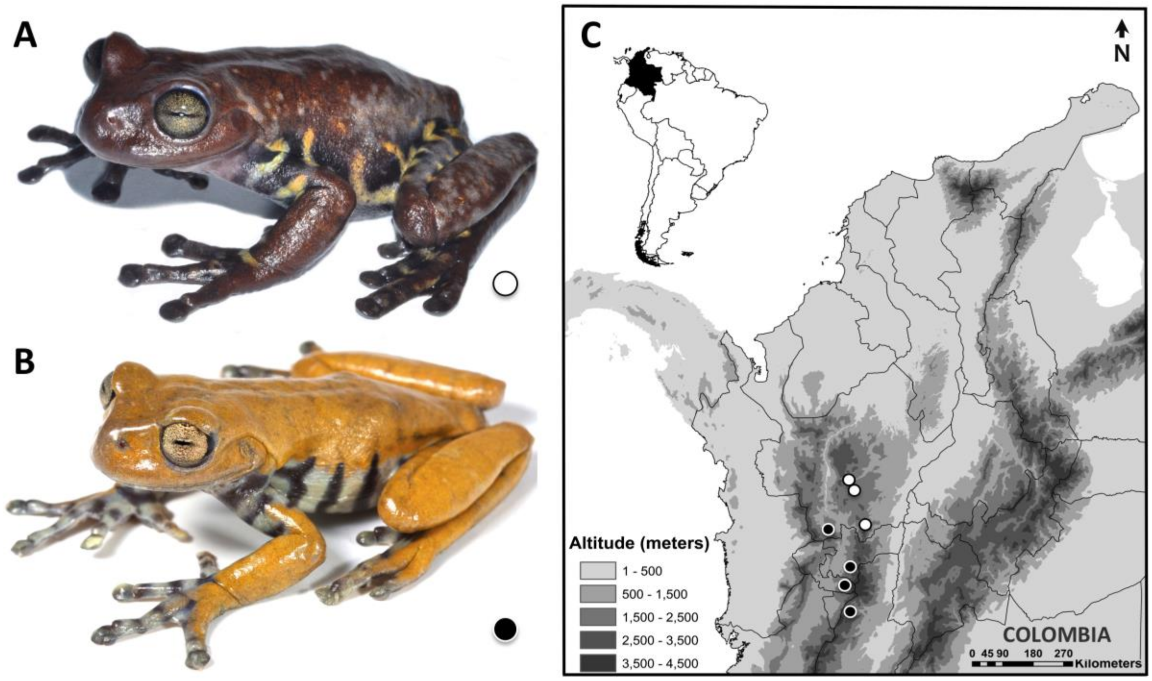

Figure 1. (A) *Hyloscirtus antioquia* in life (MHUA-A 8962, adult male, SVL 54.3 mm; photo: MRC); (B) *H. larinopygion* in life (QCAZ 41826, adult male, SVL 55.2 mm; photo: S. Ron); (C) Map showing the localities of the recorded specimens. White dots: *Hyloscirtus antioquia*; black dots: *H. larinopygion*.

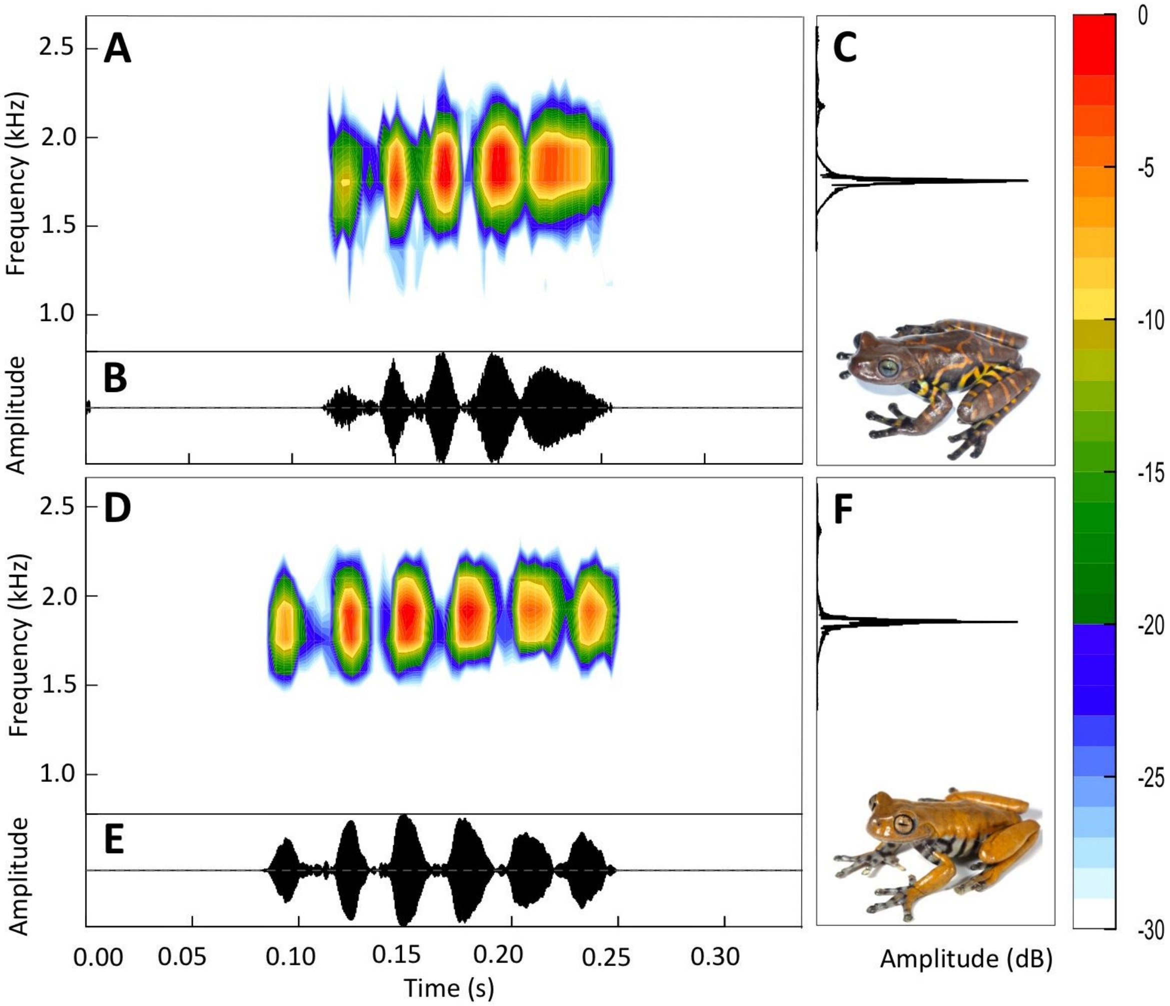

Figure 2. Advertisement call of two recorded specimens of *Hyloscirtus larinopygion* species group. *Hyloscirtus antioquia* (MHUA-A 7229): (A) Spectrogram, (B) oscillogram, (C) power spectrum. *Hyloscirtus larinopygion* (MHUA-A 9423): (D) Spectrogram, (E) oscillogram, (F) power spectrum.

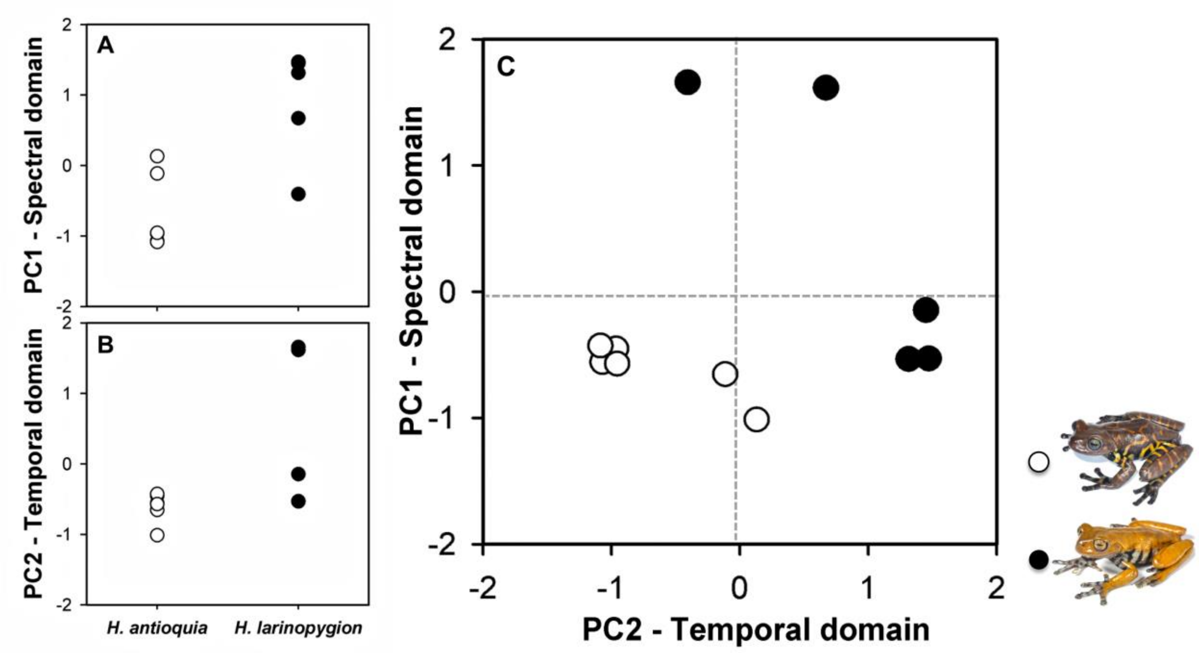

Figure 3. Differences in spectral (A), temporal (B), and both (C) advertisement call traits between *Hyloscirtus antioquia* (white dots) and *H larinopygion* (black dots). The first principal component (PC1) represents call frequency whereas the second principal component (PC2) represents call duration (see also Table 2 for values). N = 6 for both species; some dots are superimposed.

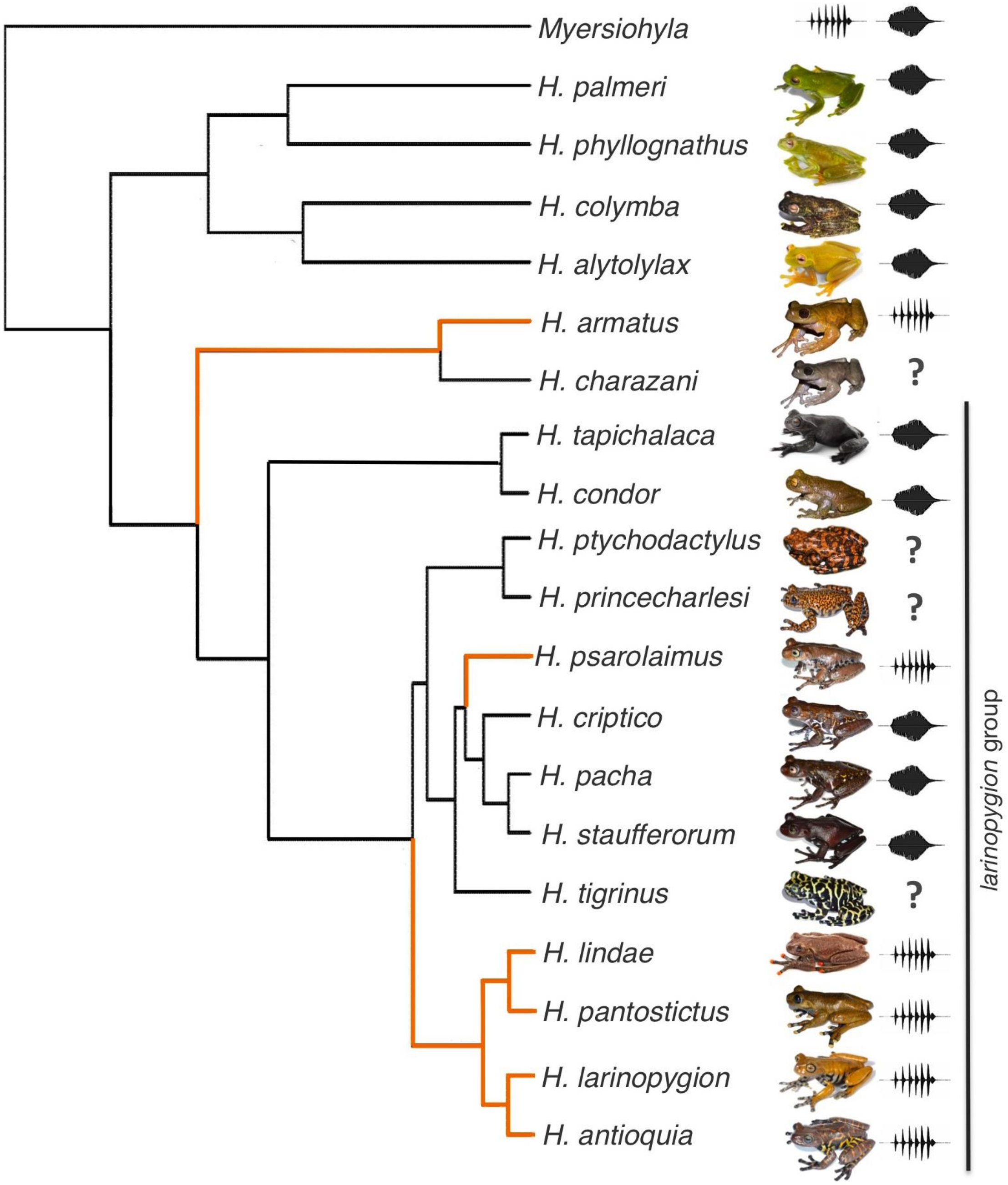

Figure 4. Phylogenetic relationship of the *Hyloscirtus larinopygion* species group, redrawn from ALMENDÁRIZ et al. (2014) and BRUNETTI et al. (2015) showing the distribution of unpulsed (black branches) and pulsed (orange branches) advertisement call structure. The advertisement call of some species of the group and phylogenetic position of *H. caucanus* and *H. sarampiona* are currently unknown (see text)

Cápitulo III

A new red-eyed of stream treefrog of *Hyloscirtus* (Anura: Hylidae) from Peru, with comments on the taxonomy of the genus.

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A new red-eyed of stream treefrog of *Hyloscirtus* (Anura: Hylidae) from Peru, with comments on the taxonomy of the genus.

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Abstract.

We describe a remarkable new species of monophyletic genus *Hyloscirtus* from northeastern Peru. The presence of an enlarged, curved, and protruding prepollical spine; hypertrophied forelimbs; large, thick supracloacal flap and supratympanic fold; large size; nuptial pad absent and iris dark red diagnose this species. We hypothesize that the new species is closely related to *H. condor* and *H. tapichalaca* of southern Ecuador due to the apparently synapomorphic occurrence in these species of an enlarged, curved prepollical spine, and small, conical vomerine odontophore processes with 3–6 prominent teeth each without contact between these. Additional research explicitly incorporating *H. diabolus* new species in a phylogenetic analysis is required to further test our hypothesis and provide a better understanding of the evolution of morphological attributes described herein. Finally we discuss some aspects concerning the taxonomy of the genus *Hyloscirtus.*

Key words. Andes Mountains; Hylidae; *Hyloscirtus larinopygion* species group; Morphology; Systematics; Taxonomy.

Introduction.

Recent contributions (Faivovich et al. 2005; Coloma et al. 2012; Rivera-Correa and Faivovich 2013; Almendáriz et al. 2014; Guayasamin et al. 2015) significant increased knowledge of the diversity, taxonomy, and phylogeny of the Neotropical genus *Hyloscirtus* Peters 1882, a monophyletic group with remarkable morphological variation and whose species are confined strictly to Andean creeks and streams (Faivovich et al. 2005; Coloma et al. 2012; Guayasamin et al. 2015). Among species of *Hyloscirtus*, the occurrence of an enlarged, curved prepollical spine is extremely rare. Until recently, *H. tapichalaca* (Kizirian et al. 2003) was the only species of the genus to exhibit this morphology. Recently, Almendáriz et al. (2014) also reported this morphology in *H. condor* Almendáriz et al. 2014, and to date these are the only two species known to possess it. Another striking attribute shared by these two species and the species of the *H. armatus* species group are conspicuously hypertophied forelimbs in adult males (see Duellman et al. 1997). In this paper we describe the third species of the *H. larinopygion* species group with a prepollical spine and hypertrophied forelimbs from remote localities in northeastern Peru. We also discuss some aspects of the morphology of these species and its systematic implications for the *H. larinopygion* species group and some aspects concerning the taxonomy of the genus *Hyloscirtus.*

Materials and Methods.

Specimens were fixed in 10% formalin and stored in 70% ethanol. All measurements were taken with digital calipers with the aid of a dissecting microscope and rounded to the nearest 0.1

mm. Measurements are those established by Duellman (1970) and Heyer et al. (1990) and modified by Rivera-Correa and Faivovich (2013). Abbreviations are SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), END (nostril–eye distance), NSD (nostril–tip-of-snout distance), IND (inter–nostril distance), AMD (distance between the anterior margins of eyes), TD (tympanum diameter), FAL (forearm length), FAB (forearm breadth), HAL (hand length), THL (thigh length), TL (tibia length), TAL (tarsus length), FL (foot length), TFD (considering the diameter of the disc of Finger III), and FTD (considering the diameter of the disc of Toe IV). Webbing formulae follow the terminology of Savage and Heyer (1967), as modified by Myers and Duellman (1982). Sex was determined by examination of secondary sexual characters (prepollical spine, vocal slits, and expanded vocal sac). Color pattern in life was described from color photos. Illustrations were made using a Zeiss stereomicroscope with a drawing tube. Information on other species was obtained from preserved specimens (listed in Appendix I), field notes, photographs of living specimens, and the literature. Institutional abbreviations used throughout this paper are CORBIDI (División de Herpetología, Centro de Ornitología y Biodiversidad), MUSA (Museo de Historia Natural de la Universidad Nacional de San Agustín, Arequipa, Perú), MHUA-A (Museo de Herpetología, Universidad de Antioquia, Medellín, Colombia), ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia), QCAZ (Museo de Zoología, Pontificia Universidad Católica del Ecuador), and DHMECN (División de Herpetología, Museo Ecuatoriano de Ciencias Naturales).

Results.

Hyloscirtus diabolus sp. nov.

(Figs. 1–3)

Holotype. CORBIDI 12885, adult male, Peru, Departamento de Amazonas, Provincia de Chachapoyas, Distrito La Jalca, km 10 from La Colpa–Rodríguez de Mendoza (-6.398944, -77.226278, 2300 m a.s.l.) Collected 14 November 2012 by Karla García-Burneo.

Paratype. MUSA 3864, adult male, Peru, Departamento de Amazonas, Provincia de Pedro Ruiz Gallo, Comunidad Shipasbama (-5.845833, -78.071944, 2180 m a.s.l.). Collected on 18 September 2011 by Roy Santa Cruz Farfán.

Diagnosis. *Hyloscirtus diabolus* (Fig. 1) can be diagnosed by the following characters: large size (SVL 82.3–89.2 mm, n = 2); vertical keel in the snout and upper lip cleft in the region of the premaxilla; vomerine odontophores with a conical-shape and a gap medially, each process with two or three prominent teeth; large, thick, supracloacal flap and supratympanic fold present; finger webbing formula: I $2^{½}$–$2^{½}$ II $1^{2/3}$–$2^{2/3}$ III 2^{+}–2 IV, toe webbing formula: I $1^{1/2}$ –2^{-} II 1^{-}–2 III $1^{1/2}$ –$2^{1/2}$ IV $2^{1/2}$–1^{-} V; nuptial pad absent; hypertrophied forelimbs and enlarged, curved and protruding spine prepollex present; fleshy calcar tubercle present; dorsum, thighs, shanks, finger, toes and discs brown with abundant tiny yellow marks; iris dark red without reticulation.

Comparison. The only species of the *H. larinopygion* species group with hypertrophied forelimbs in adult males and broad elliptical prepollex, modified as a projecting spine, are *H. tapichalaca* (Kizirian et al. 2003), *H. condor* Almendáriz et al. 2014, and *H. diabolus.* The remaining species of the group lack the hypertrophied forelimbs and their prepollex is trapezoidal (Kizirian et al. 2003; Rivera-Correa and Faivovich 2013; Table 1). *Hyloscirtus diabolus* differs from *H. condor* by having a strongly well developed, fleshy, heel tubercle (absent in *H. condor*), dark red iris (iris golden with fine tan reticulations in *H. condor*), gular region and belly dark reddish gray with pink marks in preservation (belly olive without marks and chest is light gray in *H. condor*), vertical keel in the snout and upper lip cleft in the region of the premaxilla (absent in *H. condor*) and greater adult male SVL (*H. condor*: 64.8–73.8 mm, n = 7 in). The new species differs from *H. tapichalaca* in dorsal and lateral color pattern: dorsum gray or brown, flanks gray brown, bearing pink-orange marks more conspicuous in axillary and inguinal regions, lateral and lower margin of cloaca, knee, elbow, and outer margin of forearm white in *H. tapichalaca*; and ventral color pattern: gular region and belly dark reddish gray with pink marks in preservation to *H. diabolus* (belly gray-blue and chest is light gray in *H. tapichalaca*), digital discs brown (digital discs white in *H. tapichalaca*), iris dark red (iris yellow-gold with fine black reticulations in *H. tapichalaca*), upper lip cleft in the region of the premaxilla (absent in *H. tapichalaca*) and greater adult male SVL (*H. tapichalaca*: 59.1–63.8 mm, n = 8). For a summary of others diagnostic characters of the *H. larinopygion* species group, see Table 1.

Description of holotype. Adult male, 82.3 mm SVL (Fig. 2). Body robust. Head 13% wider than long; head as wide as body; head width 35% of SVL; head length 27% of SVL. Snout rounded in dorsal view, truncate in profile (Fig. 3A–B); canthus rostralis rounded, distinct; loreal region weakly concave, nearly vertical; lips rounded, faintly flared; nostrils slightly protuberant, directed anterolaterally, located at level of anterior margin of lower jaw. Vertical keel on snout and upper lip cleft in region of premaxilla. Dorsal surface of internarial region concave. Interorbital distance slightly greater than upper eyelid width. Eye prominent, diameter greater than eye–nostril distance. Tympanum barely visible, round, positioned vertically such that is not visible from above; diameter 51% of eye diameter; tympanic annulus inconspicuous. Supratympanic fold prominent, extending from posterior to eye to posterior margin of arm insertion, slightly covering dorsal edge of tympanic membrane. Region between head and suprascapula depressed. Vomerine odontophores conical, separated by gap medially, positioned posterior to choanae; each process with 2–3 prominent teeth. Choanae small, ovoid, separated by a minimum distance greater than 3.5 times their maximum diameter. Tongue cordiform, barely free around lateral and posterior margin. Vocal slits longitudinal, originating on lateral to tongue and extending extending to the corner of the mouth. Vocal sac evident externally, large, single, median, subgular. Mental gland absent.

Upper arms and forearms hypertrophied; axillary membrane absent; ulnar fold and tubercles absent. Fingers short, thick, bearing large, ovoid discs, circumferential groove of each disc clearly defined; width of disc on Finger III 46% greater than tympanum diameter. Relative lengths of fingers I < II < IV < III.; webbing formula: I – II $1^{2/3}$–$2^{2/3}$ III 2^{+}–2 IV (Fig. 3C). Distal and proximal subarticular tubercles large, single, rounded, conical; distal tubercles on Finger IV globular. Palmar surfaces with deep folds. Large supernumerary tubercles, high and round, distributed over base of fingers. Outer metacarpal tubercle diffuse. Inner metacarpal tubercle large, its shape contouring underlying distal prepollex that is modified into an enlarged, protruding spine. Nuptial pad absent. Hind limbs robust; tibia length 50% of SVL; foot length 47% of SVL. Calcar tubercle large, fleshy; tarsal fold or tubercles absent; metatarsal fold thick. Inner metatarsal tubercle large, ovoid; outer metatarsal tubercle small, round. Toes short, lateral fringes absent, discs smaller than those of fingers, slightly wider than digit. Relative length of toes: I < II < V < III < IV; toe webbing formula: I $1^{1/2}$ –2^{-} II 1^{-}–2 III $1^{1/2}$ –$2^{1/2}$ IV $2^{1/2}$–1^{-} V (Fig. 3D). Subarticular tubercles large, round, conical; single row of smaller supernumerary tubercles along axis of each toe. Cloacal opening directed posteriorly at upper level of thighs; supracloacal flap large, thick; margins of vent with numerous small folds; cloacal sheath short. Dorsal skin, gular region, pectoral region and flanks smooth; belly and proximal ventral region of thighs granular.

Color of holotype in life. Dorsum, flanks, anterior and posterior surfaces of thighs and shanks, calcar tubercle, dorsal surfaces of fingers and toes brown with an abundant tiny yellow marks; discs dark bluish gray ventrally. Gular region and ventral surfaces dirty gray with an abundant small yellow flecks and spots; ventral surfaces of hands, feet, and webbing dirty gray. Pericloacal region light gray. Iris solid dark red, without reticulations.

Color of holotype in preservative. Dorsum, flanks, anterior and posterior surfaces of thighs and shanks, dorsal surfaces of fingers and toes dark gray with an abundant light gray marks. Gular region and belly, ventral surfaces of thighs and shanks dark reddish gray with pink flecks and spots; hands, feet, webbing, and pericloacal region dirty gray.

Measurements of holotype (in mm). SVL 82.3; HL 22.4; HW 28.5; ED 9.0; END 5.7; NSD 3.7; IND 5.8; AMD 12.1; TD 2.7; FAL 14.3; FAB 12.0; HAL 29.7; THL 39.8; TL 41.0; TAL 22.0; FL 38.7; TFD 5.0; FTD 3.8.

Variation. The paratype MUSA 3864 (SVL = 89.2 mm) is remarkably larger than the holotype, possesses fewer yellow flecks and spots on the dorsum but more conspicuous on the loreal region, digits, flanks, thighs (Fig. 4), and belly in life. Foot webbing is more extensive: I 2–$2^{2/3}$ II $1^{2/3}$–$2^{2/3}$ III 2–2 IV and I 1^{-} –$1^{1/2}$ II 1–2 III $1^{1/3}$ –2^{-} IV 2^{-}–1^{-} V. Other measurements of the paratype are: HL 23.9; HW 28.7; ED 8.8; END 6.0; NSD 3.9; IND 5.8; AMD 12.8; TD 2.7; FAL 14.3; FAB 11.8; HAL 30.5; THL 40.7; TL 44.0; TAL 25.6; FL 42.4; TFD 5.9; FTD 4.8.

Distribution and natural history. *Hyloscirtus diabolus* is currently known only from two localities approximately 100 km straight-line distance apart in the highlands on the eastern flank of the Cordillera Central, northern Peruvian Andes (Fig. 5). The two known individuals were found in dense cloud forest adjacent to very rocky streams with strong flow and 30–35° slope.

The specimens were collected while calling at night, perched on tree branches ca. 1.80 m above the ground and ca. 2 m from the edge of the stream. When handled, they clung tightly to the substrate, acquired a position with the head directed toward the belly and limbs pressed to the abdomen, and secreted a white, viscous, sticky, liquid over the entire body. Advertisement calls, reproductive biology, and tadpoles are unknown. No other anuran species was found syntopically with *H. diabolus*

Etymology. The specific epithet *diabolus* is Latin for devil and is used as a noun in apposition. The devil according to Christianity is the spirit of evil and enemy of God, often represented as the ruler of hell and depicted as a human figure with horns, cloven hoofs, fire-red eyes, trident, and tail. The species name is in allusion to the hypertrophied forelimbs, enlarged prepollex with a projecting spine, trident vomers, and dark red iris in life.

Discussion.

A noteworthy difference between the new species, *H. condor*, and *H. tapichalaca* and all other species of the *H. larinopygion* species group is the morphology of the prepollex. Most species of the *H. larinopygion* species group possess an enlarged, broad, elliptical prepollex (Duellman and Hillis, 1990; Faivovich et al. 2005; Figs. 6A–B), whereas in these three species it is modified into an enlarged protruding spine (Kizirian et al. 2003; Almendáriz et al. 2014; this study, Figs. 6C–D). Although in most anurans the prepollical spine has not been associated with any behavior, in some treefrogs—particularly large species such as *Hypsiboas faber*, *H. pardalis*, and *H. rosenbergi* (Lutz 1960; Lutz 1973; Kluge 1981; Martins and Haddad 1988; Martins et al. 1998)—it is related to territoriality and combat whereby the prepollical spine is used to slash opponents during prolonged wrestling bouts (Wells 2007), sometimes resulting in fatal injuries (Kluge 1981). Two specimens of *H. tapichalaca* (both paratypes), have scars that might have resulted from combat (Kizirian et al. 2003). One of the two known males of *H. diabolus* also possesses scars in the dorsum (MUSA 3864); although they differ from the scars observed in *H. tapichalaca*, they might also owe to male-male combat. Nevertheless, additional field observations are needed to understand the functional and behavioral significance of this morphological structure in *H. diabolus*.

Although *H. diabolus* has yet to be included in a phylogenetic analysis, several lines of evidence lead us to hypothesize a close relationship with *H. condor* and *H. tapichalaca*. These three species share some character states, absent in the other species of the *H. larinopygion* species group: curved prepollical spine, markedly hypertrophied forelimbs (also present in the *H. armatus* species group) and small, conical vomerine odontophore processes with 3–6 prominent teeth each and never in contact between them (Kizirian et al. 2003; Almendáriz et al. 2014; this study). Additional research explicitly incorporating *H. diabolus* in a phylogenetic analysis is required to further test our hypothesis and provide a better understanding of the evolution of these morphological characters.

On the taxonomy of *Hyloscirtus*. In their monumental study of the systematics of Hylidae, Faivovich et al. (2005) resurrected *Hyloscirtus* from synonymy with *Hyla*, where it had been placed 35 years earlier by Duellman (1970). Subsequent studies have consistently corroborated the monophyly of the genus and have contributed additional molecular and morphological evidence for its recognition (Sánchez, 2010; Wiens et al., 2010; Pyron and Wiens, 2011; Faivovich et al. 2013; Guayasamin et al. 2015). With the description of *H. diabolus*, *Hyloscirtus* now comprises 36 species divided among the *H. armatus* (3 species), *H. bogotensis* (17 species), and *H. larinopygion* (16 species, including *H. diabolus*) species groups, with additional known species awaiting formal description.

Given the size of the clade and its morphological diversity and widespread geographic distribution, it might seem that the time has come to formally recognize the three groups as either full genera or subgenera. However, the apparently strong support for the recognition of these species groups reported in molecular studies is illusory due to limited taxon sampling. This is especially true of the *H. bogotensis* group, of which only seven named species were included in the most recent and largest molecular phylogenetic analysis of the genus (Guayasamin et al., 2015). Faivovich et al. (2005) referred species that lacked molecular data to the group on the basis of the synapomorphic occurrence of a mental gland in adult males (Duellman, 1972); however, it was recently shown that the mental gland arose in the common ancestor of the more inclusive clade Cophomantini (Brunetti et al. 2015; see also Faivovich and De la Riva, 2006). Consequently, the occurrence of a mental gland in adult males of *Hyloscirtus* is symplesiomorphic and there is no evidential basis to refer the 10 species (60% of the group) that lack molecular data to the *H. bogotensis* group. Similarly, although molecular data are available for a greater proportion of species of the *H. larinopygion* group (12 of the 16 named species), no morphological synapomorphies have been identified to assign the remaining species. As such, formal recognition of the existing species groups would entail a large number of species being either excluded from the newly recognized genera and treated as *incertae sedis* or included on the basis of something other than synapomorphy (e.g., symplesiomorphy, character-states of unknown polarity, impressions of overall similarity, geographic distribution), either of which would be a major step backwards in hylid taxonomy. Given that *Hyloscirtus* monophyly is not in question, we believe understanding of these frogs is better advanced by focusing efforts on gathering new evidence of relationships than by rushing new names to press in the absence of synapomorphy.

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APPENDIX I. Additional specimens examined.

Hyloscirtus antioquia*:* COLOMBIA, Antioquia, Bello, San Félix, Serranía Las Baldías, Corregimiento de San Félix, Vereda Las Huertas, Quebrada El Hato, 2660 m, Km 5 San Félix road – Truchera San Félix, MHUA-A 7227 (holotype), 7228–29 (paratypes)

Hyloscirtus caucanus*:* COLOMBIA, Cauca, Páez, Hacienda Montenegro, Km 34.5 Belalcazar–

Tacueyo, 2400 m, ICN 7071 (holotype), 7002, 7056, 7072–76, 7238, 7241–48, 7250–52, 7055, 7239, 7253.

Hyloscirtus condor: ECUADOR, Zamora Chinchipe, Cantón Nangaritza, Parroquia Nuevo Paraíso, Reserva Biológica Cerro Plateado, 2317 m, EPN 14758 (holotype), EPN 14754, 14755, 14756, 14757, 14759, 14760 (paratypes).

***Hyloscirtus criptico**:* ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2760–2885 m., QCAZ 43516–18, 43528 (paratypes).

***Hyloscirtus larinopygion**:* COLOMBIA, Caldas, Villamaría, Km. 7 Villamaría–Mariquita, ICN 34433; Caldas, Pensilvania, Km 24 Pensilvania–Arboleda, 2000 m, ICN 36518–19; Cauca, Popayán, Santa Teresa stream, 2200 m, KU 144127 (holotype); Huila, Belalcazar, Parque Nacional Natural Nevado del Huila, 2900 m, ICN 41880; Quindío, Salento, Hacienda La Caleria, 2300 m, ICN 15626–27; Risaralda, Pereira, Parque Regional Ucumarí, Las Delicias stream, 2340 m, ICN 36133–38, 34970–72; Tolima, Ibagué, Juntas, El Silencio, 2600–2820 m, ICN 9380–82, 9670; Valle del Cauca, El Cairo, Las Amarillas, 2140–2200 m, ICN 28926, 28828–29, 42779; Valle del Cauca, Farallones de Cali, Campamento Corea, 2600 m, ICN 13595. ECUADOR, Carchi, Espejo, Morán, 2452 m, QCAZ 41826.

***Hyloscirtus lindae**:* ECUADOR, Napo, Papallacta, 2660 m, KU 164402 (holotype), 155476 (paratype); Pacto Sumaco, Parque Nacional Sumaco, Refugio La Laguna, 2476–2775 m, QCAZ 41232, 41294–98, 45345–47.

***Hyloscirtus pacha**:* ECUADOR, Morona Santiago, Plan de Milagro, 2350 m, KU 202762 (holotype); Plan de Milagro, Km 8 Plan de Milagro–Cuenca, 2150–2300 m, QCAZ 48237–41.

***Hyloscirtus pantostictus**:* ECUADOR, Sucumbíos, km 5 Santa Bárbara–La Bonita, 2650 m., KU 190000 (holotype); Santa Bárbara, 2590–2800 m, QCAZ 10488,11660–67, 12171, 12174, 14084, 30529–31, 38421, 40331, 41393, 41412, 42350, 45434–38, 45443–44, 45446, 45450–53.

Hyloscirtus princecharlesi: ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2720–2794 m, QCAZ 43654, 44893 (paratypes).

Hyloscirtus psarolaimus: ECUADOR, Napo Papallacta, Km 11 Papallacta–Baeza, 2660 m., KU 164313 (holotype), QCAZ 13252, 23070; Carchi, Tulcán–Santa Bárbara road, QCAZ 15366; Morona Santiago, San Vicente, Parque Nacional Sangay, 15 Km to road Lagunas de Atillo, 2815 m, QCAZ 31671; Sucumbíos, La Sofía, Campamento Río Verde, 2726 m, DHMECN 6493–94.

Hyloscirtus ptychodactylus: ECUADOR, Cotopaxi, Pilaló, 2320 m, KU 209780 (holotype).

Hyloscirtus sarampiona: COLOMBIA, Cauca, Parque Nacional Natural Munchique, Sopladero stream, 33 Km, 2190 m, ICN 7440 (holotype), 7441 (paratype).

Hyloscirtus staufferorum: ECUADOR, Napo, 27 Km N Jondachi, 2040 m, KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m, QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m, QCAZ 45962–63, 45965–67.

Hyloscirtus tapichalaca: ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m, QCAZ 15083–85, 16704–06, 17776–77 (paratypes).

Hyloscirtus tigrinus: COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m, ICN 53804 (holotype), ICN 53805–06 (paratypes).

TABLE 1. Comparison of some diagnostic characters in species of the *Hyloscirtus larinopygion* group. Sources: (1) Almendáriz et al. 2014; (2) Ardila-Robayo et al. (1993); (3) Coloma et al. (2012); (4) Duellman (1973); (5) Duellman and Altig (1978); (6) Duellman and Berger (1982); (7) Duellman and Hillis (1990); (8) Duellman and Coloma (1993); (9) Kizirian et al.

(2003); (10) Mueses-Cisneros and Anganoy-Criollo (2008); (11) Mueses-Cisneros and Perdomo-Castillo (2011); (12) Ruiz-Carranza and Lynch (1982); (*) this study.

Species	Dorsal pattern	Forelimbs	Iris color	Vomerine teeth	Nuptial pad	Prepollex	Calcar tubercle	Source
H. antioquia	Reddish brown; with or without scattered orange blotches	No hypertrophied	Gray with burgundy reticulations	In contact or not, 12– 20 teeth/each	Medial and distal component; dark brown epidermal projections	Trapezoidal	Present	* (2)
H. caucanus	Brown or reddish brown with dark brown scattered blotches; with or without middorsal stripe	No hypertrophied	Pale yellow with brown reticulations	In contact, 10–14 teeth/each	Distal component only; creamy white	Trapezoidal	Present or Absent	* (2)
H. condor	Light brown with abundant yellow marks	Hypertrophied	Golden with minute reticulations	No contact, 3–4 teeth/each	Absent	Spine	Absent	(1)
H. criptico	Grayish brown, densely stippled with minute cream flecks and bright orange blotches	No hypertrophied	Dark gray	In contact or not, 8–15 teeth/each	Absent	Trapezoidal	Present	* (3)
H. diabolus	Brown with abundant yellow marks	Hypertrophied	Dark red, without reticulations	No contact, 2–3 teeth/each	Absent	Spine	Present	*
H. larinopygion	Light brown to dark brown; with or without dark brown reticulation	No hypertrophied	Golden or silver with black reticulations	In contact or not, 8–15 teeth/each	Medial and distal component; creamy white	Trapezoidal	Present or Absent	* (4)
H. lindae	Dark metallic brown	No hypertrophied	Dull bluish gray with minute black flecks	In contact, 11–13 teeth/each	Distal component; creamy white	Trapezoidal	Present or Absent	* (3, 5)
H. pacha	Dark brown with metallic orange flecks	No hypertrophied	Olive brown	In contact, 11–15 teeth/each	Absent	Trapezoidal	Present	* (3, 7)
H. pantostictus	Olive-brown with small orange spots	No hypertrophied	Dark gray	In contact or not, 10–14 teeth/each	Medial and distal component, light brown epidermal projections	Trapezoidal	Present or Absent	* (3, 5)
H. princecharlesi	Black with many round-oval orange blotches	No hypertrophied	Dark gray	In contact or not, 11–15 teeth/each	Medial and distal component; creamy white		Absent	* (3)
H. psarolaimus	Grayish brown with dark brown and minute cream flecks; with or without middorsal stripe	No hypertrophied	Dull bronze with black reticulation	In contact, 10–18 teeth/each	Absent	Trapezoidal	Present	* (3, 7)
H. ptychodactylus	Orange-tan to reddish brown with minute orange-tan flecks enclosed in black markings; with or without middorsal stripe	No hypertrophied	Pale blue	In contact, 11–15 teeth/each	Absent	Trapezoidal	Present	* (3, 7)
H. sarampiona	Pale olive with orange spots	No hypertrophied	Gold with thin black reticulation	In contact or not, 8–16 teeth/each	Medial and distal component; dark brown epidermal projections	Trapezoidal	Present or Absent	* (12)

H. staufferorum	Uniform dark brown	No hypertrophied	Metallic brown	In contact, 16−25 teeth/each	Creamy white		Present	* (3, 8)
H. tapichalaca	Gray or dark brown	Hypertrophied	Yellow-gold with black reticulation	No contact, 4−6 teeth/each	Absent	Spine	Present	* (9)
H. tigrinus	Yellow-green or yellowish brown with transverse black stripes, sometimes reticulated; with or without middorsal stripe	No hypertrophied	Light gray or yellow with black reticulation	In contact, 10−15 teeth/each	Absent	Trapezoidal	Present	* (10, 11)

FIGURE LEGENDS

FIGURE 1. *Hyloscirtus diabolus* sp. nov. in life (CORBIDI 12885, holotype, adult male, 82.3 mm SVL). Photos: K. García-Burneo.

FIGURE 2. Dorsal and ventral views (in preservative) of the holotype of *Hyloscirtus diabolus* sp. nov. (CORBIDI 12885; adult male, 82.3 mm SVL).

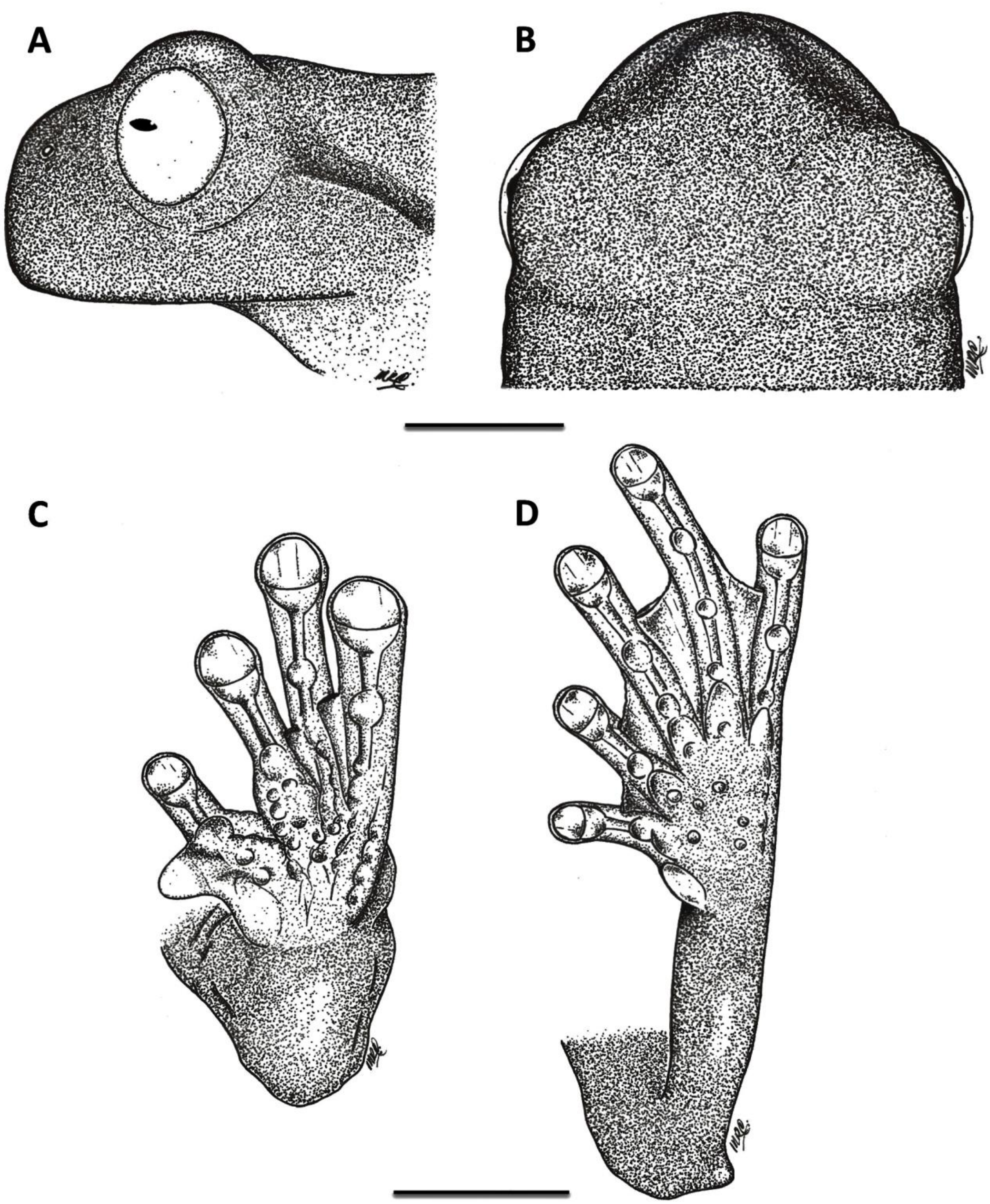

FIGURE 3. *Hyloscirtus diabolus* sp. nov. (CORBIDI 12885, holotype). (A) Head in lateral view (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bar = 10 mm.

FIGURE 4. *Hyloscirtus diabolus* sp. nov. in life (MUSA 3864, paratype, adult male, 89.2 mm SVL). Note the dark marks (scars) in the anterior region of dorsum, which are consistent with the occurrence of male combat (see text). Photo: R. Santa Cruz.

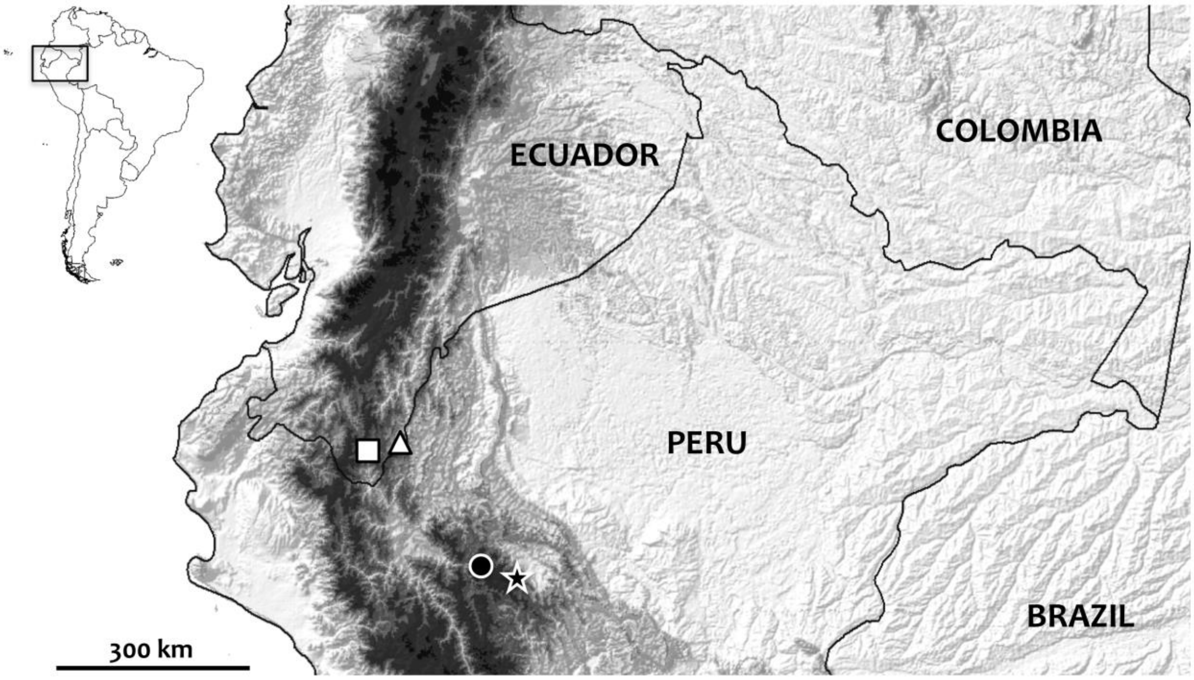

FIGURE 5. Geographic distribution of *Hyloscirtus diabolus* sp. nov. (holotype locality, star; paratype locality, dot), *H. condor* (triangle), and *H. tapichalaca* (square).

FIGURE 6. Ventral view of left hands in males of some species of the *Hyloscirtus larinopygion* species group, showing morphological diversity in the group. (A) *H. princecharlesi* (QCAZ 44893); (B) *H. lindae* (QCAZ 41298); (C) *H. tapichalaca* (QCAZ 17776); (D) *H. diabolus* (CORBIDI 12885). Scale bar = 5 mm. Photos: M. Rivera-Correa.

Cápitulo IV

A new species of *Hyloscirtus* peters, 1882 (Anura: Hylidae) with hypertrophied forelimbs from southeastern Ecuador

To be submitted to *Journal of Herpetology*

NEW SPECIES OF GENUS *HYLOSCIRTUS* PETERS, 1882 (ANURA: HYLIDAE) WITH HYPERTROPHIED FORELIMBS FROM SOUTHEASTERN ECUADOR

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ABSTRACT: We describe an extraordinary stream Andean treefrog of the genus *Hyloscirtus* from southeastern Ecuador, formerly confused with *Hyloscirtus lindae*. The new species differs from its congeners by the combination of hypertrophied forelimbs, presence of an enlarged, curved, and protruding prepollical spine, large, thick supracloacal flap and supratympanic fold, digital discs orange-red or bright-red, hidden surfaces of limbs bluish gray, and with spicules dorsally. The new taxon is sister species to *H. tapichalaca* and part of the southern clade of the *Hyloscirtus larinopygion* group, a group of four species restricted to southeastern Ecuador and northern Peru. We briefly discuss the implications of this finding on the morphology of the arms of the *Hyloscirtus larinopygion* group.

Key words: Andes Mountains; Hylidae; *Hyloscirtus larinopygion* group; Morphology; southern clade; Taxonomy.

Hyloscirtus is a stream-dwelling genus that occur from Costa Rica in Central America to Bolivia in western South America, and Venezuela, and currently includes 34 species (Frost, 2014). Currently, the species are divided into three species groups (i.e. *H. armatus*, *H. bogotensis*, and *H. larinopygion*) supported by molecular data (Faivovich et al., 2005) and larvae morphology (Sánchez 2010). The knowledge about diversity and systematics of the charismatic Andean clade *H. larinopygion* group has increased in recent years. The remarkable data come from the contributions on taxonomy, adults and tadpoles morphology, bioacoustics and phylogenetic relationships (Sánchez 2010; Coloma et al. 2012; Rivera-Correa and Faivovich, 2013; Almendáriz et al. 2014; Rivera-Correa et al. in press). Among the 16 species currently recognized in the *H. larinopygion* species group, 12 are distributed in

Ecuador (Frost 2014), being the most diverse country for this group.

Hyloscirtus larinopygion group is represented by two parapatric clades (Almendáriz et al. 2014). The northern clade is distributed from northern Andes of Colombia to the central Andes of Ecuador, and southern clade is distributed from the southeastern Andes of Ecuador to the northern Andes of Peru (Rivera-Correa and Faivovich 2013; Almendáriz et al. 2014). The southern clade, composed by *H. tapichalaca* Kizirian et al. (2003), *H. condor* Almendáriz et al. (2014) and *H. diabolus* Rivera-Correa et al. (in press). These are distinctive in having hypertrophied forelimbs associated with enlarged muscles and a prepollex modified as a prepollical spine. A fourth species with this morphological particularity has been included in previous phylogenetic analysis as *Hyla lindae* (Duellman and Hillis 1990), *Hyloscirtus lindae* (Pyron and Wiens 2011) and most recently as *Hyloscirtus "lindae"* (Almendáriz et al. 2014). This population is not *H. lindae* (Duellman and Altig1978) *sensu stricto*, consequently has been taxonomically misidentified and actually belongs to an undescribed species (Almendáriz et al. 2014). The goals of this paper include (1) the formal description of the new species, (2) a briefly discuss of the possible reasons for its misidentification in the literature and, (3) the implications of this finding on the evolution of the arm morphology of the *Hyloscirtus larinopygion* species Group.

MATERIALS AND METHODS

All measurements were taken with digital calipers and rounded to the nearest 0.1 mm. Abbreviations are SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), END (nostril–eye distance), NSD (nostril–tip-of-snout distance), IND (inter–nostril distance), AMD (distance between the anterior margins of eyes), TD (tympanum diameter), FAL (forearm length), FAB (forearm breadth), HAL (hand length), THL (thigh length), TL (tibia length), TAL (tarsal length), FL (foot length), TFD (third-finger diameter), and FTD (fourth-toe diameter). Webbing formulae follow the terminology of Savage and Heyer (1967) as modified by Myers and Duellman (1982). Sex was determined by examination of secondary sexual characters (hypertrophied forelimbs, vocal slits, and expansion of the vocal sac). Color pattern in life and natural history data was described from William E. Duellman and David M. Hillis field notes. Information on other species was obtained from preserved specimens (Appendix), notes, photo of living specimen, and literature. Institutional abbreviations used throughout this paper are CORBIDI (División de Herpetología, Centro de Ornitología y Biodiversidad), DHMECN (División de Herpetología, Museo Ecuatoriano de Ciencias Naturales), EPN (Museo de Historia Natural Gustavo Orcés de la Escuela Politécnica Nacional, ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia), MHUA-A (Museo de Herpetología, Universidad de Antioquia, Medellín, Colombia), KU (Museum of Natural History, The University of Kansas), and QCAZ (Museo de Zoología, Pontificia Universidad Católica del Ecuador).

RESULTS

Hyloscirtus **arcanus** *sp. nov.*

(Figs. 1–3)

Hyla lindae. —Duellman and Hillis (1990:16; Table 3–4; fig. 7; Appendix I; part)

Hyloscirtus lindae. —Wiens et al. (2010:877); Pyron and Wiens (2011:570)

Hyloscirtus "lindae". —Almendáriz et al. (2014:37; Table 1; fig. 2 part)

Holotype.— KU 202730, adult male, Ecuador, Provincia de Morona-Santiago, 23.3 km WSW Plan de Milagro, 2350 m a.s.l. Collected on 12 March 1984 by David. M. Hillis and Patricia. A. Burrowes

Paratopotype.— KU 202731, collected with holotype.

Paratype.— KU 202728–29, Ecuador, Provincia de Morona-Santiago, 21.6 km SW Plan de Milagro, 2425 m a.s.l. Collected on 4 March 1984 by William E. Duellman and David. M. Hillis.

Diagnosis.— We assign the new species to *Hyloscirtus* genus and *H. larinopygion* group based on the phylogenetic position inferred by Almendáriz et al. (2014) and morphological similarity with related species. *Hyloscirtus arcanus* sp. nov. can be distinguished from other species of the *H. larinopygion* group by the following combination of characters: dorsum and venter pale gray to dark gray, hidden surfaces of limbs bluish gray; digital discs orange-red to bright-red; iris dull olive bronze; enlarged, curved and protruding prepollical spine; hypertrophied forelimbs; large, thick, supracloacal flap and supratympanic fold; nuptial pad absent; vomerine odontophores process in two small bulky shape series in contact among them and carrying four teeth each; finger webbing formula: I $2^{1/2}$–$2^{1/2}$ II $1^{2/3}$–$2^{2/3}$ III 2^{+}–2 IV, toe webbing formula: I $1^{1/2}$ –2^{-} II 1^{-}–2 III $1^{1/2}$ –$2^{1/2}$ IV $2^{1/2}$–1^{-} V and spicules on snout, cantus, eyelid, supratympanic fold and shanks.

The only species of the *H. larinopygion* group with hypertrophied forelimbs in adult males and broad elliptical prepollex modified as a projecting spine, are *H. tapichalaca* (Kizirian et al. 2003), *H. condor* Almendáriz et al. 2014, and *H. diabolus* Rivera-Correa et al. in press. The remaining species of the group lack the hypertrophied forelimbs and their prepollex are trapezoidal (Kizirian et al. 2003; Rivera-Correa and Faivovich 2013; Rivera-Correa et al. in press). *Hyloscirtus arcanus* sp. nov. differs from *H. tapichalaca* in that the latter has pink orange marks in axillary and inguinal regions, thighs and shanks with brown transverse bands, lateral and lower margin of cloaca, knee, elbow, outer margin of forearm white (dorsum gray, hidden surfaces of limbs bluish gray in *H. arcanus* sp. nov.), digital discs white (digital discs orange-red to bright-red in *H. arcanus* sp. nov.), iris yellow-gold with fine black reticulations (dull olive-bronze in *H. arcanus* sp. nov.), vomerine odontophores process without contact between them and dorsal spicules absent (vomerine odontophores process contacting medially and dorsal spicules present in *H. arcanus* sp. nov.). In addition, at the molecular level, *H. arcanus* sp. nov. (KU 202728) differs from *H. tapichalaca*, its sibling species (see Almendáriz et al. 2014) by 22 (QCAZ 15083) and 29 (QCAZ 16704) nucleotide differences from 840 bp of aligned mitochondrial sequences (12S rDNA), representing between 2.84 to 3.45% of genetic divergence (uncorrected p-distances). *Hyloscirtus condor* differs by having a dark dorsum with contrasting yellow spots (dorsum gray without spots in *H. arcanus* sp. nov.), digital discs light brown, yellow brown or brown with abundant yellow blotches (digital discs orange-red to bright-red in *H. arcanus* sp. nov.), iris golden with fine tan reticulations (dull olive-bronze in *H. arcanus* sp. nov.), and vomerine odontophores process without contact between them and dorsal spicules absent. *Hyloscirtus diabolus* differs by having dorsum dark brown with abundant small yellow marks (dull olive-bronze in *H. arcanus* sp. nov.), hidden area of thighs and shanks dark brown (hidden surfaces of limbs bluish gray in *H. arcanus* sp. nov.), dark red iris (dull olive-bronze in *H. arcanus* sp. nov.), and vomerine odontophores process without contact between them and dorsal spicules absent (vomerine odontophores process with contact and dorsal spicules present in *H. arcanus* sp. nov.).

Description of holotype. – Adult male of 64.9 mm SVL (Fig. 1). Body robust. Head 16%

wider than long; head as wide as body; head width 35% of SVL; head length 27% of SVL. Snout rounded in dorsal view and truncate in profile (Fig. 2A–B); *canthus rostralis* rounded, indistinct; loreal region concave; lips rounded, faintly flared; nostrils barely protuberant, directed anterolaterally, placed at the level of the anterior margin of lower jaw. Dorsal surface of internarial region concave. Interorbital distance slightly larger than upper eyelid. Eye prominent; its diameter larger than eye–nostril distance. Tympanum and tympanic annulus inconspicuous. Supratympanic fold very prominent, starting behind the eye and extending to the posterior margin of the insertion of the arm. Region between head and suprascapulae quite depressed. Vomerine odontophores in two small fairly straight series with a bulky shape in contact medially, posterior to the choanae; each series bears four/four prominent teeth. Choanae small, ovoid, separated by a minimum distance larger than 3.5 times their maximum diameter. Tongue cordiform, attached overall except for narrow free area around postero-lateral margin. Vocal slits longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac evident externally, large, single, median and subgular. Mental gland apparently absent.

Arms and forearms hypertrophied; axillary membrane absent; a thick ulnar fold present. Fingers short, thick and bearing large, ovoid discs, with circumferential groove of each disc clearly defined by the size difference between the disc and the pad; width of disc on Finger III is 53% greater than the eye diameter. Relative lengths of fingers: 1 < 2 < 4 < 3. Fingers without dermal fringes, webbed thick, developed; webbing formula: I 2 –2 II 2^{-}–$2^{2/3}$ III $2^{1/3}$–2 IV (Fig. 2C). Subarticular distal tubercles large, single, rounded, and conic; distal tubercles on Finger IV globular. Palmar surfaces with deep folds. Large supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle differentiated, thick, elliptical, flat. Inner metacarpal tubercle large, its shape contouring the underlying distal prepollex that is modified into an enlarged, protruding spine. Nuptial pad absent.

Hind limbs robust; tibia length 48% SVL; foot length 45% SVL. Large, pyramidal and fleshy calcar tubercle present; tarsal fold absent, but tubercles along the outer margin of the tarsus present; inner fold present. Inner metatarsal tubercle large, ovoid; outer metatarsal inconspicuous. Toes short, lateral fringes absent, bearing discs smaller than those on fingers, subtly wider than the digit. Relative length of toes: 1 < 2 < 5 < 3 < 4; toe webbing formula: I $1^{1/2}$ –2 II $1^{1/2}$ –2^{+} III $1^{1/2}$ –$2^{1/2}$ IV $2^{1/2}$–2^{-} V (Fig. 2D). Subarticular tubercles large, round, and conical; single rows of smaller supernumerary tubercles along the axis of every toe. Cloacal opening directed posteriorly, at upper level of thighs; larger and thick supracloacal flap; margins of vent with numerous small folds; cloacal sheath short. Dorsal skin, gular region, pectoral region and flanks smooth, except for abundant spicules in the loreal region, eyelids, posterior region of the dorsum and tibia; belly and proximal portion of ventral surface of inner thigh granular.

Color in life of the holotype. – Dorsal surfaces and flanks dark gray, ventral surfaces pale gray. Hidden surfaces of limbs bluish gray; digital discs orange red. Iris dull olive-bronze.

Color in preservative of the holotype. – Dorsum and flanks gray purple, ventral surfaces pale gray. Hidden surfaces of limbs gray; webbing cream gray and digital discs orange pink.

Measurements of the holotype (in mm). – SVL 64.9; HL 17.7; HW 21.0; ED 7.4; END 4.0; NSD 3.0; IND 4.7; AMD 10.5; FAL 10.7; FAB 9.6; HAL 22.4; THL 31.0; TL 31.4; TAL 16.7; FL 29.4; TFD 4.0; FTD 3.6.

Variation and sexual dimorphism. – The female (KU202729) is larger than males and although her forelimbs are robust, they do not have the remarkable hypertrophy observed on the three males. The prepollex of the female is also modified as a prepollical spine, although without perceptible curvature. The tympanum is partially visible in the female, dorsally covered by the supra-tympanic fold, rounded and inclined medially towards the transversal body axis, such that almost is visible from above; its diameter equals 44% of eye diameter; in males, the tympanum is inconspicuous. The female has a thin fold, keel-shaped, perpendicular to the horizontal axis from the internarial region to the lip, undetectable in males in preservation. In life, dorsum of males dark gray and dull grayish brown in female. The belly of the female is dark gray, light gray in males. The color on the tips of the digits varies from red-orange to bright red (tomato red) and varies in extension, covering almost the entire disc in the male KU 202731 (Fig. 3). The males have spicules on snout, cantus, eyelid, supratympanic fold, the distal area of the dorsum, near the cloaca and shanks; spicules are absent in the female. The males have subtly more development of the webbing extension than the female; the webbing formula varies as follows: I $(2–2^{-})–(2–2^{-})$ II $(2–2^{-})–(2^{1/2}–2^{2/3})$ III $(2^{1/3}–2^{1/2})–(2^{+}–2^{-})$ IV / I $(2–2^{+})–(2^{+}–2^{-})$ II $1^{1/2}–(2^{+}–2^{-})$ III $(1^{1/2}–2^{-})–(2–2^{1/2})$ IV $(2–2^{1/2})–(1^{1/2} –2^{-})$ V. In addition, other secondary sexual characters (i.e. vocal slits, vocal sac) are absent in females. Morphometric measurements of the paratype series are given in Table 2.

Distribution and natural history.— Hyloscirtus arcanus sp. nov. is known from two localities in the eastern Andean slopes of southern Ecuador (Provincia Morona Santiago; Fig. 4). Elevation range is 2350 to 2425 m above sea level. Individuals KU 202728–29 were found on the night of 4 March 1984 on branches of trees in the spray zone of a waterfall. The male was calling from a cavity beneath moss; the call was whistle with three-note and a low-pitched intensity. The female was on the branch of a bush. An additional calling male (not collected) was observed at a higher height on the same waterfall. Vegetation type (according to the classification of Sierra *et al.* 1999) is Cloud Montane Forest of the Eastern Andes. The tadpoles are unknown.

Etymology.— The specific name is derived from the Latin adjective *arcanus*, meaning "hidden" or "secret". It refers to the fact that this species remained hidden in the museum and the literature under other taxonomic identity for many years.

Remarks.— The phylogenies of Duellman and Hillis (1990), Wiens et al. (2010) and Pyron and Wiens (2011) are inconsistent with the phylogeny of Coloma et al. (2012) because they recovered *H. lindae* within the southern clade of the *H. larinopygion* group (northern clade in Coloma et al. 2012). The inconsistency is a product of the misidentification of the molecular sample of the specimen KU 202728, which was reported as *Hyla lindae* or *Hyloscirtus lindae* in the earlier phylogenies, herein *H. arcanus* sp. nov. Once this misidentification is corrected, the four phylogenies are consistent with each other. Duellman and Hillis (1990) overlooked the protruding prepollical spine and hypertrophied forelimbs in the Morona-Santiago specimens (absent in *H. lindae* and all the other described species of the *H. larinopygion* group at that time). Both morphological character states were unknown in the group until the description of *H. tapichalaca* by Kizirian (2003). A possible explanation for the misidentification of *H. arcanus* sp. nov as *H. lindae* could be the presence of orange or red discs of digits in life in both species. In *Hyloscirtus* colors on discs digits are present in *H. lindae*, *H. pantostictus, H. tapichalaca* and the species described here. Thus in the context of the more comprehensive phylogenetic hypothesis of the *H. larinopygion* group (see Almendáriz et al. 2014), we infer this character state have evolved at least two times.

Discussion.— The description of *H. arcanus* sp. nov. raises to 17 the number of known species in the *H. larinopygion* group. Eight of the species are endemic to Ecuador (i.e., *H. arcanus* sp. nov., *H. condor, H. criptico, H. pacha, H. princecharlesi, H. ptychodactylus, H. staufferorum,* and *H. tapichalaca*), five species are distributed in Ecuador and Colombia (i.e., *H. larinopygion, H. lindae, H. pantostictus, P. psarolaimus,* and *H. tigrinus*); three species are endemic to Colombia (*H. antioquia*, *H. caucanus*, *H. sarampiona*) and one to Peru (*H. diabolus*).

Consequently, Ecuador maintains the highest species diversity of *H. larinopygion* group with highly restricted distribution ranges, which are reflected in the high levels of endemism to the country (Coloma et al. 2012; Frost 2013; Ron et al. 2013).

The phylogeny shown by Almendáriz et al. (2014), gives strong support to two clades within the group, a southern clade and a northern clade. The southern clade is less specious (four species currently described, including *H. arcanus* sp. nov.). The northern clade is more diverse and putatively includes the remaining 13 species (we are tentatively including the Colombian species *H. antioquia, H. caucanus* and *H. sarampiona* based on morphological and molecular evidence; MRC unpublished data). The lack of intensive fieldwork in the Andean highlands of southern Ecuador and northern Peru and poor representation of species richness possibly would indicate an underestimation of the alpha-diversity in the southern clade to the *H. larinopygion* group.

Hypertrophied forearms are characteristic of males in several groups of stream-breeding hylids, including *Bokermannohyla alvarengai, B. circumdata, B. martinsis, B. martinsi, H. armatus, Plectrohyla guatemalensis* species groups (Duellman 1970, Duellman 2001, Faivovich et al. 2005, Lugli and Haddad 2006, Kizirian et al. 2003; Faivovich et al. 2009; Rivera-Correa et al. in press). These stream-breeding species are not necessarily closely related to one another, so the enlarged forearms of males are thought to have evolved several times. However, the *H. armatus* group and the southern clade of the *H. larinopygion* group (i.e. *H. condor, H. diabolus, H. tapichalaca* and *H. arcanus* sp. nov.) are remarkable for the extreme development of their arms. In, the *H. armatus* group the arms are associated with clusters of spines on the inner surface of the prepollex (Duellman et al. 1997; Faivovich and De la Riva 2006; Rivera-Correa et al. in press), whereas in the *H. larinopygion* group they are associated with an enlarged, curved, and protruding prepollical spine. The behavior of most species of

H. larinopygion group is mysterious, so the function of the enlarged forelimbs is poorly known. However, it seems likely that males fight among themselves in at least some of these species. In *H. tapichalaca* were reported apparently anti-predatory behavior consisting in the use of the arms to cover snout and eyes (Kizirian et al. 2003; Rivera-Correa, personal observation). It has also been reported that the prepollical spine could scratch human skin during manipulation (Kizirian et al. 2003; Almendáriz et al. 2014). The development of the forelimbs of *Hyloscirtus* is variable; therefore, the taxonomic distribution of the relative development of the radius-ulna and lateral and medial humeral crests needs to be assessed to establish whether the level of development appreciated in the southern clade of *H. larinopygion* group is a putative synapomorphy.

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APPENDIX

Specimens Examined

Hyloscirtus antioquia: COLOMBIA, Antioquia, Bello, San Félix, Serranía Las Baldías, Corregimiento de San Félix, Vereda Las Huertas, Quebrada El Hato, 2660 m, Km. 5 San Félix road – Truchera San Félix., MHUA-A 7227 (holotype), 7228–29 (paratypes)

Hyloscirtus caucanus: COLOMBIA, Cauca, Páez, Hacienda Montenegro, Km. 34.5 Belalcazar–Tacueyo, 2400 m., ICN 7071 (holotype), 7002, 7056, 7072–76, 7238, 7241–48, 7250–52, 7055, 7239, 7253.

Hyloscirtus condor: ECUADOR, Zamora Chinchipe, Cantón Nangaritza, Parroquia Nuevo Paraíso, Reserva Biológica Cerro Plateado, 2317 m., EPN 14758 (holotype), EPN 14754, 14755, 14756, 14757, 14759, 14760 (paratypes).

Hyloscirtus criptico: ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2760–2885 m., QCAZ 43516–18, 43528 (paratypes).

Hyloscirtus diabolus: PERU, Amazonas, Chachapoyas, La Jalca, 2300 m., CORBIDI 12885 (holotype).

Hyloscirtus larinopygion: COLOMBIA, Caldas, Villa Maria, Km. 7 Villa Maria–Mariquita, ICN 34433; Caldas, Pensilvania, Km. 24 Pensilvania–Arboleda, 2000 m., ICN 36518–19; Cauca, Popayan, Santa Teresa stream, 2200 m., KU 144127 (holotype); Huila, Belalcazar, Parque Nacional Natural Nevado del Huila, 2900 m., ICN 41880; Quindío, Salento, Hacienda La Caleria, 2300 m., ICN 15626–27; Risaralda, Pereira, Parque Regional Ucumarí, Las Delicias stream, 2340 m., ICN 36133–38, 34970–72; Tolima, Ibague, Juntas, El Silencio, 2600–2820 m., ICN 9380–82, 9670; Valle del Cauca, El Cairo, Las Amarillas, 2140–2200 m., ICN 28926, 28828–29, 42779; Valle del Cauca, Farallones de Cali, Campamento Corea, 2600 m., ICN 13595. ECUADOR, Carchi, Espejo, Morán, 2452 m., QCAZ 41826.

***Hyloscirtus lindae**:* ECUADOR, Napo, Papallacta, 2.660 m., KU 164402 (holotype), 155476 (paratype); Pacto Sumaco, Parque Nacional Sumaco, Refugio La Laguna, 2476–2775 m., QCAZ 41232, 41294–98, 45345–47.

***Hyloscirtus pacha**:* ECUADOR, Morona Santiago, Plan de Milagro, 2350 m., KU 202762 (holotype); Plan de Milagro, Km 8 Plan de Milagro–Cuenca, 2150–2300 m., QCAZ 48237–41.

***Hyloscirtus pantostictus**:* ECUADOR, Sucumbios, km 5 Santa Bárbara–La Bonita, 2650 m., KU 190000 (holotype); Santa Barbara, 2590–2800 m., QCAZ 10488,11660–67, 12171, 12174, 14084, 30529–31, 38421, 40331, 41393, 41412, 42350, 45434–38, 45443–44, 45446, 45450–53.

***Hyloscirtus princecharlesi**:* ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2720–2794 m., QCAZ 43654, 44893 (paratypes).

***Hyloscirtus psarolaimus**:* ECUADOR, Napo Papallacta, Km 11 Papallacta–Baeza, 2660 m., KU 164313 (holotype), QCAZ 13252, 23070; Carchi, Tulcán–Santa Bárbara road, QCAZ 15366; Morona Santiago, San Vicente, Parque Nacional Sangay, 15 Km to road Lagunas de Atillo, 2815 m., QCAZ 31671; Sucumbios, La Sofía, Campamento Río Verde, 2726 m., DHMECN 6493–94.

***Hyloscirtus ptychodactylus**:* ECUADOR, Cotopaxi, Pilalo, 2320 m., KU 209780 (holotype).

***Hyloscirtus sarampiona**:* COLOMBIA, Cauca, Parque Nacional Natural Munchique, Sopladero stream, 33 Km., 2190 m., ICN 7440 (holotype), 7441 (paratype).

***Hyloscirtus staufferorum**:* ECUADOR, Napo, 27 Km N Jondachi, 2040 m., KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m., QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m., QCAZ 45962–63, 45965–67.

***Hyloscirtus tapichalaca**:* ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m., QCAZ 15083–85, 16704–06, 17776–77 (paratypes).

***Hyloscirtus tigrinus**:* COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m., ICN 53804 (holotype), ICN 53805–06 (paratypes).

FIGURE LEGENDS

FIG 1. – Dorsal and ventral view of the holotype of *Hyloscirtus arcanus* sp. nov. (KU 202730; adult male, SVL 64.9 mm). Photo: I. Nunes

FIG 2. – Drawings of *Hyloscirtus arcanus* sp. nov., (KU 202730, holotype). (A) Head in lateral view (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bar = 10 mm. Illustrations: M. Rivera-Correa

FIG 3. – *Hyloscirtus arcanus* sp. nov. in life. (KU 202731, paratype, adult male, SVL 67.8 mm). Photo: D. Hillis

FIG 4.— Map showing localities currently known of *Hyloscirtus arcanus* sp. nov. indicated with a white dots (Provincia de Morona-Santiago, Ecuador)

TABLE 1.—Measurements (in mm) of the type series of *Hyloscirtus arcanus* sp. nov. See text for abbreviations.

Measurement	Males (*n* = 3)				Female (*n* = 1)
	Mean	SD	Min	Max	
SVL	66.5	1.5	64.9	67.8	70.8
HL	18.6	0.8	17.7	19.3	19.6
HW	21.6	0.7	21.0	22.4	22.7
ED	7.4	0.2	7.2	7.5	7.6
END	4.2	0.2	4.0	4.3	3.8
NSD	3.3	0.3	3.1	3.6	3.6
IND	5.1	0.5	4.7	5.7	5.1
AMD	11.2	0.8	10.5	12.1	11.6
TD	–	–	–	–	3.4
FAL	10.9	0.5	10.5	11.6	11.4
FAB	9.8	0.2	9.6	10.0	6.0
HAL	22.4	1.0	21.4	23.4	22.9
THL	31.1	0.2	31.0	31.3	34.8
TL	30.7	0.7	30.1	31.4	34.0
TAL	16.6	0.1	16.5	16.7	20.0
FL	28.6	1.1	27.4	29.4	32.3
TFD	3.6	0.5	3.1	4.0	3.4
FFD	3.5	0.1	3.4	3.6	3.2

FIGURE 1

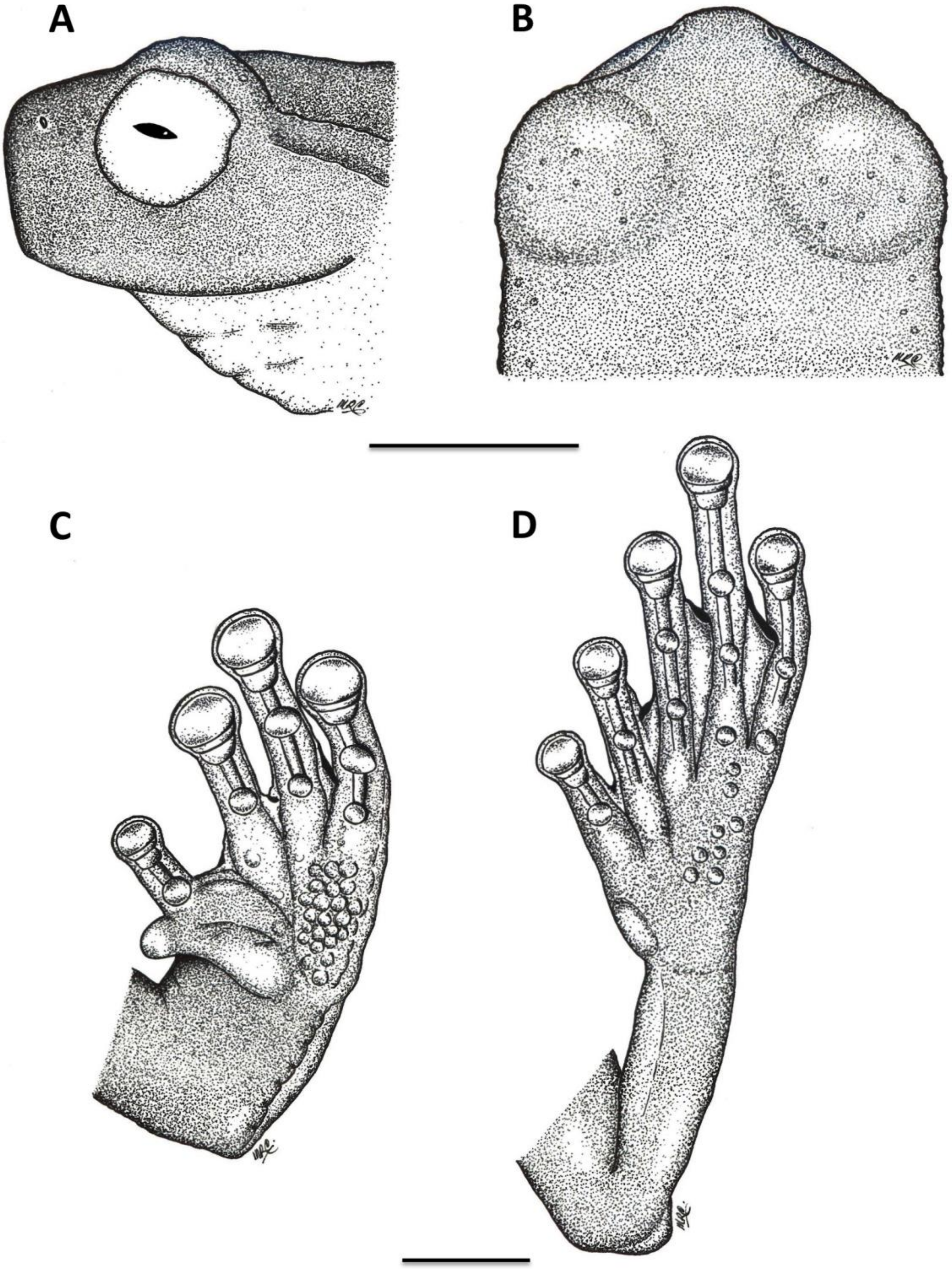

FIGURE 2

FIGURE 3

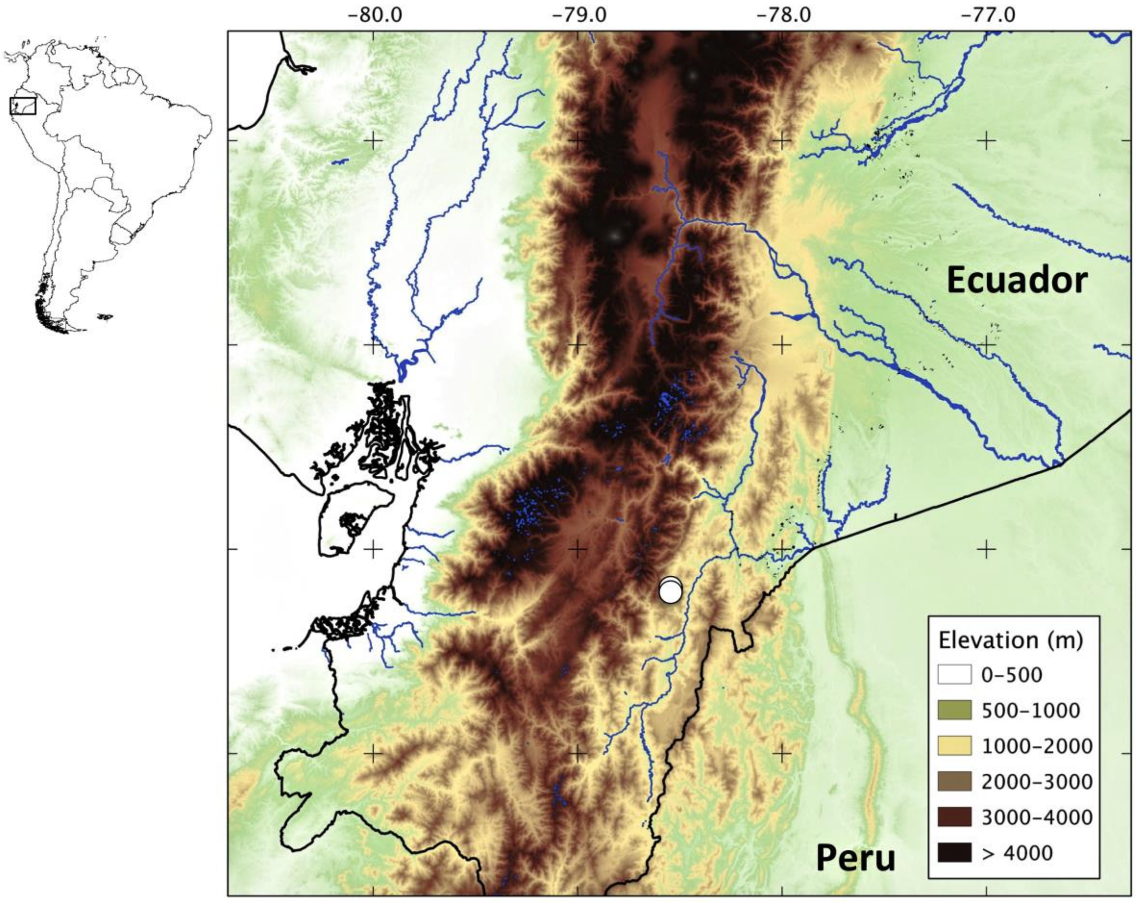
-80.0
-79.0
-78.0
-77.0
-1.0
-2.0
-3.0
-4.0
Ecuador
Peru
Elevation (m)
0–500
500–1000
1000–2000
2000–3000
3000–4000
> 4000

FIGURE 4

Cápitulo V

Evolution of the Stream Treefrogs *Hyloscirtus* (Anura: Hylidae): Phylogenetic Relationships, Taxonomic Revision and Species Limits

To be submitted to *Zoological Scripta*

EVOLUTION OF STREAM TREEFROGS *HYLOSCIRTUS* (ANURA: HYLIDAE): PHYLOGENETIC RELATIONSHIPS, TAXONOMIC REVISION AND LIMITS OF SPECIES

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ABSTRACT

The Neotropical stream-breeding frogs of the genus *Hyloscirtus,* distributed from Costa Rica to Bolivia, constitutes a significant element of the anuran fauna of the Andean region. In spite of previous studies about the systematics of *Hyloscirtus* in recent years, their phylogenetic relationships and an extensive taxonomic review within the genus have not been performed. Thus, in this thesis I present a densely sampled phylogenetic analysis and a taxonomic review of *Hyloscirtus*. Main goals of this thesis are to test the monophyly of the genus, to explore phylogenetic relationships among its species, to test the monophyly of the proposed species groups, to elucidate the potential number of putative new species in the genus on the basis of molecular data, to describe the putative new species integrating different lines of evidence available, and to interpret the evolution of the various phenotypical characters among their species in the context of the phylogenetic results. For this I present a molecular phylogenetic analysis that includes 29 of the 34 currently recognized nominal species, using up to 6,859 base pairs of DNA sequences from four mitochondrial and seven nuclear genes of 157 specimens from seven countries. Using maximum parsimony and the implementing of dynamic homology as reconstruction criterion, the phylogenetic analysis reveals that *Hyloscirtus* is monophyletic with respect to other genera of the tribe Cophomantini and that it is diagnosed by 42 transformations in nuclear and mitochondrial and ribosomal genes. Furthermore, the *Hyloscirtus bogotensis* species group and several currently recognized species within *Hyloscirtus* were recovered as non-monophyletic. In addition, the taxonomic status of *Hyloscirtus denticulentus* (Duellman, 1972) is re-evaluated. The molecular data reveals an extraordinary diversity previously not reported to *Hyloscirtus* with at least 13 lineages candidates of new species, within the lineages found seven are formally delimited and described based on phenotypic evidence and DNA data. I propose a new taxonomic re-arrangement of the genus that includes the definition of four species groups, one of them is newly proposed in this paper and supported by phenotypic synapomorphies. The unexpected phylogenetic position of *H. jahni* is discussed. The analysis resulted in a well-supported phylogenetic hypothesis within most of their clades that provides a historical framework for a discussion of the evolution of some characters associated with the morphology of the genus *Hyloscirtus*. Some morphological characters as hand morphology, nuptial excrescences and, mental gland are discussed.

INTRODUCTION

The Neotropical Andean stream-breeding frogs of the genus *Hyloscirtus* constitute a significant element of the anuran fauna of the Andean region. Their distributions range extends from wet forest Central Costa Rica to eastern and western slopes of the Andes in Colombia and Ecuador; Colombian inter-Andean valleys and Amazonian drainage of Venezuela, Colombia, Ecuador, Peru, and Bolivia (Frost, 2014; Fig. 1). Their altitudinal distribution ranges from 300 to about 3500 m a.s.l., although most species are restricted to the highlands (Duellman et al., 1997; Coloma et al., 2012; Frost, 2014; museum records). They are inhabitants of gallery forests and riparian habitats in most of their range, with sporadic encounters outside ponds and puddles near streams, particularly, species with distribution in Páramo habitat.

The genus *Hyloscirtus* includes 34 species (Frost, 2014; Rivera-Correa and Faivovich, 2013; Almendáriz et al., 2014) of stream-dwelling treefrogs whose monophyly is supported both by molecular and morphological data (Faivovich et al., 2005; Sánchez, 2010; Wiens et al., 2010; Pyron and Wiens, 2011). Currently, its species are divided in three taxonomic species groups: *H. armatus* (Duellman et al., 1997), *H. bogotensis* (Duellman, 1972), and *H. larinopygion* (Duellman and Hillis, 1990). The monophyly of each of these groups is mostly supported by molecular data (Faivovich et al., 2005; Wiens et al., 2010), further, larval morphological synapomorphies support the monophyly of the *H. armatus* group (Duellman et al., 1997; Faivovich et al., 2005; Lötters et al., 2005) and mental gland in adult males synapomorphy support the monophyly of *H. bogotensis* (Duellman et al., 1972; Faivovich et al., 2005). Although a combination of characters distinguishes to *H. larinopygion* group from two other groups, no putative phenotypic synapomorphy has yet been reported.

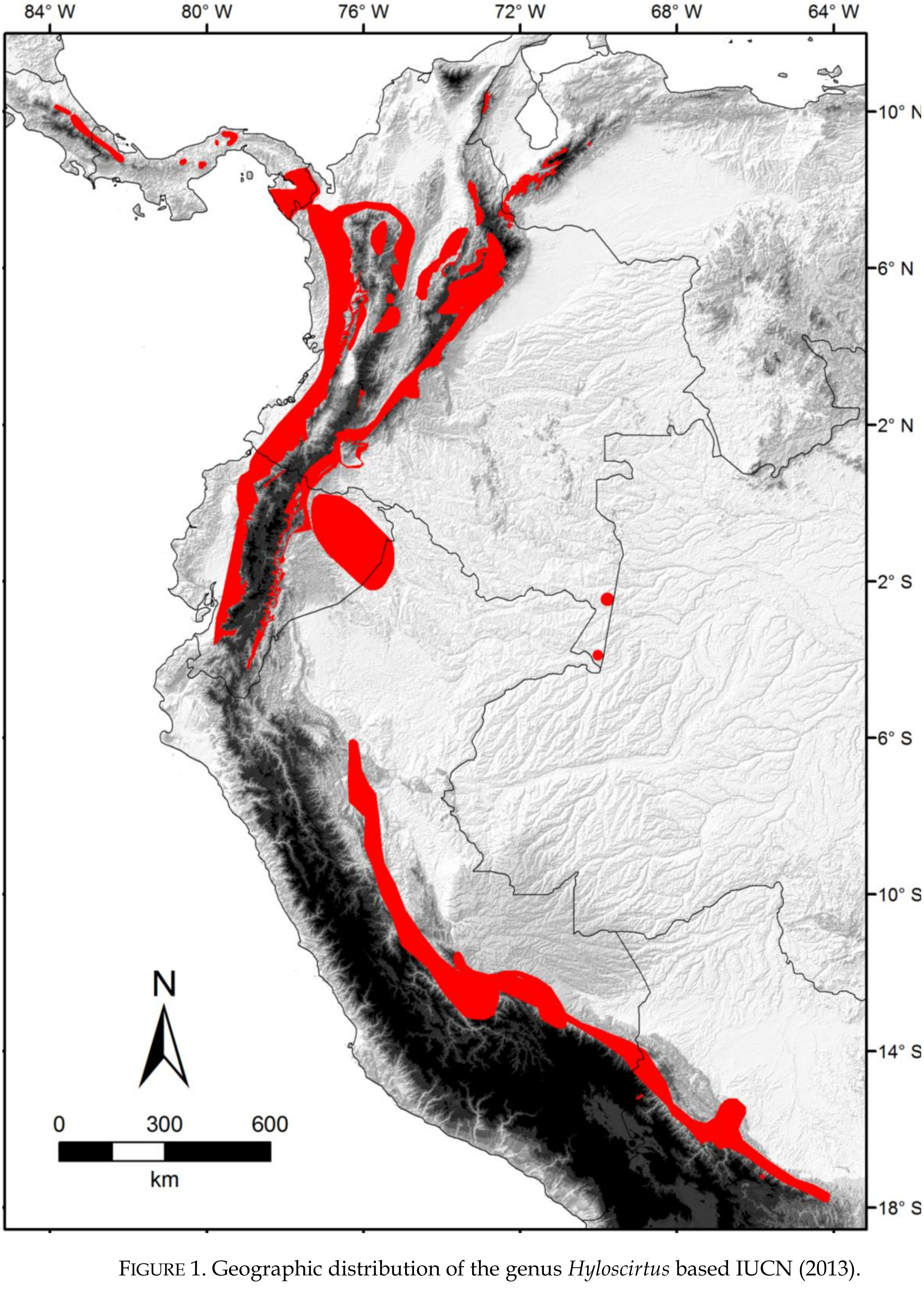


FIGURE 1. Geographic distribution of the genus *Hyloscirtus* based IUCN (2013).

The type species of the genus is *Hyloscirtus bogotensis* Peters, 1882. It was originally coined by Peters (1882) to the genus *Hylonomus*, but posteriorly is transferred to the genus *Hyloscirtus*, because it was namesake of a preexisting name *Hylonomus* Dawson, 1860. It was diagnosed by the presence of rounded sacral diapophyses. Lutz (1968) defined this genus (monotypic genus at the time of definition) by having small discs, not wider than the digits and tympanum no visible; and stated that it is related to the genera *Hyla* Laurenti, 1768 and *Aplastodiscus* Lutz, 1950. Posteriorly, *Hyloscirtus* was synonymized to *Hyla* by Duellman (1970) without additional comments.

Duellman et al. (1997) presented a phylogenetic analysis restricted to the *Hyla* species groups distributed partially or totally to the Andes. Under its most parsimonious tree, *H. armata, H. bogotensis* and *H. larinopygion* species groups together form a monophyletic clade supported by three transformations in larval morphology: the enlarged, ventrally oriented oral disc; the complete marginal papillae; and labial tooth rows formula 4/6 or more. Duellman et al. (1997) suggested also a close relationship between the *H. armata* and *H. larinopygion* groups based on the presence in the males of a greatly enlarged prepollex lacking a projecting spine. The results were congruent with Faivovich et al., (2005) and these note that males of the *H. bogotensis* group also had a prepollex with the same external morphology as those of the *H. armata* and *H. larinopygion* groups. Kizirian et al. (2003) suggested that the *H. armata* group was nested in the *H. larinopygion* group.

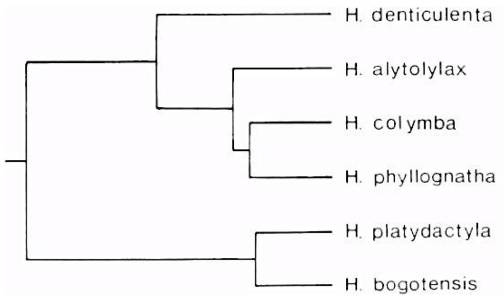
The systematic proposal by Faivovich et al. (2005) is the most complete phylogenetic hypothesis to the family Hylidae until its date. In their analysis the genus *Hyloscirtus* was resurrected (included only seven terminals) to accommodate the species traditionally allocated in the species groups *Hyla armata, Hyla bogotensis* and *Hyla larinopygion*. The genus *Hyloscirtus* was diagnosed by these authors on the basis of 56 changes in mitochondrial and nuclear ribosomal genes. Furthermore, these authors considered the wide dermal fringes on fingers and toes as a putative morphological synapomorphy (Faivovich et al., 2005). In addition, these authors allocated *Hyloscirtus* in the tribe Cophomantini (Hylinae) and their inferred relationships were: [*Myersiohyla* (*Hyloscirtus* (*Bokermannohyla* (*Aplastodiscus* + *Hypsiboas*)))]. About the same time, Wiens et al. (2005) published a phylogenetic analysis for the family Hylidae including six species of *Hyloscirtus* (*sensu* Faivovich et al., 2005) and these were recovered monophyletic and basal to a clade called by them as *Boana* Wiens, Fetzner, Parkinson, and Reeder 2005.

Sánchez (2010) provided a study of larval development of *Hyloscirtus* and evaluated some characters of the oral disc, nostrils, skin and cloacal tube of tadpoles; this author established a saccular structure associated with the vent tube that covers the limbs as a morphological synapomorphy of the genus. In addition, he proposed two morphological groups based on the formula row of teeth, upper jaw sheath morphology and shape of the nostrils of tadpoles, not congruent with the three groups of previous hypotheses. Wiens et al., (2010) and Pyron and Wiens (2011) presented a proposal of phylogenetic relationships for Hylidae and Amphibia respectively. However, they included the same number of species (11 terminals) and sequences for *Hyloscirtus* provided by Faivovich et al., (2005) and Wiens et al., (2005) and found a phylogenetic structure of the three groups, but with only weak support for the group *H. larinopygion* in Pyron and Wiens (2011). Coloma et al., (2012), expanded to 17 species of the genus making emphasis in the group *H. larinopygion* and they described two new species, provided tadpoles and osteology descriptions, and discussed divergence times and genetic distance between species. Recently, Faivovich et al. (2013) tested the monophyly and described two new species of *Myersiohyla*, a genus closely related to *Hyloscirtus*. They retrieved the relationships of *Hyloscirtus* according to inference by Coloma´s maximum parsimony, in addition, discussed some character states shared in the tribe Cophomantini.

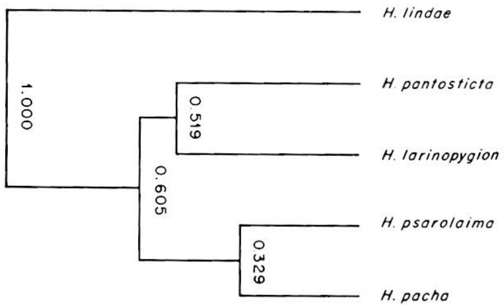
The Taxonomic Species Groups of *Hyloscirtus*

HYLOSCIRTUS ARMATUS group (Duellman et al., 1997): The *Hyla armata* group (= *Hyloscirtus armatus*) was first recognized by Duellman et al., (1997) and bearing *H. armata* as its single species. Köhler (2000) and De la Riva et al., (2000) subsequently reported *H. charazani* as the second member of this group. Duellman et al., (1997) described four synapomorphies for the *H. armatus* group: the presence in males of keratin-covered bony spines on the proximal ventral surface of the humerus, on the expanded distal element of the prepollex, and on the first metacarpal; tadpole tail long with low fins and bluntly rounded tip; forearms hypertrophied; and the presence of a ''shelf'' on the larval upper jaw sheath. Faivovich et al., (2005) diagnosed this species group by 103 transformations in nuclear and mitochondrial protein and ribosomal genes and confirmed the morphological observations of Duellman et al., (1997) as putative synapomorphies of the group. Furthermore, pigmented keratinized spicules in the body of male reproductively active specimens could be an additional synapomorphy (Faivovich et al., 2005). *Hyla chlorostea* (Reynolds and Foster, 1992) was considered by Faivovich et al., (2005) as *incerta sedis*, and subsequently appointed by Faivovich and De la Riva (2006) to the group *H. armatus* considering the presence of a skin fold of the proximal ventral portion of the arm (shared with *H. charazani* and *H. armatus*) and suggested also this condition as a putative morphological synapomorphy for the group. Currently, the *H. armatus* group includes three species: *H. armatus* (Boulenger, 1902), *H. charazani* (Vellard, 1970), and *H. chlorosteus* (Reynolds and Foster, 1992).

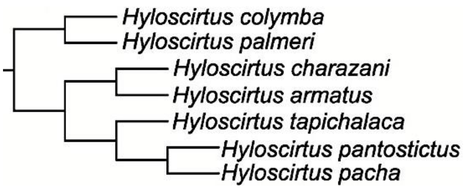
HYLOSCIRTUS BOGOTENSIS group (Duellman, 1972): The group *Hyla bogotensis* (= *Hyloscirtus*) was defined by Duellman (1972) and reviewed by Duellman (1989). *Hyla paramica* Rivero, 1961 and *H. jahni* Rivero, 1961 were allocated in synonymy of *H. platydactyla* Boulenger, 1905 by Duellman (1972). In the same work, the author presented a hypothesis of relationships of six species allocated in this group based in an analyzed of the 11 adults and five larval characters (Fig. 2); the following relationship were obtained: [((*H. denticulentus* (*H. alytolylax* (*H. colymba* + *H. phyllognathus*)) + (*H. platydactyla* + *H. bogotensis*))].



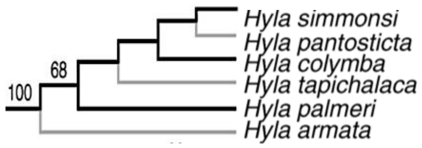
A) Duellman 1972



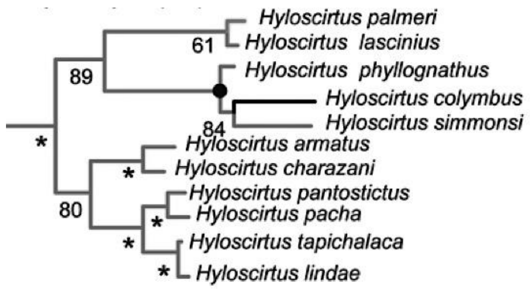
B) Duellman and Hillis 1990



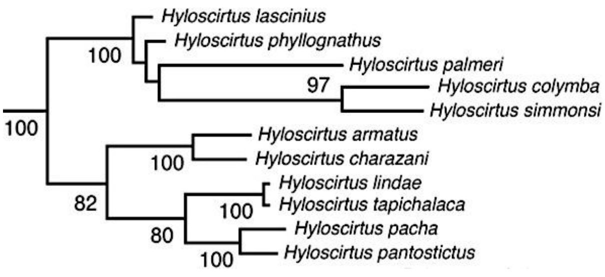
C) Faivovich et al., 2005



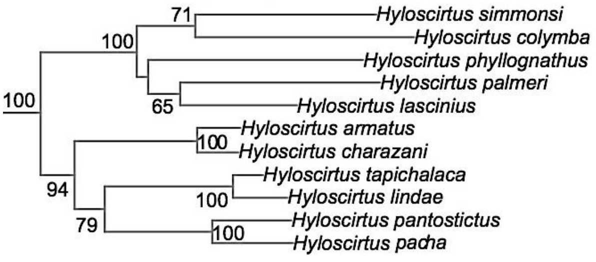
D) Wiens et al., 2005



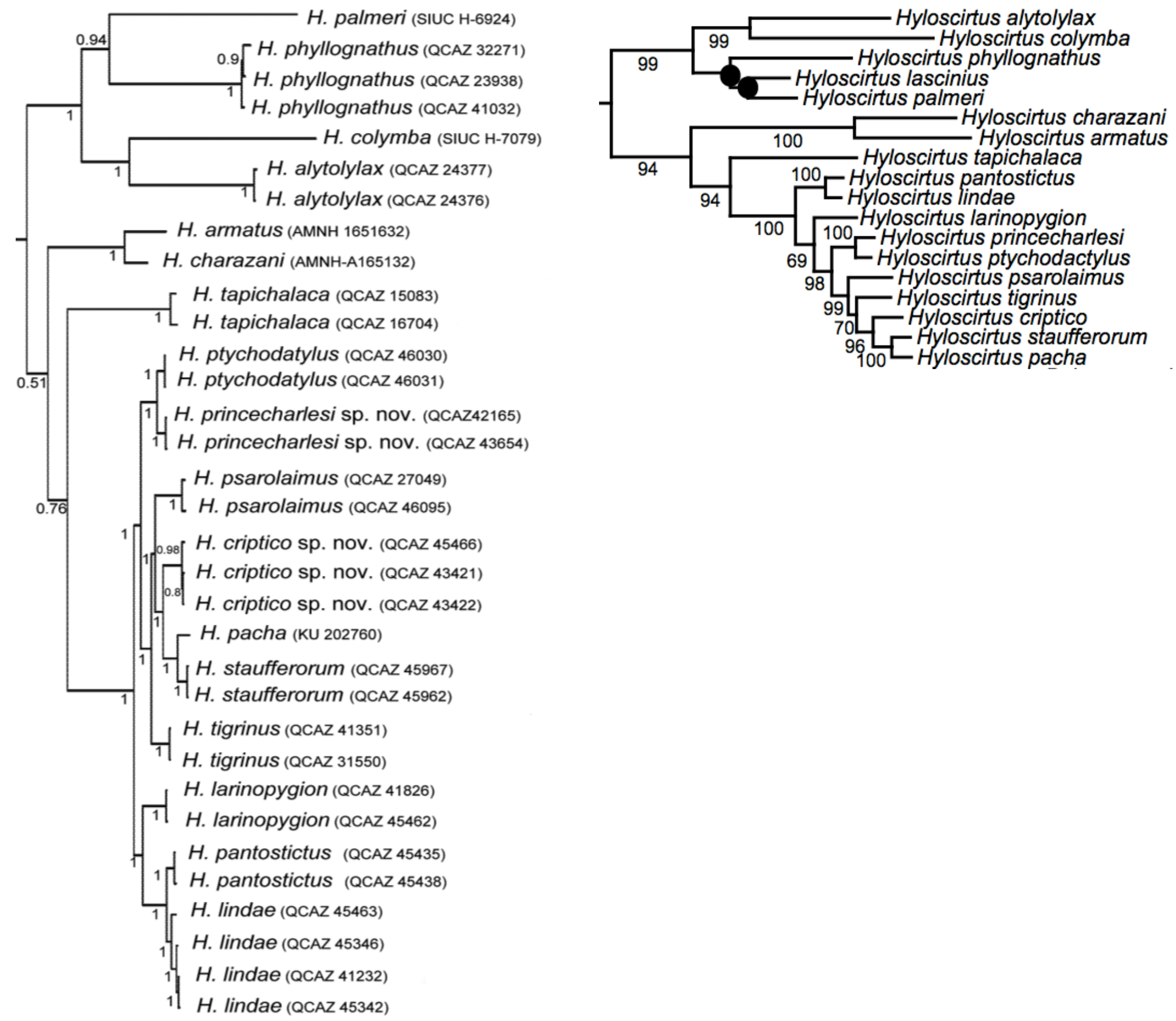
E) Wiens et al., 2006



F) Wiens et al., 2010



G) Pyron and Wiens 2011



H) Coloma et al., 2012

I) Faivovich et al 2013

FIGURE 2. Previous phylogenetic hypotheses of *Hyloscirtus*. A and B) UPGMA phenogram of genetic distances of Duellman 1972 and Duellman and Hillis 1990, obtained from isozyme analysis to some species from *H. bogotensis* and *H. larinopygion* groups respectively. All trees reproduced as appearing in the original papers. C) Section of the phylogenetic hypothesis of Faivovich et al., (2005) performed using parsimony with direct optimization. D) Section of the phylogenetic hypothesis of Wiens et al., (2005) based on Bayesian analyses. E) Section of the phylogenetic hypothesis of Wiens et al., (2006) based on Bayesian analyses. F) Section of the phylogenetic hypothesis of Wiens et al., (2010) based on parsimony analysis. G) Section of the phylogenetic hypothesis of Pyron and Wiens (2011) based on parsimony analysis. H) Majority-rule consensus tree from Bayesian analysis of Coloma et al., (2012) mainly of the *H. larinopygion* group. I) Section of the phylogenetic hypothesis of Faivovich et al., (2013), based on parsimony with direct optimization.

Myers and Duellman (1982) removed the synonymy of *Hyla palmeri* Boulenger, 1908 from *Hyla albopunctulata* Boulenger, 1882, previously proposed by Cochran and Goin (1970). La Marca (1985) revalidated *H. jahni* from *H. platydactyla* synonymy based on morphological evidence of adults, tadpoles and acoustic parameters, excluding the synonymy of *H. platydactyla*. Subsequently, *H. albopunctulata* was redescribed by Duellman and Mendelson (1995), who rejected a possible relationship with the group *H. bogotensis* as had been previously suggested by Rivero (1969) and Duellman in Frost (1985), however, these authors declared that relationships of *H. albopunctulata* were unclear. Subsequently, Duellman et al., (1997) redefined the *H. bogotensis* group including also *H. palmeri, H. lascinia* Rivero, (1970) and *H. lynchi* Ruiz-Carranza and Ardila-Robayo, (1991) and suggesting the presence of mental gland in males as a unique synapomorphy of the group.

Posteriorly, Faivovich et al., (2005) evaluated the syntypes of *H. albopunctulata* and determined to have a remarkable mental gland and suggest that the species could be related to the group *H. bogotensis*. Additionally, they evaluated the holotype of *Hyalinobatrachium estevesi* (Rivero, 1968) from the family Centrolenidae and indicated that it is a juvenile associated with the group *H. bogotensis* which could potentially be a junior synonym of *H. jahni* or *H. platydactylus*, both species recognized provisionally and pending from additional analysis.

At present, this species group is diagnosed by 95 transformations in nuclear and mitochondrial protein and ribosomal genes (Faivovich et al., 2005), and the presence of mental gland in adult males is considered as a morphological synapomorphy (Duellman 1972, Faivovich et al., 2005). Additionally, the presence of a white parietal peritoneum (*sensu* Ruiz-Carranza and Lynch, 1991), and shared with some other species of the tribe Cophomantini (i.e. *Aplastodiscus*) could possibly be an additional synapomorphy (Faivovich et al., 2005). These authors suggested the evaluation of the different character states and the taxonomic distribution of the white parietal peritoneum in *Hyloscirtus* species and other genera to Cophomantini to clarify which could be diagnosed by this synapomorphy (Faivovich et al., 2005). Later, Faivovich and De la Riva (2006) suggested a profound reassessment of the group *H. bogotensis* to establish the monophyly of the group considering that they found that the species of the group *H. armatus* also have mental gland.

Pyron and Wiens (2011), in their phylogenetic hypothesis from sequences made available by Faivovich et al., (2005) and Wiens et al., (2005), included only five species from the group *H. bogotensis* as terminals and obtained these relationships: ((*H. simmonsi* + *H. colymba*)(*H.phyllognathus* (*H. lascinius* + *H. palmeri*)). They regained the group *H. bogotensis* as sister group of *H. armatus* and *H. larinopygion*. Coloma et al., (2012) found *H. colymba* as sister species of *H. alytolylax* and *H. palmeri* sister of *H. phyllognathus*, however, they did not included *H. lascinius* and *H. simmonsi,* species evaluated in previous analysis.

The *Hyloscirtus bogotensis* group is most diverse compared to the other *Hyloscirtus* groups and currently contains 16 species: *Hyloscirtus albopunctulatus* (Boulenger, 1882), *H. alytolylax* (Duellman, 1972), *H. bogotensis* (Peters, 1882), *H. callipeza* (Duellman, 1989), *H. colymba* (Dunn, 1931), *H. denticulentus* (Duellman, 1972), *H. estevesi* (Rivero, 1968), *H. jahni* (Rivero, 1961), *H. lascinius* (Rivero, 1970), *H. lynchi* (Ruiz-Carranza and Ardila-Robayo, 1991), *H. palmeri* (Boulenger, 1908), *H. platydactylus* (Boulenger, 1905), *H. phyllognathus* (Melin, 1941), *H. piceigularis* (Ruiz-Carranza and Lynch, 1982), *H. simmonsi* (Duellman, 1989), *H. torrenticola* (Duellman and Altig, 1978).

HYLOSCIRTUS LARINOPYGION group (Duellman and Hillis, 1990): Proposed by Duellman and Hillis (1990) and reviewed by Duellman and Coloma (1993). Duellman and Hillis (1990) provided a formal definition and an analysis of isoenzymes for five species of the group, found the relationship ((*H. lindae* (*H. pantosticta* (*H. larinopygion* (*H. psarolaimus* + *H. pacha*)). Duellman et al., (1997) updated the definition but did not identify synapomorphies for the group. Considering these problems of delimitation of the group, Kizirian et al., (2003) found uncertain about placing *H. tapichalaca*, species described by them and that was considered most similar to groups *H. larinopygion*, *H. armata* and *Hyla pulchella* (*Hypsiboas pulchellus* group nowadays.) The only character state of which led Kizirian et al. (2003) to consider *H. tapichalaca* similar to the *H. pulchella* group was the presence of a prepollex extended, pointed, and curved. Subsequently, Faivovich et al., (2004) showed that *H. tapichalaca* and *H. armata* were sister species and distantly related to the *H. pulchella* group.

This species group was diagnosed by 32 transformations in nuclear and mitochondrial protein and ribosomal genes by Faivovich et al., (2005) and similar to previous papers no morphological or behavioral synapomorphies were recognized. Recently, Coloma et al. (2012) provided a comprehensive phylogenetic analysis in terms of terminal species of *H. larinopygion* group (11 species). In its maximum likelihood topology and their Bayesian analysis, they found three well-defined clades in the group *H. larinopygion* although they mentioned only two (A + B): the first and over basal clade formed by *H. tapichalaca*, the second group [(*H. princecharlesi* + *H. ptychodactylus* (*H. psarolaimus* (*H. cryptic* (*H. pacha* + *H. staufferorum*)], and the third composed by [(*H. larinopygion* (*H. lindae* + *H. pantostictus*)].

The *Hyloscirtus larinopygion* group currently comprises 15 colorful species: *H. antioquia* (Rivera-Correa and Faivovich, 2013), *H. caucanus* (Ardila-Robayo et al., 1993), *H. condor* (Almendáriz et al., 2014), *H. criptico* (Coloma et al., 2012), *H. larinopygion* (Duellman, 1973), *H. lindae* (Duellman and Altig, 1978), *H. pacha* (Duellman and Hillis, 1990), *H. pantostictus* (Duellman and Berger, 1982), *H. princecharlesi* (Coloma et al., 2012), *H. psarolaimus* (Duellman and Hillis, 1990), *H. ptychodactylus* (Duellman and Hillis, 1990), *H. sarampiona* (Ruiz-Carranza and Lynch, 1982), *H. staufferorum* (Duellman and Coloma, 1993), *H. tapichalaca* (Kizirian et al., 2003), and *H. tigrinus* (Mueses-Cisneros and Anganoy-Criollo), 2008.

In spite of the efforts mentioned above and the special attention and contribution in systematic to the *Hyloscirtus larinopygion* group in recent years, the phylogenetic relationships and an extensive taxonomic review within the genus, *Hyloscirtus* remain poorly understood. Thus, in this thesis I present a densely sampled phylogenetic analysis and taxonomic review of *Hyloscirtus.* The goals of this study are: (i) to test the monophyly of the genus, (ii) to explore relationships among its species and test the monophyly of the proposed species groups, (iii) to elucidate the potential number of putative new species in the genus on the basis of molecular data, (iv) to describe the putative new species integrating different lines of evidence available, (v) to interpret the evolution of the various phenotypical characters in the genus in the context of phylogenetic hypothesis here presented.

MATERIALS AND METHODS

TAXON SAMPLING

Ingroup: The molecular analysis included specimens of the 29 species currently recognized in *Hyloscirtus.* The species from which tissues were unavailable for this study are *H. albopuntulatus, H. caucanus, H. chlorosteus, H. estevesi* and *H. lynchi,* which are known only from their type localities. Sequences produced by Faivovich et al., (2005), Wiens et al., (2005, 2006), and Coloma et al., (2012) were downloaded from GenBank (Appendix 1). Various specimens per species were generally included, in particular for *H. armatus, H. larinopygion, H. palmeri* and *H. phyllognathus*, which have a wide geographic distribution and eventual taxonomic problem to be discussed.

Outgroups: The position of the genus *Hyloscirtus* within Hylinae and the tribe Cophomantini is well supported (Faivovich et al., 2005; Wiens et al., 2006, 2010; Pyron and Wiens 2011; Faivovich et al., 2013), thus, as outgroups I included exemplars of several genera of Cophomantini (i.e. *Aplastodiscus, Bokermannohyla, Hypsiboas* and *Myersiohyla*) on the basis of the previous results of Faivovich et al., (2005) and using *Agalychnis callidryas* from the subfamily Phyllomedusinae to root the trees.

CHARACTER SAMPLING

I have included sequences of up to 6859 bp of four mitochondrial and seven nuclear genes from 157 individuals (Appendix 1—2). The mitochondrial gene sequences analized for this thesis include portions of Cytochrome b (Cyt b), Cytochrome Oxidase I (COI), 12S, the intervening tRNAVal, 16S, and a fragment including the complete upstream section of 16S, the intervening tRNALeu and tRNAIle. The nuclear gene sequences produced include portions of 28S, Histone 3 (H3), proopiomelanocortin A gene (POMC), recombination activating gene 1 (RAG 1), Rhodopsin Exon I (Rhodopsin), seven in absentia homolog 1 (SIAH 1) and Tyrosinase.

LABORATORY PROTOCOLS

Tissue fragments were obtained from specimens preventing any damage to structures and organs used for morphological analyses. Total genomic DNA was extracted from ethanol-preserved tissue using the DNeasy blood and tissue kit (Qiagen). Amplification was carried out in a 25-lL reaction using puRe Taq Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ, USA) or Fermentas Master Mix. For all amplifications, the PCR programme included an initial denaturing step of 30 s at 94 °C, followed by 35 (mitochondrial gene fragments) or 45 (nuclear gene fragments) cycles of amplification (94°C for 30s; 48–62°C for 30s; 72°C for 60s), with a final extension step at 72 °C for 6 min. PCR amplification products were desalted and concentrated using either an Ampure (Agencourt Biosciences, Beverly, MA, USA), or GE GFX PCR purification kit and labeled with fluorescent-dye labels terminators (ABI Prism Big Dye Terminators v. 1.1 cycle sequencing kits; Applied

Biosystems, Foster City, CA, USA). The labeled PCR products were cleaned using cleanSEQ (Agencourt Biosciences, Beverly, MA, USA). The products were sequenced with an ABI 3730XL (Applied Biosystems), and all samples were sequenced in both directions to check for probable sequencing mistakes. Reading of sequencing reactions was performed by Macrogen Inc. (Seoul, Korea) and Genomic Engenharia Molecular (São Paulo, Brazil). Chromatograms obtained from the automated sequencer and sets of overlapping sequences from each sample and for each gene were assembled into contigs using Sequencher 4.5 (Gene Codes). All sequences were cross-checked and compared with GenBank sequences using NCBI's (National Center for Biotechnology Information) BLAST tool in order to identify possible sequencing and identification errors, as well as cross-contamination. Complete sequences were edited with Se-Al v. 2.0a11 (http://tree.bio.ed.ac.uk/software/seal/). Tissue samples and extractions are preserved at the public DNA and tissue collection of the Museu de Ciência e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP; Porto Alegre, Brazil). For a list and sources of the primers employed, see Table 1.

TABLE 1. Primers and gene regions used in the laboratory phase of this study

Gene	Primer	Direction	Primer sequence (5 to 3´)	Source
16S rDNA	AR	Forward	CGCCTGTTTATCAAAAACAT	Palumbi et al. 1991
	BR	Reverse	CCGGTCTGAACTCAGATCACGT	Palumbi et al. 1991
	L2A	Forward	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges, 1994
	H10	Reverse	TGATTACGCTACCTTTGCACGGT	Hedges, 1994
	L13	Forward	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller and Hedges, 1998
	Titus I	Reverse	GGTGGCTGCTTTTAGGCC	Titus and Larson, 1996
12S rDNA	MVZ59	Forward	ATAGCACTGAAAAYGCTDAGATG	Graybeal, 1997
	MVZ50	Reverse	TYTCGGTGTAAGYGARAKGCTT	Graybeal, 1997
	12s A-L	Forward	AAACTGGGATTAGATACCCCACTAT	Goebel et al. 1999
	12s F-H	Reverse	CTTGGCTCGTAGTTCCCTGGCG	Goebel et al. 1999
COI	COI 17	Forward	ACHAAYCAYAAAGAYATYGG	
	COI 18	Reverse	CCRAARAATCARAADARRTGTTG	
Cytochrome b	MVZ15	Forward	GAACTAATGGCCCACACWWTACGNAA	Moritz et al. (1992)
	Cytb2	Reverse	AAACTGCAGCCCCTCAGAAATGATATT TGTCCTCA	Kocher et al. (1989)
Rhodopsine	Rhod1A	Forward	ACCATGAACGGAACAGAAGGYCC	Bossuyt and Milinkovitch (2000)
	Rhod1C	Reverse	CCAAGGGTAGCGAAGAARCCTTC	Bossuyt and Milinkovitch (2000)
POMC	POMC-1	Forward	GAATGTATYAAAGMMTGCAAGATGGW CCT	Wiens et al. 2005
	POMC-2	Reverse	TAYTGRCCCTTYTTGTGGGCRTT	Wiens et al. 2005
Tyrosinase	Tyr 1C	Forward	GGCAGAGGAWCRTGCCAAGATGT	Bossuyt and Milinkovitch (2000)
	Tyr 1G	Reverse	TGCTGGGCRTCTCTCCARTCCCA	Bossuyt and Milinkovitch (2000)
RAG-1	R1-GFF	Forward	GAGAAGTCTACAAAAAVGGCAAAG	Faivovich et al. (2005)
	R1-GFR	Reverse	GAAGCGCCTGAACAGTTTATTAC	Faivovich et al. (2005)

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OPTIMALITY CRITERION FOR PHYLOGENETIC RECONSTRUCTION

The theoretical principle, Maximum Parsimony is one that explains the evolutionary processes with fewer assumptions. The cladistic parsimony is a research program that attempts to maximize the scientific principles of explanatory power and severity of the test. The justifications for using parsimony as an optimality criterion in cladistic was advanced by Farris (1983) and discussed by Goloboff (2003) and Goloboff and Pol (2005). Additional arguments for the use of parsimony in phylogenetic reconstruction have been widely discussed by Kluge and Grant (2006). The understanding of phylogenetic as a strictly ideographic and historical science suggests that the minimizing globally of the transformation events postulated to explain the character-states of terminal taxa and the use of the equally weighted parsimony analysis maximizes explanatory power (Kluge and Grant, 2006). In addition, the application of phylogenetic parsimony in an analysis of equally weighted evidence minimizes the total number of hypotheses of transformation required to explain the heritable variation observed among species and, as such, the most parsimonious cladogram represents the objectively optimal phylogenetic theory (Grant and Kluge, 2004). As such, in the present study I analyzed the total, equally weighted evidence under the parsimony criterion.

PHYLOGENETIC ANALYSIS

I performed the phylogenetic analyses with POY 4.1.1 (Varón et al., 2009), which implements the concept of *dynamic homology*. The phylogenetic analyses with POY maximize explanatory power by taking advantage of the analytical framework of dynamic homology. It seeks to combine the two historically disconnected processes of multiple alignment and cladogram searching into one step (Wheeler et al., 2006). Under this concept, intermediate alignment steps are avoided by directly assessing the number of evolutionary events—that is, DNA sequence transformations (Wheeler et al., 2006). This is accomplished through the generalization of existing character optimization procedures to insertion and deletion events (indels) and base substitutions. This method generates more efficient explanations of sequence variation (shorter, optimal trees) than do multiple alignments and produces multiple optimal results if more than a single optimal cladogram/alignment exists (Wheeler 1996, 2002).

Through dynamic homology, the most parsimonious solution is sought by generating different nucleotide alignments for each topology obtained in order to minimize transformation events in DNA sequences, and, thus, minimize the length of most parsimonious trees (Wheeler, 2003). Additionally, employing dynamic homology guarantees an explicit optimality criterion in generating sequence alignments while also eliminating the subjectivity of manual corrections.

Large datasets usually tends to have suboptimal trees very close in topology and length to the optimal trees; this region of the tree space is known as local optima or island. If a dataset exhibit composite optima the branch swapping by TBR usually get bogged down in large tree islands, collecting trees of equal length that differ only by minor rearrangements (Nixon, 1999). A strategy that makes the TBR algorithm more efficient is the use of multiple starting points when creating Wagner trees, this method is known as Multiple Random Addition Sequence (RAS). Large numbers of RAS + TBR generally work well in exploring islands for datasets of 50 to 150 taxa (Goloboff, 2002). The Parsimony Ratchet is a strategy that avoids getting stuck in one island and allows the examination of many islands from the tree space in a short time. This method maximizes the number of starting points and reduces the amount of time spent in the search from a particular starting point, it additionally retains the optimal tree structure found at each round while searching the improvement of other areas of the tree (Nixon, 1999).

To conduct the analysis in POY, sequences of 12S, 16S and intervening tRNA^{Val} were preliminarily delimited in sections of putative homology (Wheeler et al., 2006). Searches were performed using the command ‘Search’. This command implements a driven search building Wagner trees using random addition sequences (RAS), Tree Bisection and Reconnection (TBR) branch swapping followed by Ratchet (Nixon, 1999), and Tree Fusing (Goloboff, 1999). The command (Search) stores the shortest trees of each independent run and performs final tree fusing using the pooled trees as a source of topological diversity. One 120-h run of search were implemented in parallel at the American Museum of Natural History Cluster using 32 processors for a total of 3840 CPU hours. The resulting trees were submitted to a final round of swapping using iterative pass optimization (Wheeler, 2003a).

Although above discussed, I wanted to evaluate the effect of static homology method of maximum parsimony on the topology and discuss the differences if any with dynamic homology. For that, I performed a multiple sequence alignment obtained using the web version of the software MAFFT version 6.903 (Katoh et al., 2005) under the Q-INS-I strategy for each of the molecular markers independently. Posteriorly, all matrices were concatenated in Sequence Matrix and considering the missing data at the extremes of the sequences.

To static homology in parsimony, I employed T.N.T. (Willi Hennig Society Edition; Goloboff et al., 2008). Tree searches were performed with a driven new technology search, using 100 as the initial level. The strategy included sectorial searches, tree drift and tree fusing (Goloboff, 1999). The driven search was requested to hit the minimum length 500 times. The analyses were conducted considering gaps as a fifth state. Although I am aware that this decision may increase the length of the trees obtained. The Gaps are insertion or deletion events, thus, constitute evidence phylogenetic. A strict consensus of the most parsimonious trees found was generated in order to resume the phylogenetic hypotheses. Values of Goodman-Bremer (GB) support or patristic difference support metric (Grant and Kluge, 2008) and Parsimony Jackknife (PJ) absolute frequencies (JF; Farris et al., 1996) were estimated from the implied alignment (Wheeler, 2003b) with T.N.T. (Goloboff et al., 2008), the latter generating 100 RAS + TBR per replicate, for a total of 1000 replicates. The character optimizations and reconstructions with T.N.T. and Tree edition was performed with Winclada (Nixon, 2002) and Adobe Ilustrator CS6 (Microsoft 2012).

CHARACTER EVOLUTION

Through assessment of phenotypic characters for diagnosis of the species treated here a few were considered consistent enough (fixed within species) to be diagnostic and useful for evaluating their evolution (transformation series). I have selected three characters for understand of their evolution in *Hyloscirtus*. Characters were optimized in a summary tree, derived from the strict consensus of the most parsimonious trees, with all terminals representing the same taxon collapsed to a single terminal. Morphological characters were scored as absent or present. Character optimization was accomplished using the parsimony algorithm in Mesquite 2.74 (Maddison and Maddison, 2007).

SPECIES CONCEPT AND OPERATIONAL CRITERIA

The species concept is fundamental in the definition and understanding of species as natural units of evolution. Given the large diversity of forms and the great variation shown by different groups of organisms their history, biology, and dynamic, it is not surprising to find a variety of concepts and views on the issue of problem species. Consequently, I recognize that it is important to use a conceptual framework to structure empirical studies. According to de Queiroz (2007) alternative species concepts agree in treating existence as a separately evolving metapopulation lineage as the primary defining property of the species category, but they disagree in adopting different properties acquired by lineages during the course of divergence (e.g., intrinsic reproductive isolation, diagnosability, monophyly) as secondary defining properties (secondary species criteria). Therefore, I consider a species as the single lineage segment of ancestor-descendant populations or metapopulations delimited by one splitting event (de Queiroz, 2005, 2007). This theoretical position, species exist and evolve regardless of our ability to discover them, and are discoverable to the degree that footprints of their evolutionary history allow us to infer their existence (Ghiselin, 1975; Hull, 1976; Wiley, 1978; Frost and Kluge, 1994; Padial et al., 2012). Under the previous framework, the different operational criteria used to infer species limits herein are:

Morphological evidence: species delimitation supported by morphological evidence was when it exhibits at least one fixed character state relative to its sister species or when the combination of total diagnostic characters are unique to that lineage and character states are not shared with other lineage. The underlying assumption is that fixed differences in morphology is strong evidence of reduced or absent gene flow between populations or metapopulations (Frost and Hillis, 1990; Padial and de la Riva, 2010 and citations included by them). The fixed diagnostic traits across populations are indicative of lineage divergence, because character fixation across populations requires limited or absent gene flow (see review by Padial et al., 2010).

Molecular evidence: Monophyly of genealogies is considered evidence of species divergence on the assumption that character fixation in gene genealogies across populations is indicative of lineage independence (Padial et al., 2010). To infer the existence of distinct species using DNA data and to guide the recognition of candidate species was when I found evidence of monophyly. Including the uncorrected pairwise distance or genetic distance (number of base mismatches divided by total sequence length), is what provides clues for an initial search for species identification without conducting a complete phylogenetic analysis, however, and according to Grant et al., (2006), I do not advocate using pairwise distances only to delimit species. There is no justification for setting some arbitrary distance (e.g., 3% in Fouquet et al., 2007)—phenetic or otherwise as sufficient for granting species status (Grant et al., 2006; Padial et al., 2009; Rivera-Correa and Orrico, 2013).

Given variation in evolutionary rates and sampling density, it is expected that intraspecific variation may be greater in some species than others and as two-taxon statements, pairwise distances do not distinguish between symplesiomorphy and synapomorphy and therefore fail to explain the observed variation (Grant et al., 2006). Nevertheless, because they do not require extensive sampling or detailed phylogenetic analysis, pairwise comparisons are extremely fast and simple and therefore highly heuristic, and as such they are a useful starting point in examining species identity.

Acoustical signals evidence: The use of acoustic signals as diagnostic features in identifying species is a common practice in a several organism groups that communicate acoustically. Thus, specific hypotheses on anurans species often rely on differences in acoustical signals of mating calls as evidence for prezygotic reproductive barriers (Vences and Wake, 2007). Specifically, the advertisement calls are important in species and mate recognition (Blair, 1964; Ryan, 1988; Ryan and Rand, 1993), and constitute important premating isolating mechanisms, thus, they may play an important role in speciation events.

The recorded calls were advertisement calls based on the behavior of observed frogs (no other event or external stimulus was detected). Other call types are mostly the result of modifications in individual motivation or interactions, while advertisement calls are emitted continuously under appropriate conditions with the goal of mate attraction (Duellman and Trueb, 1986; Ryan and Rand, 1993; Angulo and Reichle, 2008). The advertisement call variation in temporal and spectral components, namely, in the quantitative and qualitative variables are indicative of divergence between populations and therefore, reproductive isolation mechanism evidence; constituting a criterion for independent evolutionary lineages at behavioral level.

When a population shows acoustical, genetic or morphological divergence from the other populations within the same nominal species, but the evidence of lineage divergence is inconclusive, I consider the lineage at the population level as an unconfirmed candidate species (UCS; Vieites et al., 2009; Padial et al., 2010). If evidence is conclusive about the existence of a new species but the available material is considered insufficient to provide an accurate species description, I consider the lineage at the population level as a confirmed candidate species (CCS; Vieites et al., 2009; Padial et al., 2010). Both CCS and UCS are flagged following the proposal of Padial et al., (2010). Candidate species are referred through the combination of the binomial species name of the most similar or closely related nominal species (or just the genus name), followed (in square brackets) by the abbreviation "Ca" (for candidate) with an attached numerical code referring to the particular candidate species, followed by the GenBank accession number for the sequence of reference deposited in GenBank, and terminating with the author name and year of publication of the article in which the respective lineage was first discovered.

MORPHOMETRIC DATA

I collected morphological data from Alcohol-preserved specimens. Institutional abbreviations follow Sabaj-Perez (2013). Morphometric data were taken only by one researcher (with digital calipers, Mitutoyo 0.01 mm) to minimize interobserver bias (Hayek et al., 2001; Lee, 1982), rounded to the nearest 0.1 mm and I calculated simple univariate summary statistics for males and females separately. Character definitions follow Duellman (1970) and Heyer et al., (1990). Abbreviations are SVL (snout-vent length), HL (head length), HW (head weight), ED (eye diameter), END (nostril-eye distance), NSD (nostril-tip of snout distance), IND (inter-nostril distance), AMD (distance between the anterior margins of eyes), TD (tympanic diameter), TL (tibia length), FL (foot length), TFD (third finger disc diameter) and FTD (fourth toe disc diameter). Webbing formulae follow terminology of Savage and Heyer (1967) as modified by Myers and Duellman (1982). Sex was determined by examination of secondary sexual characters (i.e. gonadal inspection when possible, direct observations of vocalizing behavior in males, and visibility of eggs through the body wall of females, mental gland, vocal slits, expansion of the vocal sac, and nuptial excrescences). Color pattern in life was described from field notes and color photographs. Drawings were made using a Zeiss stereomicroscope with a drawing tube or directly from a digital photo of the specimen for accuracy of body proportions. Information on other species were taken from preserved specimens, field notes and living photos. According to Wägele et al. (2011) once mentioned a species name or taxonomic identity, the authority that describes this species, will be reported in references, because species names are scientific contributions.

GENETIC DISTANCES

Genetic distances are usually based in molecular analysis of a short fragment of the 16S rRNA gene that is commonly used as the barcode for amphibians to identify species, assess the monophyly of species, and to discover divergent lineages (Vences et al., 2005; Padial et al., 2009; Vieites et al., 2009; Crawford et al., 2010). To evaluate uncorrected pairwise distances (p-distances, or number of base mismatches divided by total sequence length) was conducted an initial static alignment of all 16S gene to establish homologous positions of all terminals of *Hyloscirtus* exclusively. Posteriorly a block of 505 bp of 16S rRNA was selected, in this case delimited almost entirely by the AR-BR gene primers (Palumbi et al., 1991) and were calculated through the construction of a matrix of distances in PAUP* 4.0b10 (Swofford, 2002). I designated paragenetypes following the suggestion of Chakrabarty (2010), by sequencing the fragment of the 16S rRNA.

BIOACOUSTICS ANALYSES

I analyzed 42 recordings, which equates to 428 advertisement calls obtained from 32 males recorded at several localities in Colombia, Venezuela, Ecuador, Perú and Bolivia. Calls were recorded with a digital recorder Sony PCM-D50 or Marantz PMD-660 and a Sennheiser K6-ME66 directional microphone. All recordings were made at a distance of 1.0–3.2 m approximately from the signaller. I measured the body size (snout-vent length, SVL) and the air temperature at the end of the recording. Additionally, some records were retrieved from audio files of P. M. Ruiz-Carranza and M. C. Ardila-Robayo (Instituto de Ciencias Naturales, Universidad Nacional de Colombia). Recordings were analyzed employing Raven Pro 1.5 software for Mac OS X (Bioacoustics Research Program 2013), with a FFT of 256 points, at a sampling rate of 44.1 kHz and 16-bit precision, overlap 90%, Hann's sampling window and spectrogram resolution of 10,000 samplings. Sound graphs were obtained with Seewave 1.6 (Sueur et al., 2008), R 2.15.1 package (R Development Core Team 2012). Seewave settings, window name (Fourier transform window) = Hann; window length = 256 samples; overlap = 90%. I analyzed oscillograms (waveforms), spectrograms, and power spectra. Call parameter definitions follow Cocroft and Ryan (1995) and Duellman and Trueb (1994).

The following temporal parameters were measured from the waveform: call duration (in some cases equal to note duration), interval between call, number of pulses per call, and pulse duration. From a mechanistic and behavioral point of view, each act of sound emission corresponds to a cycle of exhalation–inhalation (Robillard et al., 2006). The notion of ''note'', which refers to the total amount of sound energy generated during a single airflow cycle (McLister et al., 1995), is an appropriate acoustic unit to use when comparing the sound production in frogs (Robillard et al., 2006). The peak frequency (= dominant frequency), and frequency bandwidth (as the difference between the 5% and 95% frequencies, hereinafter, lowest frequency and highest frequency) were obtained from spectrograms. The frequency bands and dominant frequencies were obtained from spectrograms. The note rate (notes per second) and the pulse rate (pulses per second) were calculated. Specimens collected and sound voucher for the present study are deposited in the Museo de Herpetología Universidad de Antioquia (MHUA), Medellín, Colombia and the Instituto de Ciencias Naturales, Universidad Nacional, Bogotá, Colombia.

RESULTS

PHYLOGENETIC RELATIONSHIPS

The data set included 157 terminals and 6859 aligned molecular bases (MAFFT alignment), from which 4330 were constant, 598 were variable but parsimony uninformative, and 2005 were parsimony informative. An ambiguously aligned 16S region was detected, therefore 46 bp were excluded from the analysis to avoid incorporation of "spurious" gaps. Using equal weights for substitutions and insertion / deletion events, the analysis using direct optimization in POY resulted in four equally parsimonious trees of length 16306 (Fig. 4). For the analysis using static alignments in TNT and considering gaps as fifth state, was obtained 68 equally parsimonious trees of length 16783 (Appendix 3).

Relationships among outgroups do not differ from previous analyses and the monophyly of *Aplastodiscus*, *Bokermannohyla* and *Hypsiboas* were corroborated, though relationships within each outgroups varies in some cases, compared to the previous hypotheses (Faivovich et al., 2005; Wiens et al., 2006, 2010; Pyron and Wiens, 2011; Faivovich et al., 2013). Most striking result is that *Myersiohyla* was not recovered as monophyletic in both dynamic and static alignments analysis. This result is in contrast with Faivovich et al. (2013), who recently included more sequences and terminal species in a taxonomic revision of the genus and it was recovered as monophyletic with high support values.

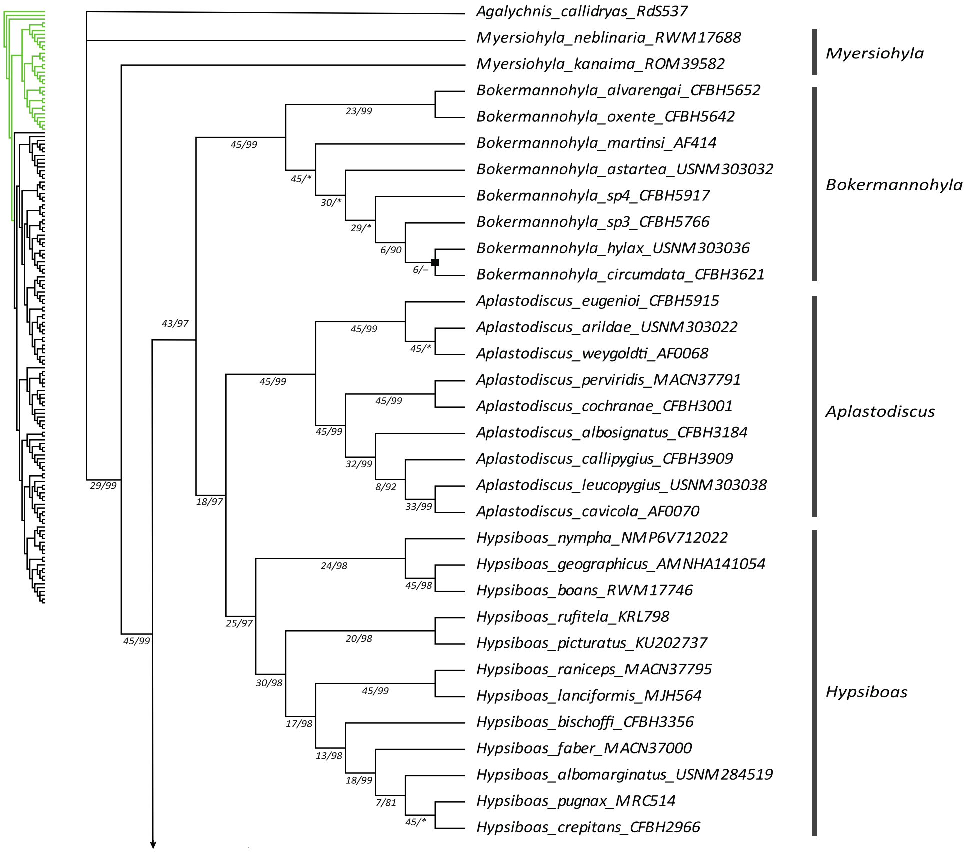

Figure 4. Phylogenetic relationships (outgroups) of *Hyloscirtus* inferred from maximum parsimony analysis under dynamic homology. The topology reflects consensus tree (length 16306 steps). Black squares on nodes indicate clades collapsed in the strict consensus tree of the static parsimony analysis. The taxonomy proposed in this work and distribution of clades mentioned in the main text is shown. Numbers on branches are Goodman-Bremer support and parsimony jackknife absolute frequencies respectively and calculated for the implicated parsimony analysis. Asterisks indicate parsimony jackknife frequencies of 100%; dashes indicate frequencies ≤ 50%. The *"x"* indicate that the relationship was not recovered in the analysis to establish jackknife support. A skeletal topology for reference shown on the left side and the magnified section is marked in bright color. Green asterisks indicate that the localities voucher come from the holotype or paratypes

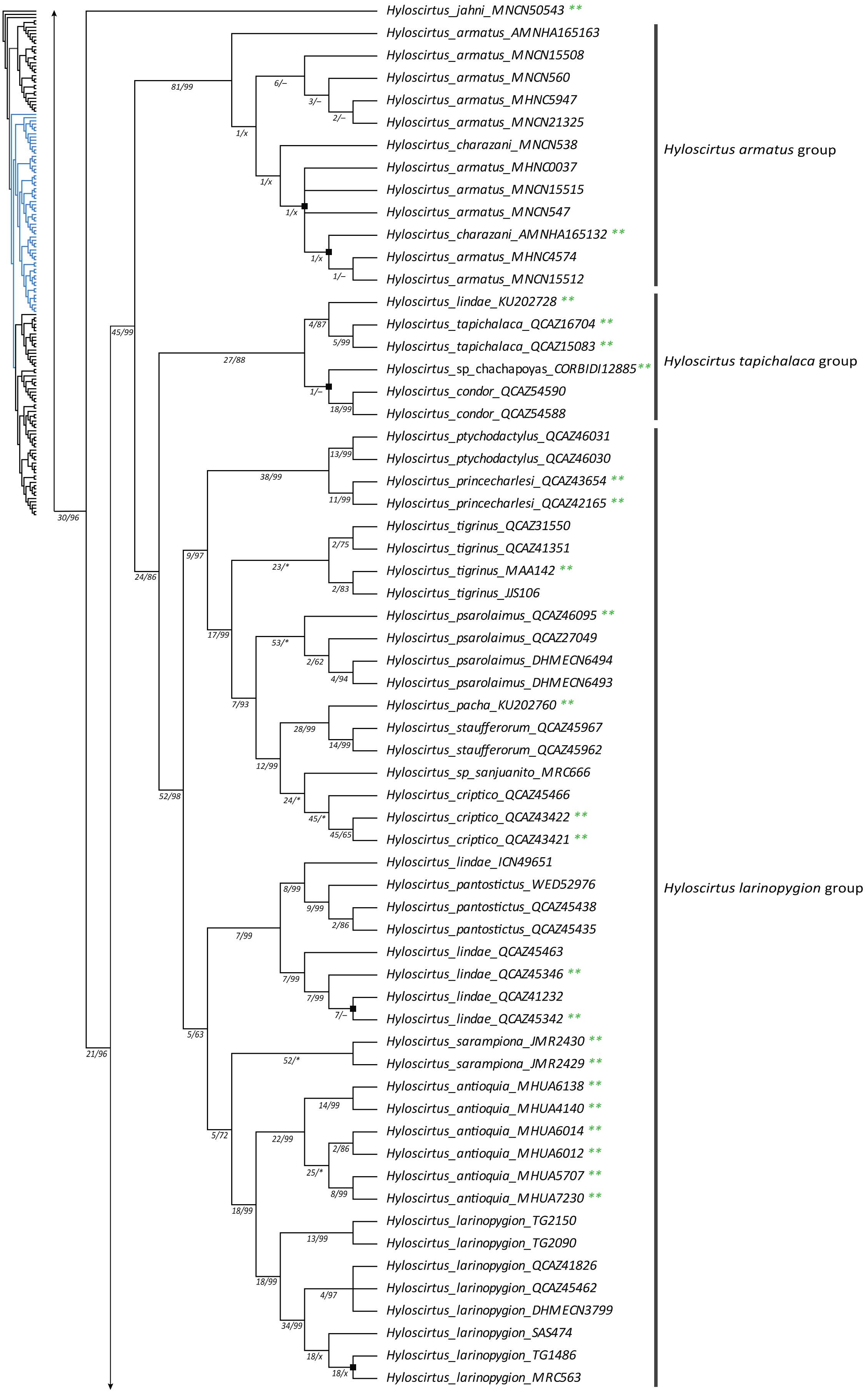

FIGURE 4. Continued

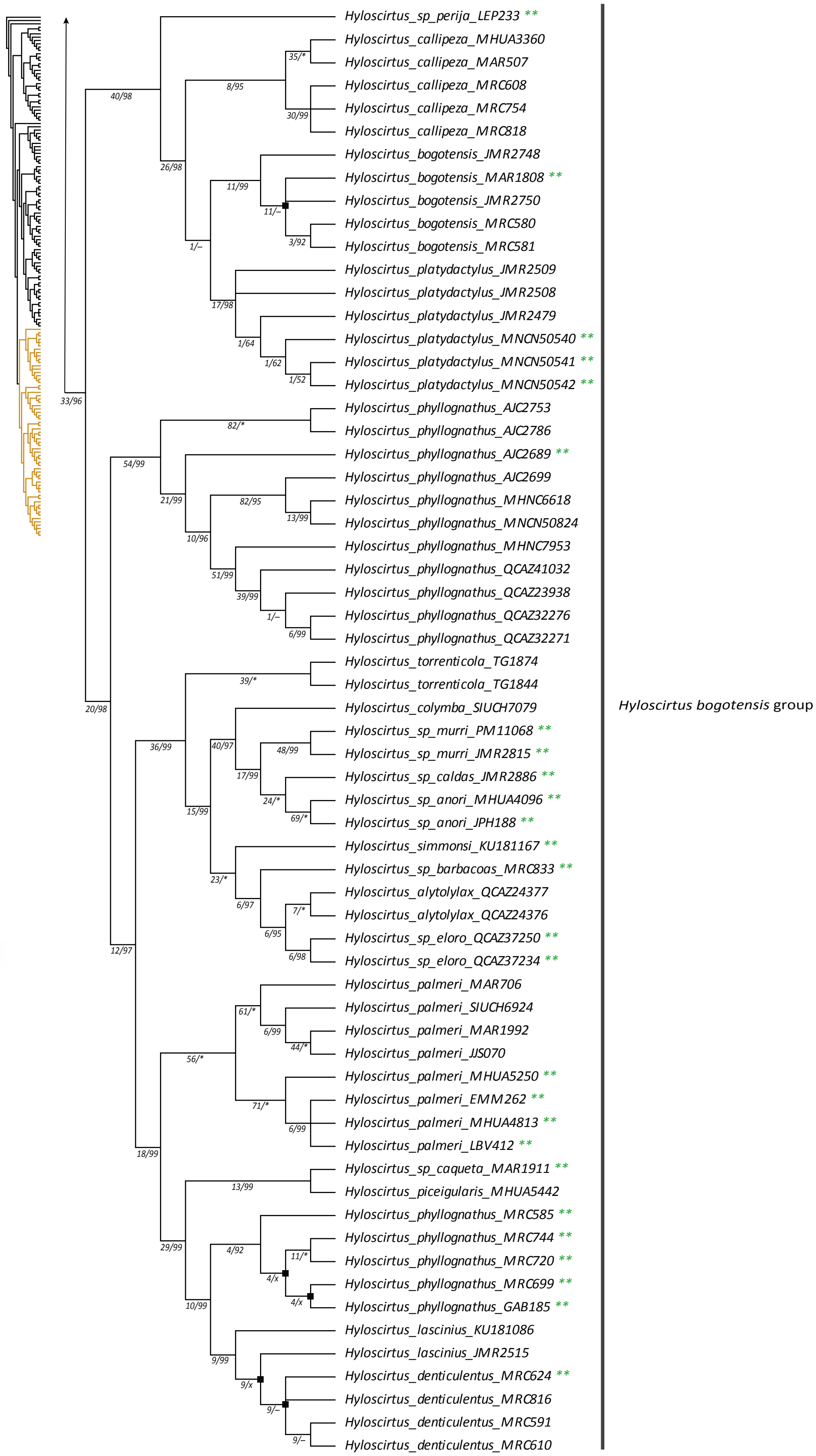

FIGURE 4. Continued

The strict consensus of the best hypotheses indicates that *Hyloscirtus* is monophyletic with high support values (GB = 30, PJ = 96 to dynamic homology and GB = 25, PJ = 96 to static alignments). Parsimony Jackknife values for most clades (i.e. 86.1% of the 144 clades present in the strict consensus) were relatively high (≥ 75%) and only 8.3% of the clades in the strict consensus received low jackknife values (< 50%). The species groups as currently defined were retrieved as monophyletic and received high support in all trees (not shown). Most of the conflict between both dynamic and static alignments analysis was among shallow clades of closely related terminals and the phylogenetic position of *H. jahni* (Fig. 4).

In dynamic homology topology, *H. jahni* was recovered as the most basal species of the ingroup (Fig. 4). This species is the sister taxon of a clade composed of *H. armatus* group, *H. tapichalaca* group (new group, see below), *H. larinopygion* group and *H. bogotensis* group. Each of these five clades is in general well supported, but the relationships among some of them received poor support. While in the static homology analysis, *H. jahni* is the most basal and sister to all remaining species of the *H. bogotensis* group, but with poor support value (GB support = 1). In general terms, dynamic homology analysis showed higher support values compared with static homology (Fig. 4). See systematics implications section for definitions and diagnoses of the above groups.

SPECIES DIVERSITY AND GENETIC DIVERGENCE

Phylogenetic inferences presented here suggest the non-monophyly of some nominal species (i.e. *H. armatus*, *H. charazani, H. lindae, H. phyllognathus, H. lascinius*) and a remarkable genetic divergence expressed as pairwise distances among and within recovered clades. Interestingly the results suggest various patterns in terms of genetic variability in *Hyloscirtus*. The species of the clade formed by the species of the *H. bogotensis* group have considerable genetic divergence exceeding 24.0%, for example, *H. colymba* (SIUCH 7079) comparatively with *Hyloscirtus* sp "perija" (LEP 233). The clade containing all species of the *H. larinopygion* group, excluding the clade geographically of southern Ecuador and northern Peru (see systematics implications section), possesses genetic divergences among species from 0.6% to 8.5%. While for those species from southern clade (i.e *Hyloscirtus tapichalaca* group) the genetic divergence is from 7.0% to 8.2%. *Hyloscirtus armatus* was retrieved as non-monophyletic with *H. charazani* nested within this, predominantly in a clade with many polytomies.

Hyloscirtus lindae is recovered as paraphyletic with three independent origins. The population of *H. lindae* represented by the specimens from Sucumbios, Ecuador (QCAZ voucher's) is sister species to *H. pantostictus* and this clade is the sister to *H. lindae* (ICN 49951) from Putumayo, Colombia. Furthermore, *H. lindae* from Plan de Milagro (Ecuador, specimen KU202728) was recovered as sister species of *H. tapichalaca.* The 16S sequences were not obtained from the population of Plan de Milagros, however, *H. lindae* series (KU 202728–31) was reviewed and is morphologically distinct therefore does not correspond to the same entity and constituting a taxonomic misidentification and is therefore an undescribed species (see species account section, for further discussion). Intraspecific genetic divergences of *H. lindae* from Putumayo and Sucumbios populations ranged from 0.0% to 1.7%, and between them and *H. pantostictus* from 0.6% to 1.9%. Remarkably *H. pantostictus* and *H. lindae* of Colombia have a genetic divergence of 0.8%.

Hyloscirtus phyllognathus was recovered as non-monophyletic, into two clades showing high support values and having very variable interpopulational genetic divergences (0.0–20.1%) partially associated with allopatric geographic regions (Appendix 4).

Hyloscirtus lascinius was recovered as paraphyletic. The sample KU 181056 from the type locality at Delicias (Tachira, Venezuela) and a sample (JMR 2515) from Chinacota (Norte de Santander, Colombia) about 15 kms in a straight line between the two localities are clustered together with *H. denticulentus* from Santander, Colombia and forming the most recent clade of *Hyloscirtus*. The samples of *H. denticulentus* including one specimen (MRC 624) from its type locality have a genetic divergence of 0.2% with the *H. lascinius*, suggesting that both are conspecifics. These results are also supported by morphological data that indicates no variation or diagnostic characters to differentiate these two populations.

NEW CANDIDATE SPECIES

Despite recent contributions in taxonomy (Coloma et al., 2012; Rivera-Correa and Faivovich 2013; Almendáriz et al., 2014), the results herein indicate that *Hyloscirtus* is more diverse than previously suggested, and have a series of fifteen candidate species. Available morphological and/or bioacoustical data, allowed confirming the state of nine species as undescribed.

As mentioned above, the population of *H. lindae* (KU202728) from Plan of Milagros-Ecuador, which is a confirmed candidate species [(CCS), Ca1] and in addition to *H. condor* recently described by Almendáriz et al. (2014). In addition, *Hyloscirtus* sp "chachapoyas". (CORBIDI 12885) from Chachapoyas-Amazonas, Perú is a confirmed candidate species [(CCS), Ca2], morphologically distinct from *H. condor* (QCAZ 54588 and QCAZ 54590) and with genetic divergence between the two species from 8.2%. Added to *H. tapichalaca* this clade is now comprised of four species, two of which are described herein (Fig. 5)

The samples of *Hyloscirtus* sp from San Juanito-Meta, Colombia is a recently metamorphosed specimen, thus, the morphological diagnostics attributes were unavailable. However, the phylogenetic position and the very low genetic divergence (ranging from 1.3%

to 1.7%) suggest that could be conspecific with *H. criptico* (Appendix 5). Thus, no taxonomic decision is taken until further adult sample are available.

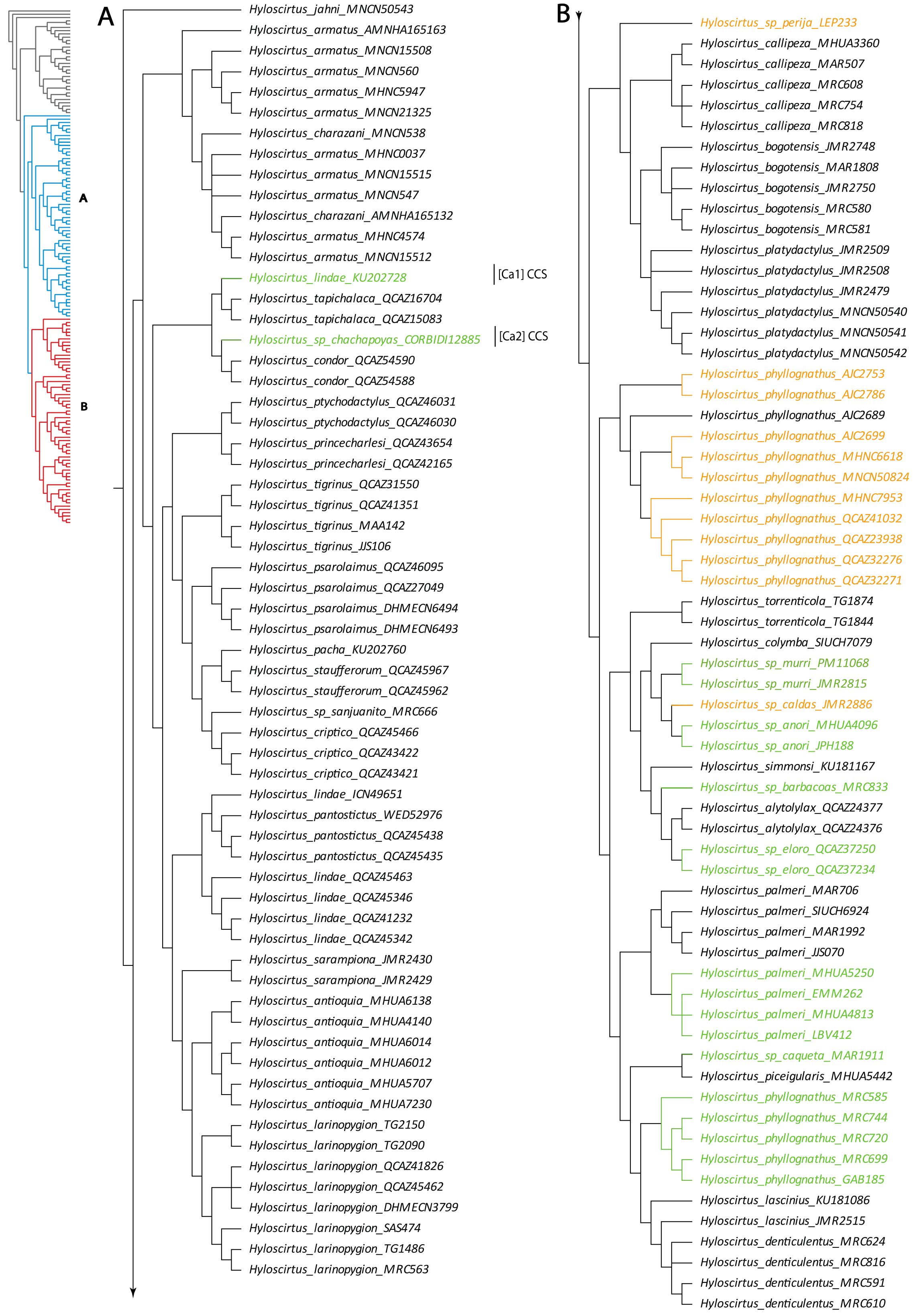

FIGURE 5. Phylogenetic relationships of *Hyloscirtus* inferred from maximum parsimony analysis under dynamic homology. The topology reflects consensus tree (length 16306 steps). Candidate new species are highlighted color: Confirmed (CCS, green), Unconfirmed (UCS, orange). A skeletal topology for reference shown on the left side and the magnified section is marked in bright color.

Within the *H. bogotensis* species group, the sample *Hyloscirtus* sp. (LEP 233), (UCS), Ca3) from Perijá, northern Colombia isolated in the Serranía del Perija was recovered as sister species to the clade formed by [*H. callipeza* (*H. platydactylus* + *H. bogotensis*)]. The specimen have high interspecific genetic divergences (ranging from 9.5% to 11.1%) with respect to *H. callipeza* and *H. platydactylus,* plus a phylogenetic position suggests that is a candidate species to be described. However, it remains unconfirmed at the time because vouchers specimens were unavailable to establish whether there are diagnostic morphological attributes and provide a formal description.

Despite morphological conservatism between the two clades of *H. phyllognathus*, suggests at least two independent genetic lineages. A "southern" clade from Ecuador and Peru and a "northern" clade from Colombia. The genetic divergence within clades is relatively high, to the southern clade reaches 15.4%. The sample (AJC 2689) from Tarapoto-San Martin, Perú to 40 Kms away from the type locality (Roque, San Martin), may belong to *H. phyllognathus* sensu stricto. Thus, this further suggests the probable existence of various unnamed species within of southern clade. In some cases, these results are supported by bioacoustical data (S. Ron and A. Catenazzi pers. comm.). Because I have not had access to the vouchers, I would rather suggest these lineages as unconfirmed candidate species, therefore no taxonomic decision is taken.

Hyloscirtus colymba with distribution in Central America was recovered as sister species to a clade formed by three independent lineages of Cordillera Central and Cordillera Occidental in Colombia. Two of these lineages consist into two confirmed candidate species and one unconfirmed candidate species. *Hyloscirtus hydrophilus* sp nov. [(CCS), Ca10] from

Anori-Antioquia, Colombia is recovered as sisters species from *Hyloscirtus* sp. "caldas" [(UCS), Ca9] with a genetic divergence from 6.6%, but devoid of phenotypic characteristics to differentiate between them. The latter clade is closely related to *Hyloscirtus murri* sp nov. [(CCS), Ca8] other confirmed candidate species and with a genetic divergence from 12.0% to 14.6%. The results here recover a clade geographically located in southwestern of Colombia and Central West of Ecuador clustering four species, *H. alytolylax* and *H. simmonsi* and two confirmed candidate species. *Hyloscirtus baios* sp nov. [(CCS), Ca11] was recovered as sister species to the clade grouping *H. alytolylax* and *Hyloscirtus oro* sp nov. [(CCS), Ca12] with genetic divergence from 8.5% to 8.7% and while the genetic distance between latter species range from 5.1% to 5.3% (Appendix 3).

The specimens of *H. palmeri* form two clades with high support, one from Choco-Darien biogeographic region with four samples, two of them near the type locality in Valle del Cauca, Colombia, cluster together with a second clade from the Middle Magdalena River valley, Colombia, and that comprised of four samples also. The geographically restricted, large geographic distance among these and large genetic divergences (11.7% to 15.2%) separate these two clades, further phenotypic evidence allows to assert that the inter-Andean lineage is a confirmed candidate species [(CCS), Ca13] and traditionally associated with *H. palmeri.*

Hyloscirtus hyalinus sp nov. [(CCS), Ca14] sister lineage of *H. piceigularis* and morphologically distinct is a confirmed candidate species, show a 6.1% genetic divergence between them, the latter is also distributed in the Cordillera Oriental in Colombia. The northernmost lineage of *Hyloscritus phyllognathus*, represented by five specimens from the Cordillera del Oriental, Boyacá, northeastern Colombia is a confirmed candidate species [(CCS), Ca15] with molecular diagnostics and bioacoustic characters. Sequence divergence between the Colombian clade of *H. phyllognathus* and (*H. lascinius* + *H. denticulentus*) was

4.4% and 5.9%. The figure 7 shows the geographical distribution where new species are located.

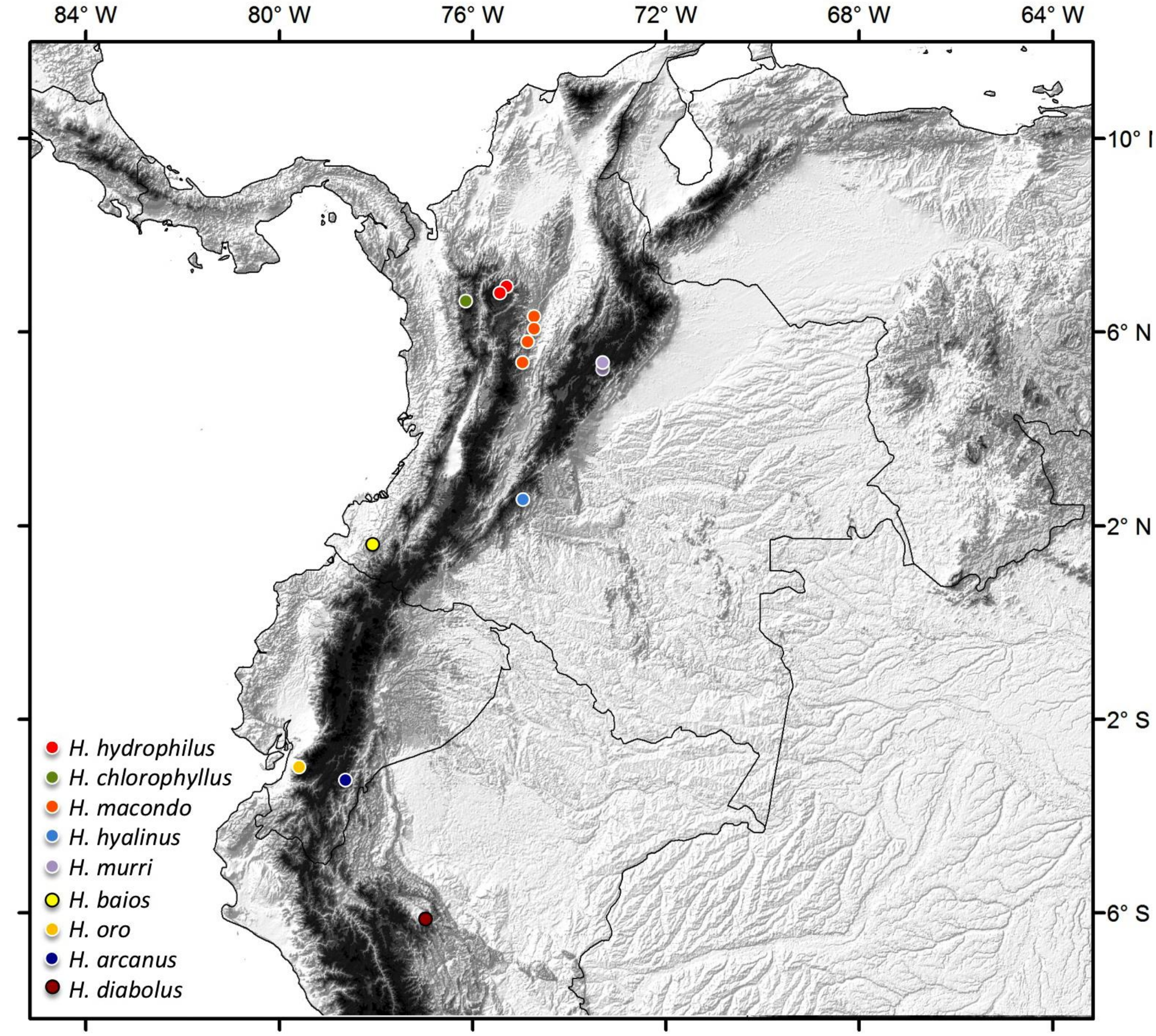

FIGURE 7. Geographic distribution of nine new species of *Hyloscirtus*. Dots corresponding to localities from which tissue samples were sequenced for this study.

TAXONOMIC STATUS OF *HYLOSCIRTUS LASCINIUS* (RIVERO, 1970) AND *HYLOSCIRTUS DENTICULENTUS* (DUELLMAN, 1972)

Hyloscirtus lascinius was described by Rivero (1970) based on seven specimens obtained at Tabor, above Delicias, region of Páramo de Tamá, 1700 m., Estado de Tachira, Venezuela. This species was diagnosed as a species of medium sized; green in life; almost vertical loreal region; anteriorly converging vomerine odontoids; 1/3 webbed fingers; disks larger than the tympanum; a distinctive transverse cloacal fold; a distinct fold along the forearm, a large, triangular lappet at the heel; a tarsal fold; fully webbed toes (with the exception of the fourth, where two phalanges are left free) and black-margined nostrils. Subsequently, Duellman (1972) published a review of the *Hyloscirtus bogotensis* group. In that work he described *H. denticulentus* based on two individuals at Municipio de Charta, Departamento de Santander and Municipio de Valdivia, Departamento de Antioquia, both localities in Colombia. *Hyloscirtus denticulentus* (Fig. 8) was diagnosed by having webbing extending beyond distal subarticular tubercle on fourth finger; distal subarticular tubercle on finger bifid and flat; ulnar and tarsal folds present; calcar large; snout in profile anteriorly inclined; tympanum distinct; mental gland absent; dorsolateral stripe absent; tarsal and cloacal stripes white above, brown below; and tadpoles having 7-8/10 tooth rows, tooth rows of labial papillae and no serrations on beaks.

Limited information has been published for both species since its original publication. Ruiz-Carranza and Lynch (1982) extended the geographical distribution of *H. denticulentus* to the Municipio de Tona, Departamento de Santander, Colombia. Meanwhile, Mijares-Urrutia (1992) described the larva of *H. lascinius*. Ruiz-Carranza, et al., (1996), noted that the paratype

(KU 133452) of this species is referable to *H. palmeri*. Faivovich et al., (2005) include *H. lascinius* in *H. bogotensis* group. Posteriorly, Sánchez (2010) evaluated the morphological variation in tadpoles of both species in different stages of development, grouping them in the same phenetic group. Finally, it is likely that the manuscripts of both species were being evaluated at the same time, due to this; Duellman´s did not include a comparison with *H. lascinius* in the original description of *H. denticulentus*. Given this, I present the inferences derived below:

FIGURE 8. *Hyloscitus denticulentus* dorsal and ventral view. Holotype, adult male, KU 133451, SVL 36.6 mm. Photos: M. Rivera-Correa.

Morphology: I compared the holotype of *H. lascinius* (Rivero, 1970) and holotype and paratype of *H. denticulentus* and I analyzed additional material of both species and no there evidence to consider both species as independent entities. I examine the diagnostic characters proposed by Duellman (1972: 22) to distinguish *Hyloscirtus denticulentus* from other conspecific species and demonstrate how these are also present in *H. lascinius:* (1) *Hyloscirtus denticulentus* and *H. lascinius* having webbing extending beyond distal subarticular tubercle on fourth finger, however, this character is polymorphic, because in a topotype specimen (MRC 624) only reaches to the distal subarticular tubercle; (2) in both entities the distal subarticular tubercle on finger is bifid, but the flat condition could be the result of fixing, since some specimens evaluated have this globular structure; (3) both species have ulnar and tarsal folds and this is conspicuous; (4) the snout is anteriorly inclined in lateral view both in *H. denticulentus* and *H. lascinius,* although in juveniles this may be less obvious and in some cases the snout is truncated; (5) both species have calcar tubercle development and more prominent in adult females; (6) the tympanum is distinct to two species, however, Rivero suggested that the tympanum is not distinctive, this is present and noticeable in the female holotype and other specimens reviewed, it is possible that he was referring to the tympanic annulus, because it is not visible; (7) the mental gland is present to both species, Duellman suggested absence of mental gland, however, the holotype and paratype are females contrary to that observed by him who determined of paratype as an adult male; (8) the dorsolateral stripe (= supratympanic pale stripe) is absent both in *H. denticulentus* and *H. lascinius*; (9) both species having the tarsal and cloacal stripes (herein supracloacal fold) white above and brown below; (10) Duellman reported tadpoles with tooth rows of labial papillae and beaks without serrations in *H. denticulentus*, this character states is also present in *H. lascinius; H. denticulentus* have 7-8/10 tooth rows, nevertheless Mijares-Urrutia (1992) found LTRF variation to *H. lascinius* of 6-8 (6-8)/7-11(1), depending on the state of development of the larvae.

Genetic: I compared mitochondrial partial sequence of 12S rRNA gene from a topotype specimen available in genbank (KU 181086; DQ380359) to *H. lascinius* with a fragment of the same length (452 bp) of the type locality to *H. denticulentus* (MRC 684). At the molecular level, *H. denticulentus* differs from *H. lascinius* by 3 fixed nucleotide differences in 12S rDNA, this represents 0.6% of genetic divergence (uncorrected p-distances) between both species. This genetic divergence is very low between species of the *H. bogotensis* group (Appendix 4).

Taxonomic implications: In light of our morphological and genetic results, there no evidence for supporting the specific status of *Hyloscirtus denticulentus*, therefore we consider *H. denticulentus* (Duellman, 1972) a junior synonym of *H. lascinius* (Rivero, 1970).

Hyloscirtus lascinius (Rivero, 1970)
Hyla lascinia Rivero, 1970
Hyloscirtus lascinius (Faivovich et al., 2005)
Boana denticulenta (Wiens et al., 2005)
Hyloscirtus denticulentus (Duellman, 1972)

Holotype: MCZ 65901, (adult female) from Tabor, above Delicias (opposite Herran, Colombia), region of Páramo de Tamá, 1700 m., Estado Táchira, Venezuela.

Description: This species is placed in the *Hyloscirtus bogotensis* group (Duellman, 1972; Faivovich et al., 2005) because adult males have mental gland. The description provided by Rivero (1970) is adequate; however, it was based only on few individuals and some character state were ignored or misinterpreted, additionally more specimens have been collected in the last years; therefore deserves an update. These new specimens indicate that males of this species have a large mental gland as in all adult males from the *Hyloscirtus bogotensis* group; it is elliptic, covers the gular area and extending about the half-length of the throat.

Diagnosis: Hyloscirtus lascinius differs from all other species of *H. bogotensis* group by the following combination of character states: Snout truncate or slightly sloping in lateral view; loreal region nearly flat; upper lip flared and white with brown markings; black nostril margin; ulnar, tarsal and supracloaclal fold white above and brown below present; calcar tubercle prominent; and white warts ventrally extending 3/4 of the length of the thigh. Some individuals, especially males with large pink patches on dorsum and flanks, unique pattern in the group.

Characterization: The species is characterized by the following combination of characters: (1) medium size (SVL 36.7–42.6 mm in males, n = 14; 45.5–55.6 in females, n = 8); (2) body slender, head wider than body; (3) snout rounded in dorsal view and truncate or slightly sloping in lateral view; (4) dorsal and flanks green with scattered black spots, white markings and pink blotches, gular region light green and ventral surfaces yellowish in life (Fig. 9); (5) axillar region light blue and inguinal light orange; (6) large mental gland in males present, light yellow; (7) upper lip flared white with brown markings and black nostril margin; (8) parietal peritoneum white present; (9) iris light pink with abundant tiny maroon reticulation; (11) nuptial pad absent; (13) tympanum rounded upper edge of covered by a supratympanic fold and tympanic annulus only visible in females; (14) canthal striped and supratympanic pale stripe absent; (15) ulnar fold and tarsal fold present, white above and brown below; (16) calcar tubercle present, prominent; (17) supracloacal fold white above and brown below present; (18) white warts around and below cloaca, ventrally extending 3/4 of the length of the thigh; (19) green bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) large dentigerous processes of vomers, anteromedially inclined, with discernible gap, with 7–11 teeth each; (22) the advertisement call consists of one unpulsed note, 0.072–0.221 s of duration and a dominant frequency of 2239.5 to 2411.7 Hz.

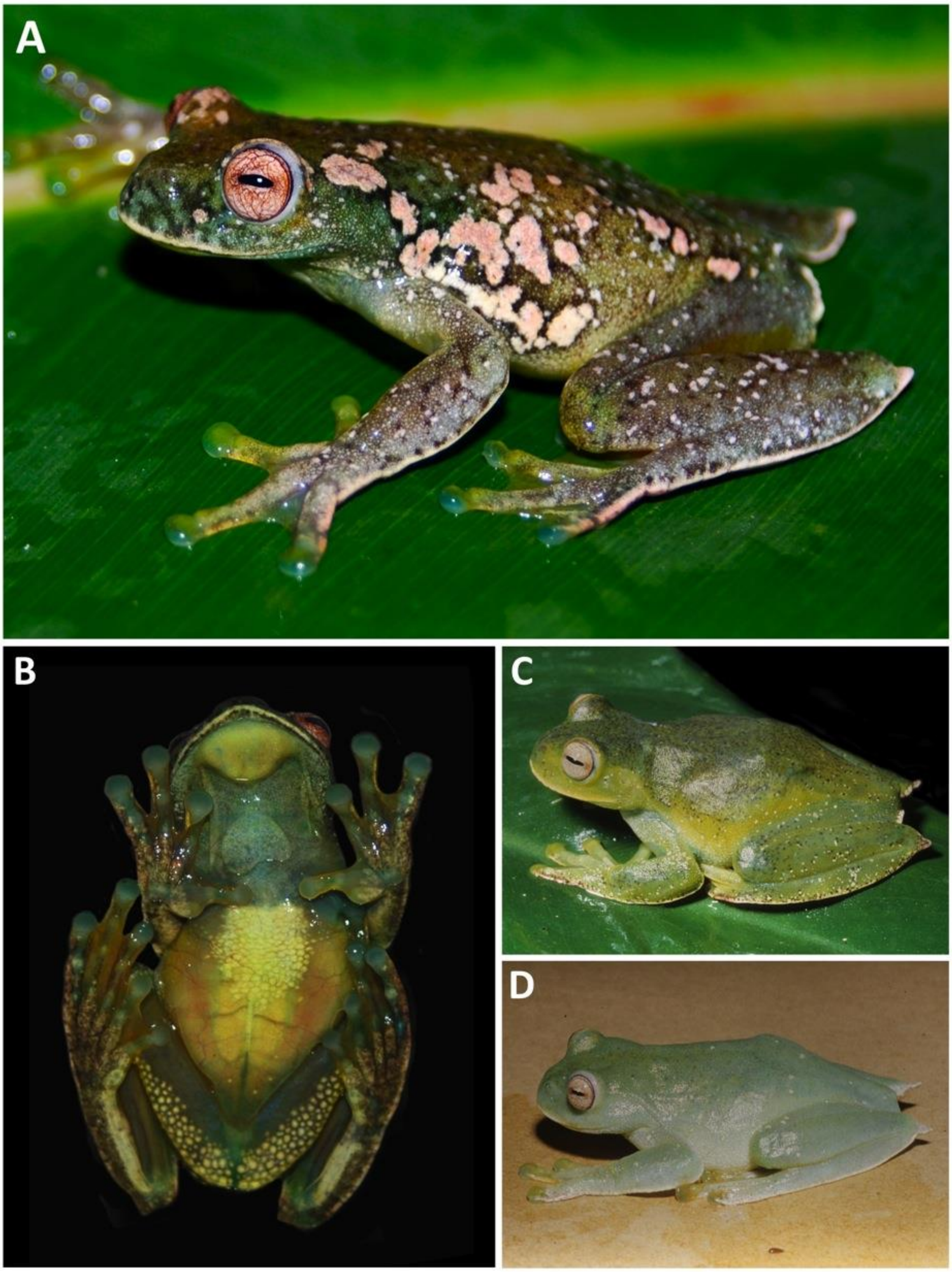

FIGURE 9. *Hyloscirtus lascinius* in life. (A and B) Lateral and ventral view (MRC 760, adult male, SVL 42.6 mm,); (C) lateral view (ICN 15031, adult female, SVL 54.7); (D) lateral view (ICN 15055, adult female, SVL 53.6). Photos: M. Rada (A-B); J. Lynch (C-D).

Distribution and natural history: Hyloscirtus lascinius is distributed in the buffer zone of the

Tamá Natural Park, Colombian-Venezuelan border, where is type locality, Estado de Tachira in Venezuela; there are also records in both the western and eastern flank of the cordillera Orienta, Departamento de Norte de Santander and Santander in Colombia. The natural history of *Hyloscirtus lascinius* was briefly discussed by Rivero (1970) describing that the species inhabits mountain streams, in bushes between 0.90-1.20 m from the surface of the water; the males were observed calling and not dwell in syntopy with other *Hyloscirtus* in Venezuela. However, in some populations of Colombia is sympatric with *H. callipeza* and tadpoles of both species coexist in the same streams. I detected only two adult males calling in a creek at a distance of 500 m. approximate each other at Santander, Colombia. The ovarian oocytes in two females (ICN 15070) are cream unpigmented, 2.6–3.4 mm diameter (*n* = 24).

Advertisement call: Two calling males of *H. lascinius* were recorded at 19:00 h and 22:00 on leafs or branch of bush between 0.30 to 1.50 m high approximately over stream. The advertisement call (calls = 40) is always composed of one acoustic and semi-pulsed note with shorter mean call length 0.130 ± 0.011 s (0.116–0.148) repeated at a low rate (22–30 calls/min). The peak (dominant) frequency coincides with the fundamental frequency 2296.9 ± 86.10 Hz (2239.5–2411.7), and the call may have at least two additional harmonically related frequencies with mean to (4681.3–7166.7 Hz), respectively.

The highest energy input was on the second semi-pulse. The amplitude modulation of each note has a pattern consisting of ascending intensities from the beginning up to a one-quarter of the note and decreasing rapidly and rises again to the middle of the call, where has visibly higher wave amplitude and decreasing towards the end (Fig. 10).

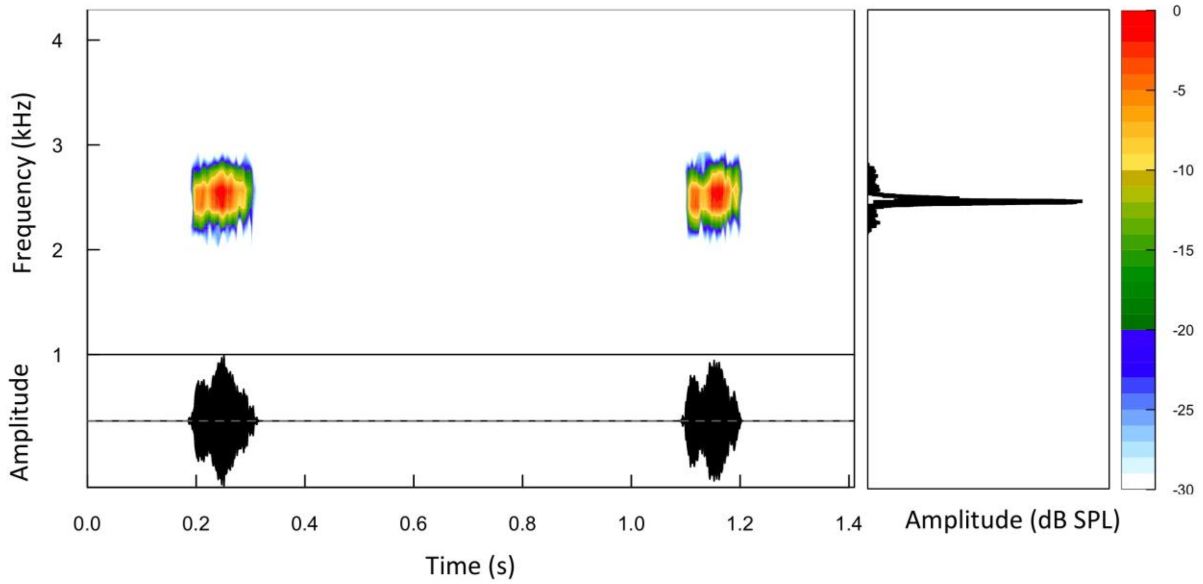

FIGURE 10. Spectrogram, oscillogram and power spectrum showing two advertisement call of *Hyloscirtus lascinius* (MRC 760; SVL 42.6) from Pie de Cuesta, Departamento de Santander, Colombia.

HYLOSCIRTUS BAIOS SP. NOV

Holotype: PR 16275, adult male, Colombia, Nariño, Municipio de Barbacoas, Corregimiento de Altaquer, Inspección de Policia, Quebrada La Carbonera (1º14′45″ N, 78º06′54″ W, 1100 m a.s.l.), collected on 14 March 1995 by Maria C. Ardila-Robayo and Claudia Vélez.

Paratypes: All adults. PR 16276, 16278, 16280, males, collected with the holotype.

Paragenetype: MRC 833 (16S rRNA)

Referred specimens: PR 16161, subadult female, Colombia, Nariño, Municipio de Barbacoas, Corregimiento de Altaquer, Quebrada la Carbonera (1º14′45″N, 78º06′54″W, 1020 m a.s.l.).

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 29.9–31.0 mm in males, n = 5); (2) body slender, head as wide as the body; (3) snout acuminate in dorsal view and slightly protruding in lateral view; (4) dorsal and flanks yellow with maroon spots, pattern of spots forming an interorbital and transversal band with T-shape, gular region yellowish green and ventral surfaces yellowish in life (Fig. 11); (5) axillar and inguinal region light yellow; (6) small mental gland in males present; (7) upper lip without white stripe, not flared and immaculate nostril margin; (8) parietal peritoneum white present; (9) iris brown; (11) nuptial pad absent; (13) tympanum rounded subtly conspicuous, upper edge covered by a weak supratympanic fold and, tympanic annulus inconspicuous; (14) canthal striped absent and supratympanic pale stripe present; (15) ulnar and tarsal fold absent; (16) calcar tubercle absent; (17) supracloacal fold absent (18) tubercles scattered around and below cloaca absent; (19) green bones in life; (20) small elliptical prepollex, not modified as a projecting spine; (21) small dentigerous processes of vomers, slightly curved, separated, with 5–10 teeth each; (22) the advertisement call consists of three to ten notes, all of same duration, 0.240–0.713 s of duration and a dominant frequency of 3186.9 to 3445.3 Hz.

FIGURE 11. *Hyloscirtus baios* sp. nov., in life (PR 16275, holotype, adult male, SVL 30.1 mm) Photo: P. Ruiz-Carranza

Comparison with other species: At the molecular level, *H. baios* sp. nov., differs from *H. alytolylax* and *H. oro* sp. nov., their sister clade by 43 to 44 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 8.5-8.7% of genetic divergence (uncorrected p-distances) between both. The new species is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group by its small size. *Hyloscirtus baios* having supratympanic pale stripe (absent in *H. albopunctulatus*, *H. bogotensis*, *H. chlorophyllus* sp. nov., *H. hyalinus* sp. nov., *H. lascinius, H. lynchi, H. macondo* sp. nov., *H. palmeri, H. platydactylus, H. piceigularis, H. phyllognathus*, *H. simmonsi* and *H. torrenticola*), ulnar fold absent (present in *H. albopunctulatus, H. alytolylax, callipeza, H. colymba, H. hyalinus* sp. nov., *H. hydrophilus* sp. nov., *H. jahni, H. lascinius, H. macondo* sp. nov., *H. palmeri, H. phyllognathus, H. piceigularis, H. oro* sp. nov., *H. torrenticola*), supracloacal fold absent (present in *H. albopunctulatus, H. alytolylax*, *callipeza, H. chlorophyllus* sp. nov., *H. colymba, H. hyalinus* sp. nov., *H. hydrophilus* sp. nov., *H. macondo* sp. nov., *H. palmeri, H. phyllognathus, H. piceigularis*)

Hyloscirtus chlorophyllus sp. nov., *H. hyalinus* sp. nov., *H. lascinius*, *H. macondo* sp. nov., *H. palmeri*, *H. piceigularis*, and *H. phyllognathus*, also differ by having calcar tubercle and white labial stripe (absent in *H. baios* sp. nov.). *Hyloscirtus baios* sp. nov. is most similar to *H. alytolylax,* but differs from them by being smaller and lacking supracloacal fold. Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

Description of holotype: An adult male of 30.1 mm SVL (Fig. 12). Body slender. Head as wide as long; head as wide as body; head width 34% SVL; head length 34% SVL. Snout acuminate in dorsal view and slightly protruding in profile (Fig. 13); *canthus rostralis* rounded, slightly distinct; loreal region concave; lips rounded, not flared. Canthal stripe absent. Nostrils slightly protuberant, directed anterolaterally, at level to anterior margin of lower jaw. Internarial region and top of head concave. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum barely visible and, rounded and slightly discernible from above; its diameter equals 29% eye diameter; tympanic annulus inconspicuous. Supratympanic fold weakly developed, starting in the anterior region of tympanum, slightly covering the dorsal margin of this and without reaching the posterior margin of the insertion of the arm; supratympanic stripe present. Mental gland present, medium, semicircular, covering partially of the gular area and extending about the third length of the throat.

FIGURE 12. *Hyloscirtus baios* sp. nov., dorsal and ventral view. Holotype, adult male, PR 16275, SVL 30.1 mm. Photo: M. Rivera-Correa.

Dentigerous processes of vomers slightly curved, separated and posterior ovoid choanae; each series bears five (right) and seven (left) teeth. Choanae separated by a distance larger than 2.5 times their maximum diameter, equal diameter regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

Forearm slender; axillary membrane absent. Outer ulnar fold absent. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III larger than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers without dermal fringes; webbing formula I – II 2^{-}–3 III $2^{1/3}$–2^{+} IV (Fig. 13). Subarticular distal tubercles large and elliptical. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine; nuptial excrescencies absent.

Hind limbs slender; tibia length 50% SVL; foot length 41% SVL. Outer tarsal fold and calcar tubercle absent; inner tarsal fold indistinct. Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 < 5 < 4; extensive toe webbing, formula I 1^{+}–1 II 1^{+} – 2 III 1^{+}–2 IV 2 –1 V (Fig. 13). Inner metatarsal tubercle elliptical, barely visible from above; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive. Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent without tubercle; supracloacal fold absent; sheath very short; tubercles scattered around and below cloaca absent. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

Color in life of the holotype: Dorsal surfaces and flanks yellow with maroon spots, pattern of spots forming an interorbital and transversal band with T-shape. Vocal sac and ventral surfaces yellowish green and medial area with a white parietal peritoneum. Yellow mental gland with tiny brown spots; ventral surfaces of fingers, toes, webbing on hands and feet yellow-green; axillar and inguinal region light yellow. Iris brown; bones green.

Color in preservative of the holotype: Dorsal surfaces creamy with dark scattered brown spots, some forming a T-shape pattern; flanks, fingers, toes, webbing and shanks creamy yellow with tiny brown spots. Ventral surfaces creamy white and gular region creamy white with tiny brown spots on mental gland. Supratympanic stripe creamy-white and, peritoneum white.

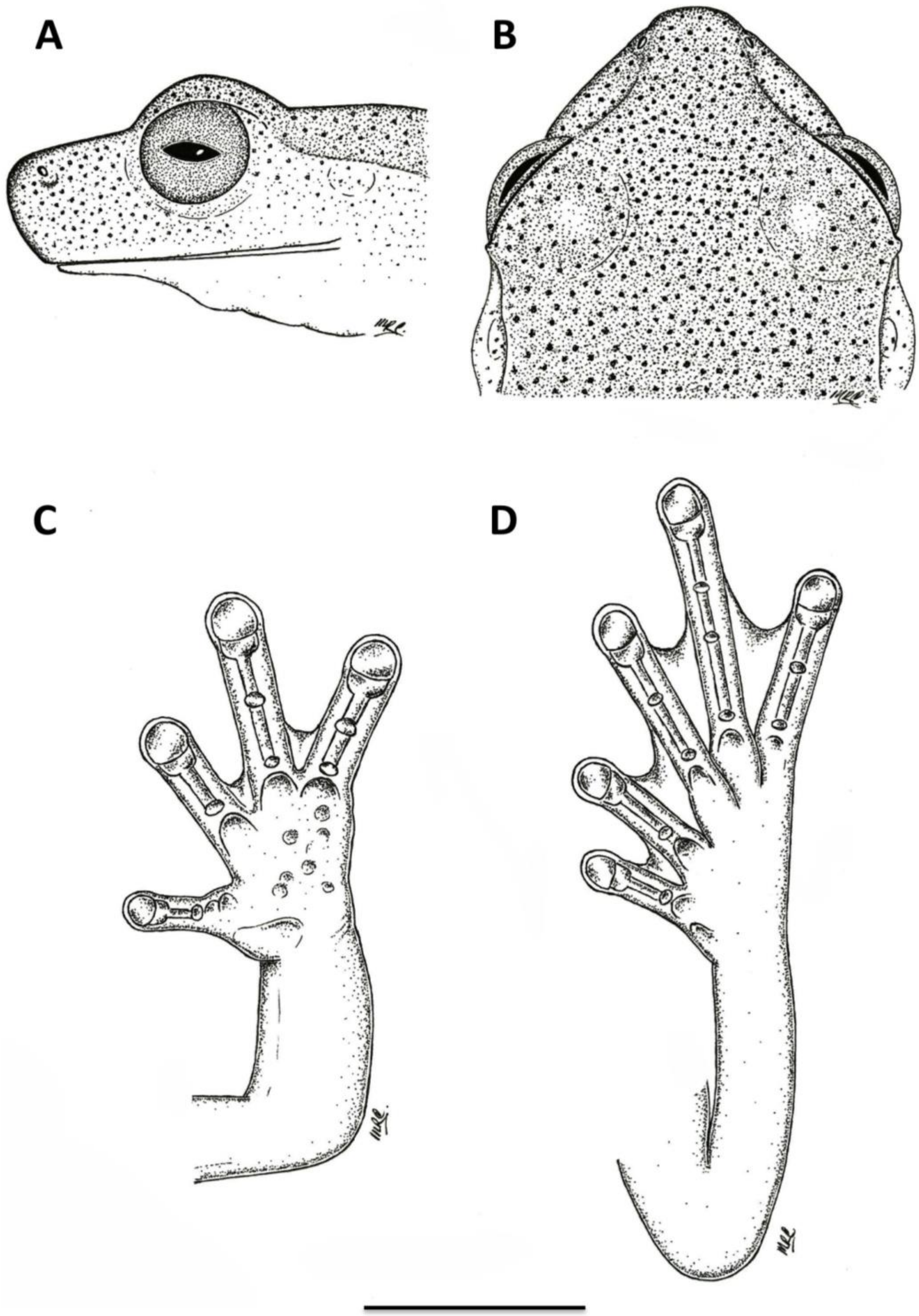

FIGURE 13. *Hyloscirtus baios* sp. nov., holotype, PR 16275. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Measurements of the holotype (in mm): SVL 30.1; HL 10.2; HW 10.3; ED 3.5; END 2.1;

NSD 1.4; IND 2.5; AMD 5.5; TD 1.0; FAL 5.5; FAB 2.3; HAL 9.1; THL 14.8; TL 15.2; TAL 8.2; FL 12.5; TFD 1.5; FTD 1.4.

Variation: Measurements are given in Appendix 7. Size males between 29.2 to 31.0, n = 5) of SVL. The number the maroons spots vary among individuals, in PR 16276 are more abundant. The vomerine odontophores process is narrower in holotype regarding the other individuals. Number of vomerine teeth 5–8 / 7–10. The finger-webbing formula varies as follow I – II ($1^{1/2}$–2^{-}) – (3^{+}–3^{-}) III (2– $2^{1/2}$) – (2–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–$1^{1/3}$) – (1^{+}–$1^{1/2}$) II (1^{+}– $1^{1/3}$) – (1^{-} – $1^{1/2}$) III (1^{+}–$1^{1/3}$) – (2–2^{-}) IV (2–2^{-}) – (1^{+}–1) V.

Distribution and natural history: Hyloscirtus baios sp. nov. is known from type locality between 1020–1400 m, on the southwestern of the Cordillera Occidental from Colombia. Individuals of *H. baios* sp. nov. inhabit streams; calling males were observed on bushes along the streams at night, the only specimen recorded was on a leaf to 2.5 m high.

Advertisement call: The calling male (PR 16275) of *H. baios* was recorded at 19:30 h on leaf of shrub to 2.5 m high above the creek. The advertisement call (25 calls) is composed three to eight acoustic notes (6 ± 1.61) with call length between 0.240–0.713 s (0.569 s ± 0.15) repeated at a low call rate and (10–12 calls/min) and 62 to 66 notes per minute. The dominant (peak) frequency is equal to the fundamental frequency (3186.9–3445.3 Hz; 3333.3 ± 101.15), and the call may have at least two additional harmonically related frequencies (not shown) with mean to (9978.5–16643.9 Hz), respectively (Fig. 14)

Etymology: The specific name is from the Greek word *baios* meaning “small” and is used here as an adjective. *Hyloscirtus baios* is the smallest species in the genus to now known.

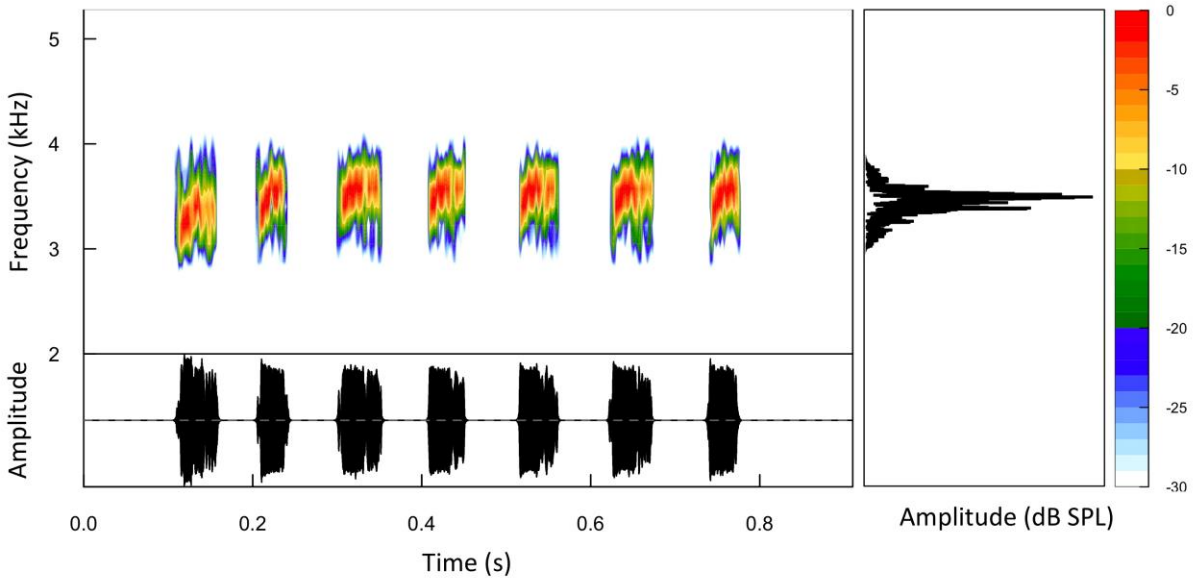

FIGURE 14. Spectrogram, oscillogram and power spectrum showing one advertisement call of *Hyloscirtus baios* sp. nov. (PR 16275, holotype; 21 ºC) from Altaquer, Barbacoas, Nariño, Colombia.

HYLOSCIRTUS CHLOROPHYLLUS SP. NOV.

Hyla phyllognathus. – Ruiz-Carranza and Lynch (1982: ; part).

Hyla phyllognathus. – Ruiz-Carranza et al., (1996:386; part).

Hyla phyllognathus. – Acosta-Galvis et al., (2006:299).

Holotype: MRC 744, adult male, Colombia, Boyacá, Municipio de Miraflores, Inspección Guayabal, Vereda Tunjita, Finca San José (05º06'42.8"N 73º12'27.8"W, 2190m a.s.l.), collected on 17 May 2010 by Mauricio Rivera-Correa and Marco Rada.

Paratypes: One adult. MRC 720, male, Colombia, Boyacá, Municipio de Miraflores, Inspección Guayabal, Vereda Tunjita, Quebrada Agua Blanca (05º05'43.8"N 73º14'6.6"W, 1710 m a.s.l.), collected on 16 May 2010 by Mauricio Rivera-Correa and Marco Rada.

Paragenetype: MRC 744 (16S rRNA)

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 34.8–36.6 mm in males, n = 2); (2) body slender, head barely wider than long; (3) snout truncate dorsal view and truncate or slightly sloping in lateral view; (4) dorsal and flanks green without or with scattered brown and black spots and blotches; gular and ventral surfaces region yellowish green in life (Fig. 15); (5) axillar and inguinal region light blue; (6) mental gland in males present; (7) upper lip white with brown markings, not flared and black nostril margin; (8) parietal peritoneum white present; (9) iris cream with abundant red reticulation; (11) nuptial pad absent; (13) tympanum rounded, inconspicuous and upper edge of tympanic annulus covered by a weak supratympanic fold; (14) canthal striped and supratympanic pale stripe absent; (15) ulnar fold and tarsal fold present, white above and brown below; (16); calcar tubercle present; (17) supracloacal fold white above and brown below present; (18) white warts around and below cloaca, ventrally extending 1/4 of the length of the thigh; (19) green bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) large dentigerous processes of vomers, anteromedially inclined, with discernible gap and with 6–8 teeth each; (22) the advertisement call consists of one note with one to three semi-pulses with 0.087–0.176s of duration and a dominant frequency of 2411.7 to 2928.5 Hz.

Comparison with other species: At the molecular level, *H. chlorophyllus* sp. nov. differs from *H. lascinius* its sister species by 21 to 29 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 4.2-5.7% of genetic divergence (uncorrected p-distances) between both species. The new species is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having calcar tubercle (absent in *H. alytolylax, H. baios* sp. nov, *H. bogotensis*, *H. callipeza*, *H. colymba, H. hydrophilus* sp. nov., *H. jahni*, *H. lynchi*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi, H. torrenticola*), white labial stripe present (absent in *H. alytolylax*, *H. baios* sp. nov, *H. bogotensis*, *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. lynchi*, *H. jahni*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi*), supracloacal fold present (absent in *H. baios* sp. nov., *H. bogotensis*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*).

Hyloscirtus alytolylax, H. baios sp. nov., *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. jahni, H. oro* sp. nov and *H. simmonsi* also differ by having supratympanic pale stripe (absent in *H. chlorophyllus* sp. nov). *Hyloscitus chlorophyllus* is most similar to *H. hyalinus* sp. nov *H. lascinius* and *H. phyllognathus* but differs from them by having white warts ventrally extending only to 1/4 of the length of the thigh (3/4 in *H. hyalinus* sp. nov and *H. lascinius*). Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

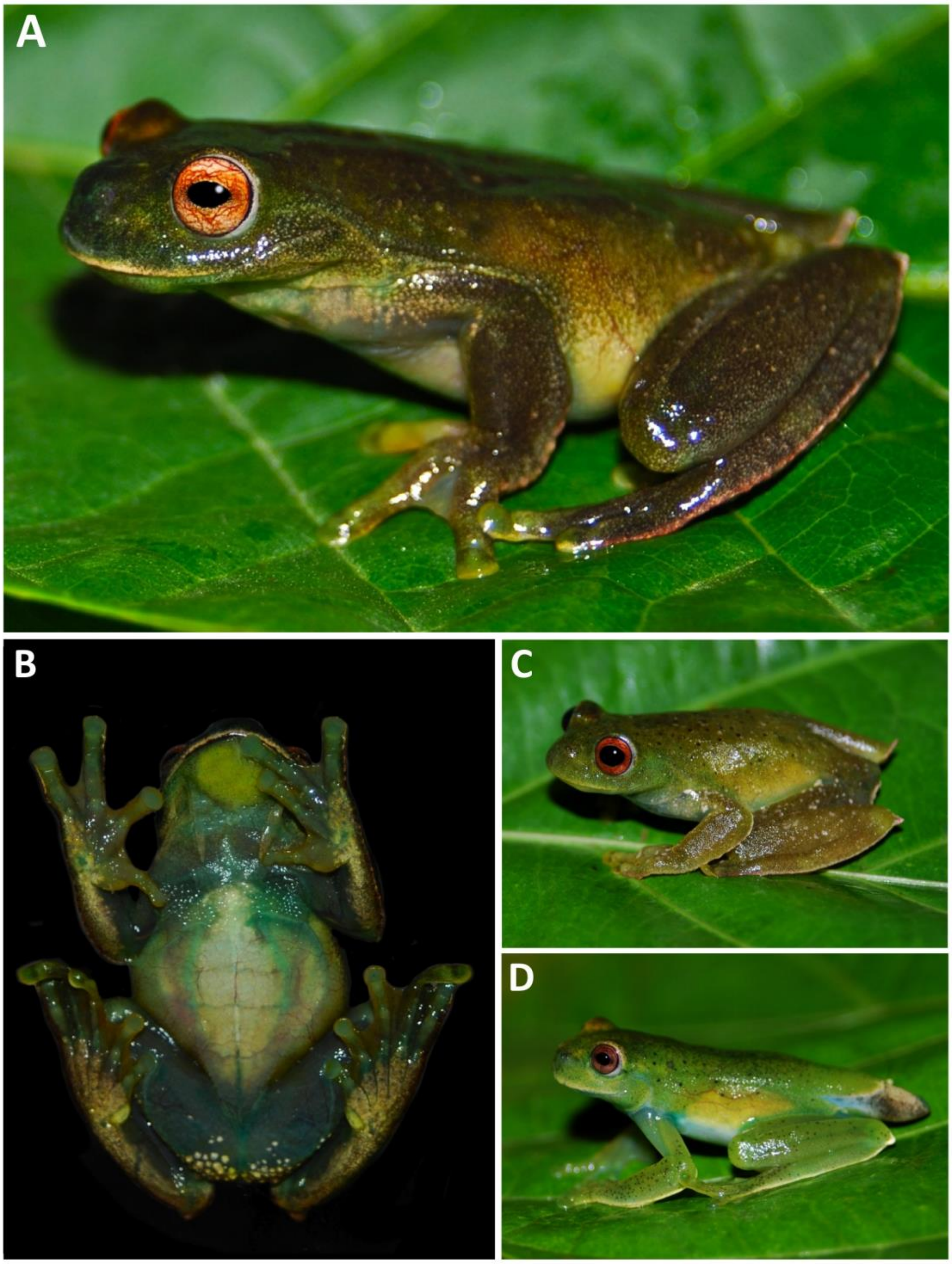

FIGURE 15. *Hyloscirtus chlorophyllus* sp. nov., in life. (A and B) Lateral view and ventral view (MRC 744, holotype, adult male, SVL 36.6 mm); (C) lateral view (MRC 720, paratype, adult male, SVL 34.8); (D) Lateral view (MRC 719, froglet, SVL 18.8). Photos: M. Rada

Description of holotype: An adult male of 36.6 mm SVL (Fig. 16). Body slender. Head barely wider than long; head as wide as body; head width 33% SVL; head length 30% SVL.

Snout truncate in dorsal and lateral view (Fig. 17); *canthus rostralis* rounded, slightly distinct; loreal region slightly concave to flat; lips not flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, at the level of the anterior margin of the lower jaw. Internarial region and top of head flat. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum and tympanic annulus inconspicuous. Supratympanic fold weakly developed, starting in the posterior upper corner of the eye and reaching the anterior margin of the insertion of the arm; supratympanic stripe absent. Mental gland present, medium, semicircular, covering partially of the gular area and extending about the third length of the throat.

Large dentigerous processes of vomers anteromedially inclined, separated and posterior ovoid choanae; each series bears eight (right) and seven (left) teeth. Choanae separated by a distance larger than 2.5 times their maximum diameter, smaller regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular. Forearm moderately robust; axillary membrane absent. Outer ulnar fold present. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is equal than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers without dermal fringes; webbing formula I – II 2^{+}–3 III $2^{1/3}$ –2 IV. Subarticular distal tubercles large and elliptical. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, elliptical; elliptical prepollex, not modified as a spine; nuptial excrescencies absent.

FIGURE 16. *Hyloscitus chlorophyllus* sp. nov., dorsal and ventral view. Holotype, adult male, MRC 744, SVL 36.6 mm. Photos: M. Rivera-Correa.

Hind limbs moderately robust; tibia length 47% SVL; foot length 37% SVL. Outer tarsal fold and calcar tubercle present; inner tarsal fold indistinct. Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 < 5 < 4; extensive toe webbing, formula I 1–1$^{1/2}$ II 1 – 2^{+} III 1–2 IV 1$^{2/3}$–1 V. Inner metatarsal tubercle elongate, elliptical, visible from above; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive. Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent with tubercles; supracloacal fold present; sheath short; white warts around and below cloaca, ventrally extending 1/4 of the length of the thigh. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

Color in life of the holotype: Dorsal surfaces dirty green with scattered small dark brown spots and gray blotches; yellowish-cream on the flanks; brown pigmentation around the nostrils. White upper lip with brown spots. White above and brown below ulnar and tarsal fold; calcar tubercle and white supracloacal fold white above and brown below. White warts around and below cloaca and on the proximal region of the thighs. Vocal sac and ventral surfaces yellowish green; belly with a yellowish-white parietal peritoneum. Yellow mental gland; ventral surfaces of fingers, toes, webbing on hands and feet yellow-green; axillar and inguinal region light blue. Iris cream with fine red reticulation. Bones green.

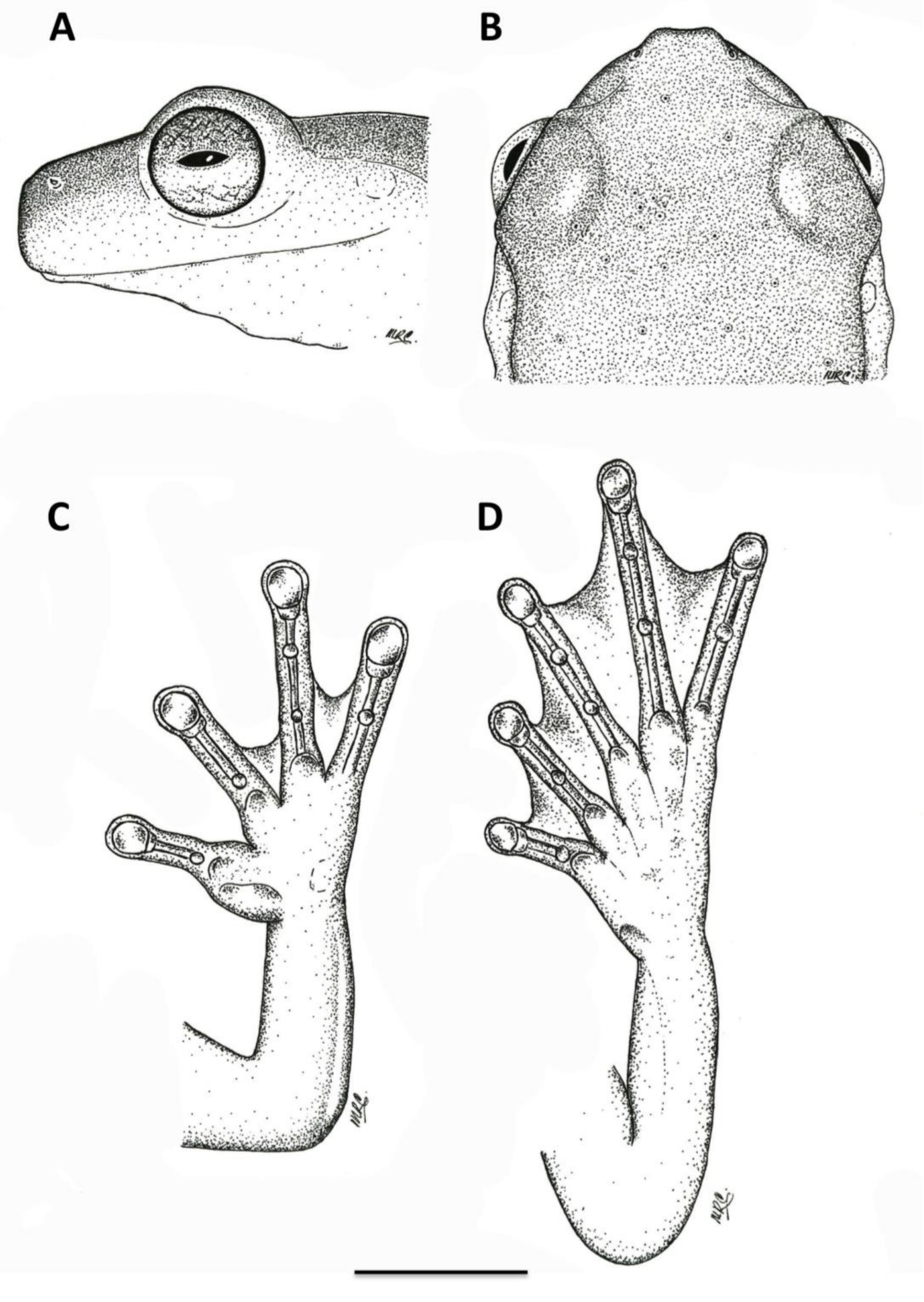

FIGURE 17. *Hyloscitus chlorophyllus* sp. nov., holotype, MRC 744. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm.

Drawings: M. Rivera-Correa

Color in preservative of the holotype: Dorsal surfaces and flanks brown with scattered small dark brown spots. Brown pigmentation around the nostrils and cream upper lip with brown spots. The ulnar and tarsal fold, calcar tubercle and white supracloacal fold cream above and brown below. Cream warts around and below cloaca and and in the ventral region of the thighs. Mental gland, vocal sac and ventral surfaces, cream. Ventral surfaces of fingers, toes, webbing on hands cream, except external region of finger IV and toe V, with brown spots, forming a band from the wrist and tibia respectively.

Measurements of the holotype (in mm): SVL 36.6; HL 11.2; HW 12.0; ED 3.8; END 2.4; NSD 1.3; IND 3.0; AMD 6.5; FAL 7.1; FAB 3.5; HAL 11.7; THL 16.6; TL 17.2; TAL 10.6; FL 17.2; TFD 1.7; FTD 1.4.

Variation: The snout is slightly sloping in MRC 699 and MRC 720. MRC 700 has dark brown crosslinked pattern in the dorsum. In preservation, the dorsum in MRC 699 and MRC 720 is cream, the latter has abundant brown dorsal spots, while in the others are brown. In MRC 703, the superior lip is less pigmented dark spots. Number of vomerine teeth 6–8 / 6–7. The finger-webbing formula varies as follow I – II ($1^{2/3}$–2) – (3^{+}–3^{-}) III ($2^{1/2}$ – $2^{1/3}$) – (2–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–$1^{1/2}$) – (1^{+}–$1^{1/2}$) II (1– $1^{1/3}$) – (2^{-} – 2) III (1–$1^{1/3}$) – ($1^{2/3}$ – 2) IV (2^{+}–2) – (1–1^{-}) V.

Distribution and natural history: Hyloscirtus chlorophyllus sp. nov. is known from some localities at elevations of 1500–2190 m, on the eastern flank of the Cordillera Oriental, in the Municipio de Pajarito, Chivor and Miraflores, all these municipalities in the Departmento de Boyacá, Colombia; the linear distance between the localities is approximately 110 km. *Hyloscirtus chlorophyllus* inhabits flowing waters, males were observed vocalizing always on leaves of shrubs between 0.50 and 1.50 m in height, during light rain.

Advertisement call: Four calling males of *H. chlorophyllus* were recorded at 20:00 h and 01:00 on leafs or branch of bush between 0.50 to 1.50 m high approximately over stream.

The advertisement call (calls = 68) is always composed of one acoustic note with one pulse to three semi-pulses, shorter. The call has a mean length 0.130 ± 0.022 s (0.087–0.176) repeated at a low rate (16–20 calls/min). The peak (dominant) frequency coincides with the fundamental frequency 2690.3 ± 158.82 Hz (2411.7–2928.5)

The call presents a slight modulation of note, the last pulse with most highest frequently although the highest energy input was on the first semi-pulse. The amplitude modulation of each note consisting of a rapid rise in intensity from the beginning up to a one-quarter of the note and decreasing rapidly and again increases in second pulse but does not reach the peak of the first pulse; posteriorly, again increases to the middle of the call, where has visibly higher wave amplitude and decreasing towards the end (Fig. 18).

Etymology: The specific name is from combination of the Greek word *chloros*, which means “green” and the Greek word *phillon*, which means, “leaf”. *Hyloscirtus chlorophyllus* is named in reference to the coloration of this species that is easily confused with the leaves often found. The name is used here as a noun in apposition.

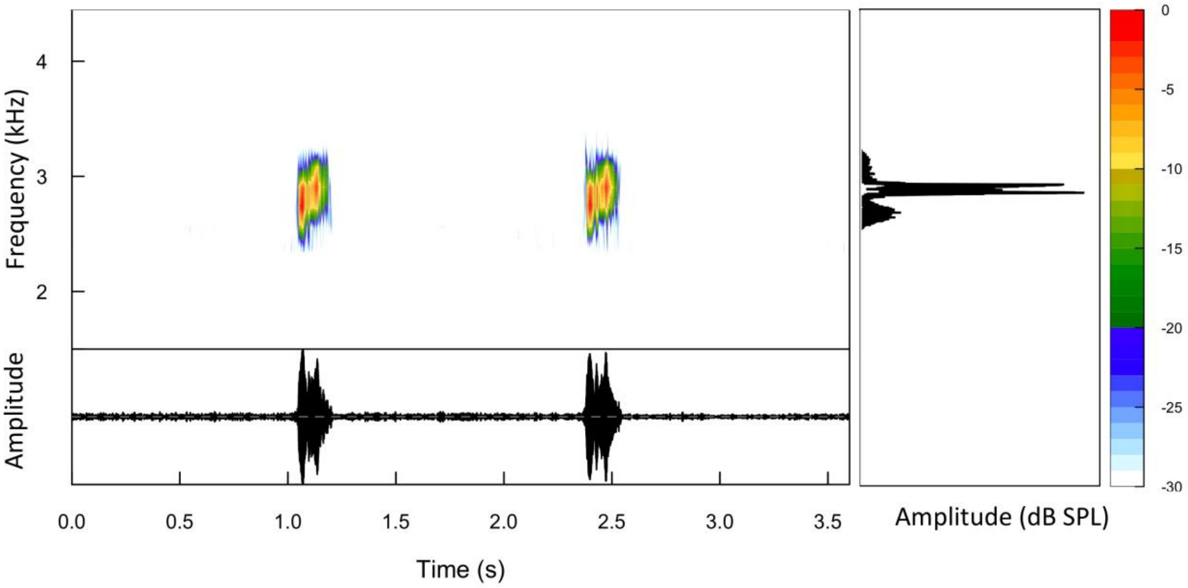

FIGURE 18. Spectrogram, oscillogram and power spectrum showing two advertisement call of *Hyloscirtus chlorophyllus* sp. nov. (MRC 744, holotype; 18.0 ºC) from Miraflores, Boyacá, Colombia.

HYLOSCIRTUS HYALINUS SP. NOV.

Holotype: MC 9751, adult male, Colombia, Caquetá, Municipio de San Vicente del Caguan, Inspección Guayabal, Vereda Cristo Rey, Hacienda Andalucía (2º40′11″N, 74º53′18″W, 1560-1600 m a.s.l.), collected on 27 November 1997 by Maria C. Ardila-Robayo and Fabio Quevedo.

Paratypes: Six adults. MC 9747, 9750, males, collected with holotype. MC 9109–9110, males, Colombia, Caquetá, Municipio de San Vicente del Caguan, Inspección Guayabal, Río Pato (2º48′9″N, 74º50′30.6″W, 1600 m a.s.l.), collected on 16 November 1997 by Maria C. Ardila-Robayo and Fabio Quevedo. MC 9413, female, Colombia, Caquetá, Municipio de San Vicente del Caguan, Inspección Guayabal, Vereda Cristo Rey, Hacienda Andalucía (2º47′49″N, 74º51′19″W, 1590 m a.s.l.), collected on 22 November 1997 by Maria C. Ardila-Robayo and Fabio Quevedo. MAR 1911, female, Colombia, Caquetá, Municipio de Florencia, Vereda Tarqui, from kilometer 47 road Altamira — Florencia (1º51′16.8″N, 75º40′0.7″W, 1850 m a.s.l.), collected on 22 March 2010 by Marco Rada.

Paragenetype: MAR 1911 (16S rRNA)

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 33.7–36.2 mm in males, n = 7; 40.8–42.4 in females, n = 2); (2) body slender, head barely wider than long; (3) snout rounded in or truncate dorsal view and truncate or slightly sloping in lateral view; (4) dorsal and flanks light green to olive green without or with scattered brown and gray blotches, some gray blotches with brown edge; gular and ventral surfaces region yellowish green in life (Fig. 19); (5) axillar and inguinal region light blue; (6) mental gland in males present; (7) upper lip flared white with brown markings and black nostril margin; (8) parietal peritoneum white present; (9) iris cream with abundant maroon reticulation; (11) nuptial pad absent; (13) tympanum rounded and upper edge of tympanic annulus covered by a weak supratympanic fold; (14) canthal striped and supratympanic pale stripe absent; (15) ulnar fold and tarsal fold present, white above and brown below; (16); calcar tubercle present; (17) supracloacal fold white above and brown below present; (18) white warts around and below cloaca, ventrally extending 3/4 of the length of the thigh; (19) green bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) large dentigerous processes of vomers, anteromedially inclined, with discernible gap and with 6–12 teeth each.

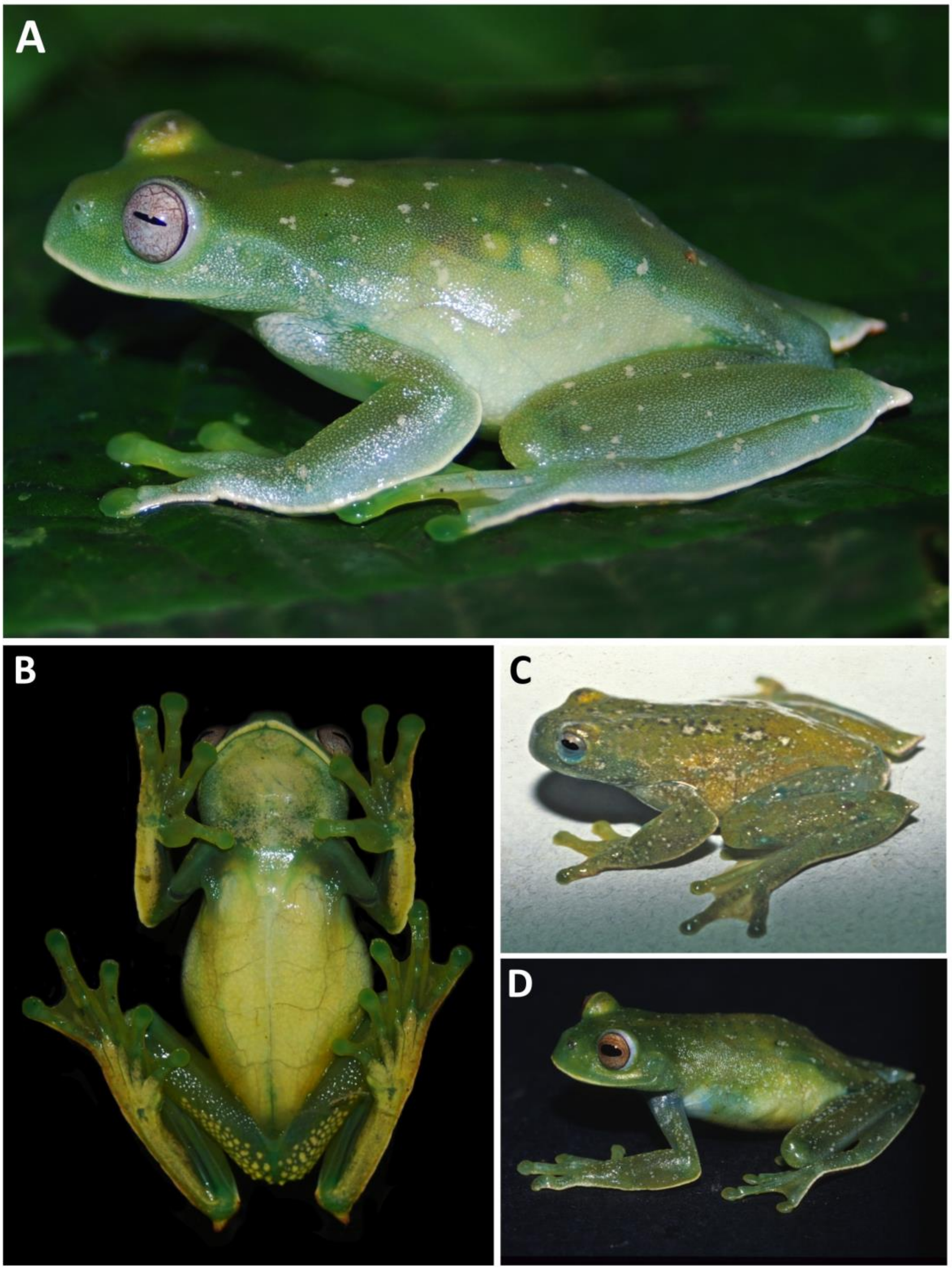

FIGURE 19. *Hyloscirtus hyalinus* sp. nov., in life. (A and B) Lateral view and ventral view (MAR 1911, paratype, adult female, SVL 42.4 mm); (C) lateral view (MC 9747, paratype, adult male, SVL 35.6); (D) Lateral view (MC 9413, paratype, adult female, SVL 40.8). Photos: M. Rada (A-B); P. Ruiz-Carranza (C-D).

Comparison with other species: At the molecular level, *H. hyalinus* sp. nov. differs from *H. piceigularis* its sister species by 31 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 6.10% of genetic divergence (uncorrected p-distances) between both species.

Hyloscirtus hyalinus is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having calcar tubercle (absent in *H. alytolylax, H. baios* sp. nov, *H. bogotensis*, *H. callipeza*, *H. colymba, H. hydrophilus* sp. nov., *H. jahni*, *H. lynchi*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi, H. torrenticola*), white labial stripe present (absent in *H. alytolylax*, *H. baios* sp. nov, *H. bogotensis*, *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. lynchi*, *H. jahni*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi*), supracloacal fold present (absent in *H. baios* sp. nov., *H. bogotensis*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*). *Hyloscirtus alytolylax, H. baios* sp. nov., *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. jahni, H. oro* sp. nov and *H. simmonsi* also differ by having supratympanic pale stripe (absent in *H. hyalinus* sp. nov.). *Hyloscitus hyalinus* is most similar to *H. chlorophyllus* sp. nov but differs from them by having white warts ventrally extending only to 3/4 of the length of the thigh (1/4 in *H. chlorophyllus* sp. nov.). Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

Description of holotype: An adult male of 34.7 mm SVL (Fig. 20). Body slender. Head barely wider than long; head as wide as body; head width 32% SVL; head length 29% SVL. Snout rounded in dorsal view and truncate in lateral view; *canthus rostralis* rounded, slightly distinct; loreal region slightly concave to flat (Fig. 21); lips slightly flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, at the level of the anterior margin of the lower jaw. Internarial region and top of head flat. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum barely visible, rounded and slightly discernible from above; its diameter equals 34% eye diameter, tympanic annulus inconspicuous. Supratympanic fold weakly developed, starting in the posterior upper corner of the eye, slightly covering the dorsal margin of this and reaching the anterior margin of the insertion of the arm; supratympanic stripe absent. Mental gland present, medium, semicircular, covering partially of the gular area and extending about the third length of the throat.

FIGURE 20. *Hyloscirtus hyalinus* sp. nov., dorsal and ventral view. Holotype, adult male, MC 9751, SVL 34.7 mm (top); paratype, adult female, MAR 1911, SVL 42.4 mm (down). Photos: M. Rivera-Correa.

Large dentigerous processes of vomers anteromedially inclined, separated and posterior ovoid choanae; each series bears 12 (right) and 10 (left) teeth. Choanae separated by a distance larger than 2.5 times their maximum diameter, smaller regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

Forearm moderately robust; axillary membrane absent. Outer ulnar fold present. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is equal than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers without dermal fringes; webbing formula I – II 2^{-}–3 III $2^{1/3}$–2 IV. Subarticular distal tubercles large and elliptical. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine; nuptial excrescencies absent.

Hind limbs moderately robust; tibia length 50% SVL; foot length 41% SVL. Outer tarsal fold and calcar tubercle present; inner tarsal fold indistinct. Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 < 5 < 4; extensive toe webbing, formula I 1^{+}–$1^{1/2}$ II 1 – 2 III 1–2^{-} IV $1^{2/3}$–1 V (Fig. 3D). Inner metatarsal tubercle elongate, elliptical, visible from above; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive. Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent without tubercle; supracloacal fold present; sheath short; white warts around and below cloaca, ventrally extending 3/4 of the length of the thigh. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

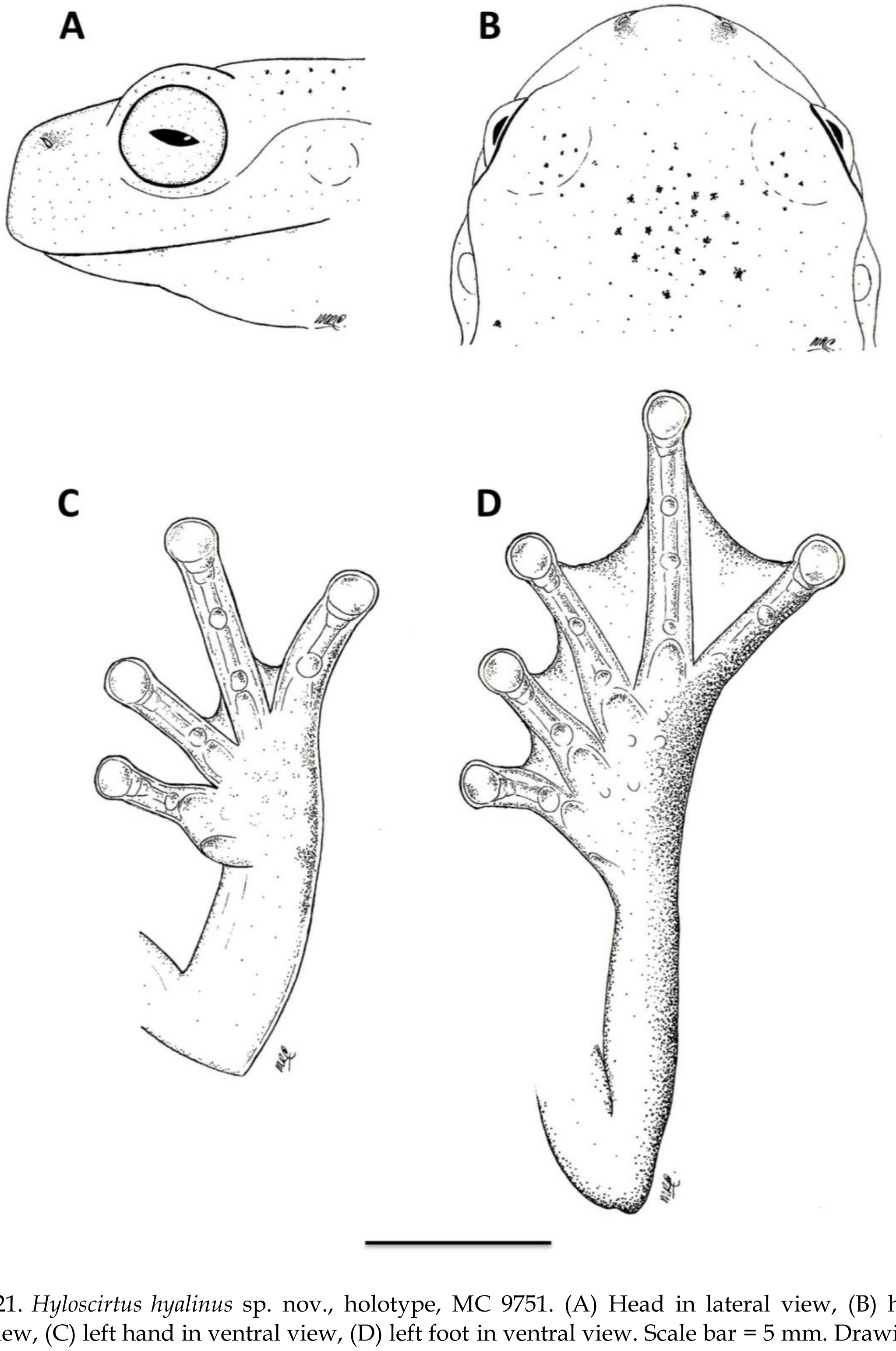

FIGURE 21. *Hyloscirtus hyalinus* sp. nov., holotype, MC 9751. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Color in life of the holotype: Dorsal surfaces and flanks light green with scattered small dark brown spots, in the anterior region of the body, specifically, between the eyes and over the eyelids; small white blotches on the flanks; brown pigmentation around the nostrils.

White upper lip with brown spots. The ulnar and tarsal fold extending to the heel white above and brown below; calcar tubercle and white supracloacal fold white above and brown below. White warts around and below cloaca and on the ventral region of the thighs. Vocal sac and ventral surfaces yellowish green; belly with a white parietal peritoneum. Yellow mental gland; ventral surfaces of fingers, toes, webbing on hands and feet yellow-green, except finger IV and toe V, with abundant brown spots, forming a band from the wrist and tibia respectively; axillar and inguinal region light blue. Iris cream with fine maroon reticulation. Bones green.

Color in preservative of the holotype: Dorsal surfaces and flanks cream with scattered small dark brown spots, in the anterior region of the body, specifically, between the eyes and over the eyelids; small white blotches on the flanks; brown pigmentation around the nostrils. White upper lip with brown spots. The ulnar and tarsal fold, calcar tubercle and white supracloacal fold white above and brown below. White warts around and below cloaca and and in the ventral region of the thighs. Mental gland, vocal sac and ventral surfaces, cream. Ventral surfaces of fingers, toes, webbing on hands cream, except finger IV and toe V, with abundant brown spots, forming a band from the wrist and tibia respectively.

Measurements of the holotype (in mm): SVL 34.7; HL 10.2; HW 11.3; ED 3.5; END 2.5; NSD 1.4; IND 3.1; AMD 6.7; TD 1.2; FAL 7.5; FAB 3.2; HAL 10.7; THL 15.9; TL 17.5; TAL 10.4; FL 14.4; TFD 1.8; FTD 1.7.

Variation and sexual dimorphism: Females are larger than males (males SVL 33.7–36.2, n = 7; females SVL 40.8–42.4, *n* = 2). The snout in females is slightly sloping, while in males is truncate. Females have wider head than the males. The number the brown tiny spots in the dorsum varies among individuals. The dorsum in MC 9747 and MC 9109 is light brown with blotches. The female MAR 1911 lacks the ventral brown band in the external finger, external toe and tibia. The gular region in males having brown pigmentation (MC 9109 and 9750).

The white peritoneum is visible ventrally and the extension varies among individuals, apparently more extensive in females. Number of vomerine teeth 6–12 / 6–11. The finger-webbing formula varies as follow I – II ($1^{2/3}$–2) – (3^{+}–3^{-}) III ($2^{1/2}$ – $2^{1/3}$) – (2–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–$1^{1/2}$) – (1^{+}–$1^{1/2}$) II (1– $1^{1/3}$) – (2^{-} – 2) III (1–$1^{1/3}$) – ($1^{2/3}$ – 2) IV (2^{+}–2) – (1–1^{-}) V. Secondary sexual characters (mental gland, vocal slits, vocal sac) in females are absent.

Distribution and natural history: Hyloscirtus hyalinus sp. nov. is known from some localities at elevations of 1560–1850 m, on the eastern flank of the Cordillera Oriental, in the Municipio de San Vicente del Caguan and Municipio de Florencia, both municipalities in the Departmento de Caquetá, Colombia; the distance between the localities is approximately 120 km. Very little is known about the natural history of *H. hyalinus* only this species inhabits flowing waters, males were vocalizing at the time of collection, but the calls was not recorded and were perched on leaves between 1.20 and 2.50 in height over the creek. The tadpole is currently unknown.

Etymology: The specific name given from the Greek adjective *hyalinos* meaning of glass. The epithet is in allusion to the translucent skin on the flanks of the females.

HYLOSCIRTUS HYDROPHILUS SP. NOV.

Holotype: MHUA-A 5042, adult male, Colombia, Antioquia, Municipio de Anorí, Vereda El Retiro, Finca el Chaquiral (6º59′00″ N, 75º08′05″ W, 1730 m a.s.l.), collected on 14 October 2004 by Paul D.A. Gutiérrez-Cárdenas.

Paratypes: All adults. MHUA-A 5041, male, collected with the holotype; MHUA-A 3494–5, females, MHUA-A 3496–97, males, Colombia, Antioquia, Municipio de Amalfi, Vereda Guayabito, Bosque Caracolí, Finca Costa Rica (6º52′25″ N, 75º05′55.7″ W; 1840 m a.s.l.), collected on 28 October 2004 by Lucas Barrientos. MHUA-A 4096–98, males, Colombia, Antioquia, Municipio de Anorí, Vereda El Retiro, Finca el Chaquiral (6º58.8′00″ N, 75º7.83′00″ W, 1730 m a.s.l.), collected on 26 November 2005 by Paul D.A. Gutiérrez-Cárdenas, Taran Grant and Claudia Molina. MHUA-A 4306, female, Colombia, Antioquia, Municipio de Anorí, Vereda El Retiro, Finca el Chaquiral (6º58.8′00″ N, 75º7.83′00″ W, 1730 m a.s.l.), collected on 10 January 2006 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 4786, male, Colombia, Antioquia, Municipio de Anorí, Vereda El Retiro, Finca el Chaquiral (6º58.8′00″ N, 75º7.83′00″ W, 1730 m a.s.l.), collected on 1 October 2006 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 5016–19, adult males, MHUA-A 5022, female, Colombia, Antioquia, Municipio de Anorí, Vereda El Retiro, Finca el Chaquiral (6º59.0′00″ N, 75º08′05″ W, 1730 m a.s.l.), collected between 12–14 August 2003 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 5024–27, males, Colombia, Antioquia, Municipio de Anorí, Vereda Cañadahonda, Quebrada El Claro, collected between 22–24 September 2003 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 5032–33 male and female respectively, Colombia, Antioquia, Municipio de Anorí, Vereda Cañadahonda, Quebrada El Claro, collected between 8–10 November 2003 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 5039–40, male and female respectively, Colombia, Antioquia, Municipio de Anorí, Vereda El Roble, Bosque La Forzosa (6º59′16″ N, 75º08′36″ W, 1820 m a.s.l.), collected between 20–21 July 2004 by Paul D.A. Gutiérrez-Cárdenas. MHUA-A 5360, male, Colombia, Antioquia, Municipio de Anorí, Vereda El Nevado, collected on 17 September 2007 by Laura Bravo, Claudia Molina and Natalia Silva. MHUA-A 7010–11, adult female and adult male respectively, Colombia, Antioquia, Municipio de Valdivia, Vereda San Fermín, (7º05′20″ N, 75º28′26″ W, 1890 m a.s.l.), collected on 4 November 2011 by Diego Rivera.

Paragenetype: MHUA-A 4096 (16S rRNA)

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 33.2–36.7 mm in males, n = 18; 39.6–43.0 in females, n = 6);

(2) body robust, head wider than body; (3) snout truncate in lateral view and rounded in males and rounded or truncate in females in dorsal view; (4) dorsal and flanks light green to lemon green with scattered brown spicules, gular region yellowish green and ventral surfaces yellowish in life (Fig. 22); (5) axillar and inguinal region light blue; (6) mental gland in males present; (7) upper lip without white stripe, not flared and immaculate nostril margin; (8) parietal peritoneum white present; (9) iris light gray with abundant maroon reticulation; (11) nuptial pad absent; (13) tympanum rounded and upper edge of tympanic annulus covered by a weak supratympanic fold; (14) canthal striped absent and supratympanic pale stripe present; (15) ulnar fold and tarsal fold white present; (16) calcar tubercle absent; (17) supracloacal fold white present (18) tubercles scattered around and below cloaca absent; (19) white bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) small dentigerous processes of vomers, slightly curved, narrowly separated, with 5–10 teeth each; (22) the advertisement call consists of two notes, the first longest, 0.231–0.251 s of duration and a dominant frequency of 2.411 to 2.584 Hz.

Comparison with other species: At the molecular level, *H. hydrophilus* sp. nov. differs from *Hyloscirtus* sp. "caldas", its sister species by 34 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 6.6 of genetic divergence (uncorrected p-distances) between both species. *Hyloscirtus hydrophilus* is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having supratympanic pale stripe (absent in *H. albopunctulatus*, *H. bogotensis*, *H. chlorophyllus* sp. nov., *H. hyalinus* sp. nov., *H. lascinius*, *H. lynchi*, *H. macondo* sp. nov., *H. palmeri*, *H. platydactylus*, *H. piceigularis*, *H. phyllognathus*, *H. simmonsi* and *H. torrenticola*), ulnar fold present (absent in *H. bogotensis*, *H. callipeza*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*), supracloacal fold present (absent in *H. bogotensis*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*). *Hyloscirtus chlorophyllus* sp. nov, *H. hyalinus* sp. nov., *H. lascinius*, *H. macondo* sp. nov, *H. palmeri*, *H. piceigularis*, and *H. phyllognathus*, also differ by having calcar tubercle and white labial stripe (absent in *H. hydrophilus* sp. nov.). *Hyloscirtus hydrophilus* is most similar to *H. alytolylax* and *H. colymba* but differs from them by not having pale canthal stripe (present in *H. alytolylax* and *H. colymba*), and the spectral and temporal parameters of the advertisement call. Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

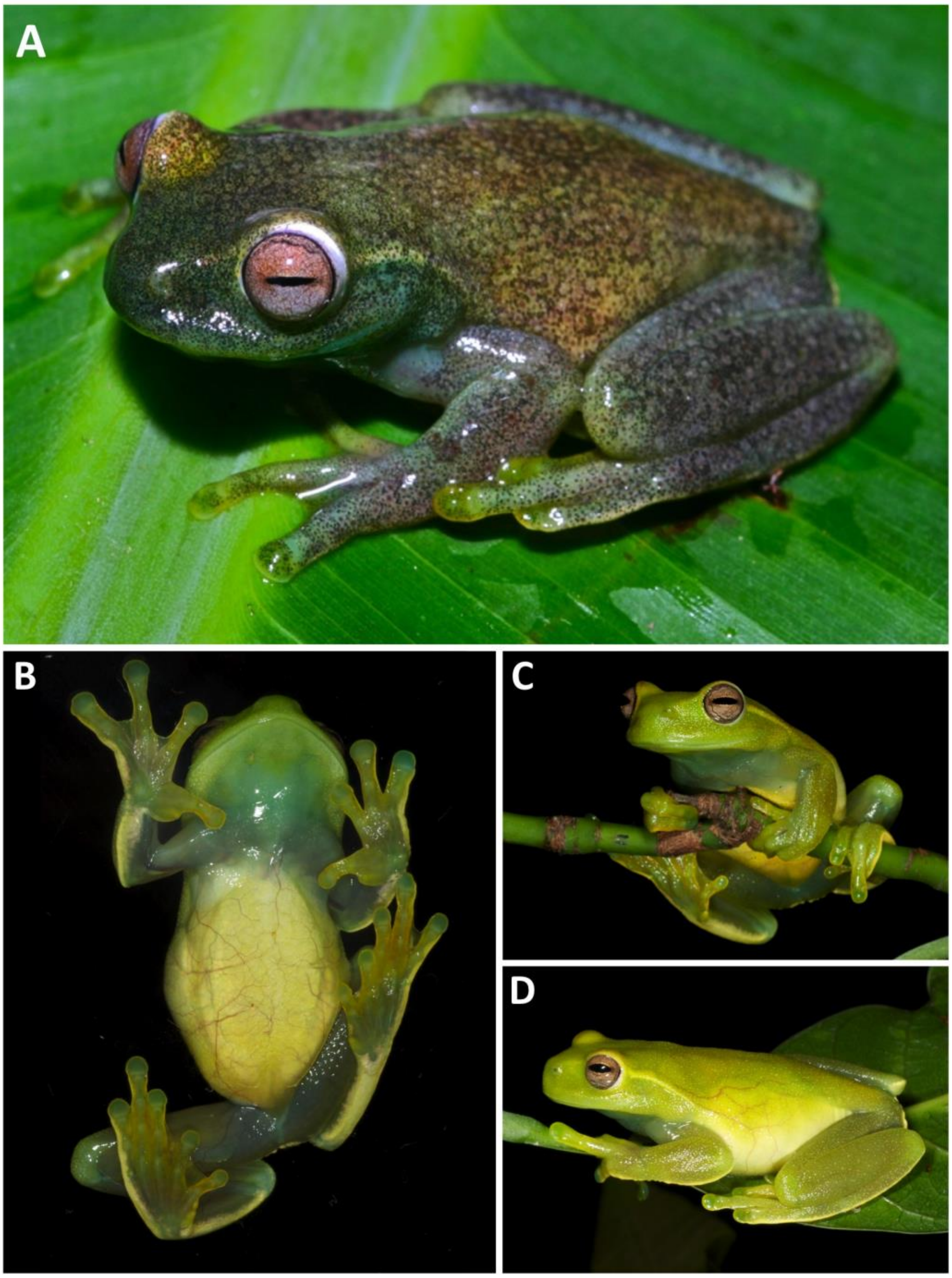

FIGURE 22. *Hyloscirtus hydrophilus* sp. nov., in life. A) Lateral view (MHUA-A 4096, paratype, adult male, SVL 35.2 mm); (B-D) ventral, frontal and lateral view (female, specimen not collected). Photos: T. Grant (A) and M. Rivera-Correa (B-D)

Description of holotype: An adult male of 36.2 mm SVL (Fig. 23). Body robust. Head barely wider than long; head as wide as body; head width 34% SVL; head length 32% SVL. Snout rounded in dorsal view and truncate in profile (Fig. 24); *canthus rostralis* rounded, slightly distinct; loreal region concave; lips rounded, not flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, posterior to anterior margin of lower jaw. Internarial region and top of head flat. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum and tympanic annulus barely visible, rounded and no discernible from above; its diameter equals 40% eye diameter. Supratympanic fold weakly developed, starting in the anterior region of tympanum, slightly covering the dorsal margin of this and without reaching the posterior margin of the insertion of the arm; supratympanic stripe present. Mental gland present, medium, semicircular, covering partially of the gular area and extending about the third length of the throat.

Dentigerous processes of vomers slightly curved, separated and posterior ovoid choanae; each series bears eight (right) and six (left) teeth. Choanae separated by a distance larger than three times their maximum diameter, smaller regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

Forearm moderately robust; axillary membrane absent. Outer ulnar fold present. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is equal than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers without dermal fringes; webbing formula I – II 2^{-}–3^{-} III $2^{1/2}$ –2^{+} IV. Subarticular distal tubercles large and elliptical. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine; nuptial excrescencies absent.

FIGURE 23. *Hyloscirtus hydrophilus* sp. nov., dorsal and ventral view. Holotype, adult male, MHUA-A 5042, SVL 36.2 mm (top); paratype, adult female, MHUA-A 5040, SVL 45.2 mm (down). Photos: M. Rivera-Correa

Hind limbs moderately robust; tibia length 53% SVL; foot length 45% SVL. Outer tarsal fold and calcar tubercle absent, but pale tarsal stripe evident; inner tarsal fold indistinct. Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 = 5 < 4; extensive toe webbing, formula I 1–1^{+} II 1 – $1^{1/2}$ III 1–$1^{2/3}$ IV $1^{2/3}$ –1 V.

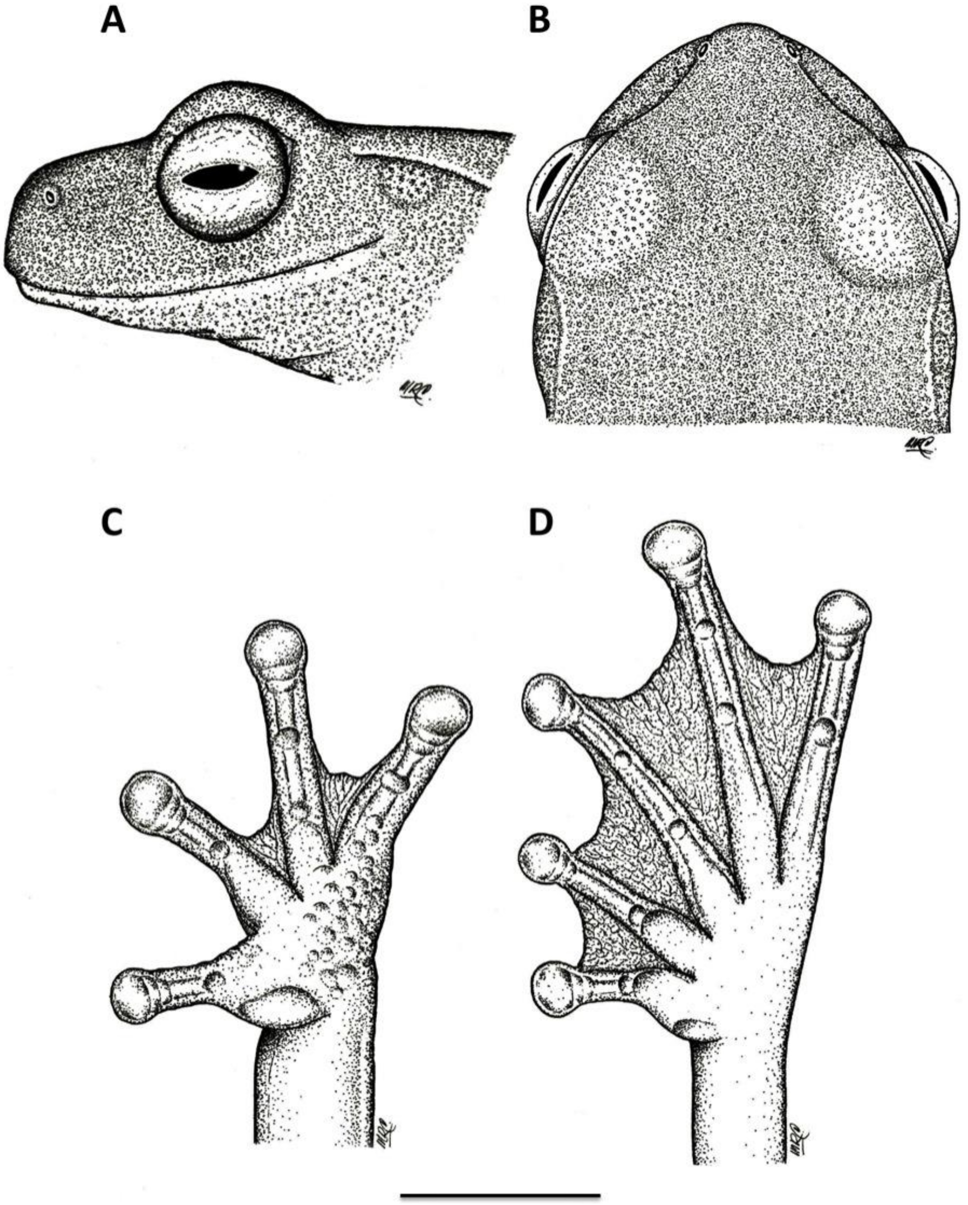

FIGURE 24. *Hyloscirtus hydrophilus* sp. nov., holotype, MHUA-A 5042. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Inner metatarsal tubercle elongate, elliptical, visible from above; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive.

Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent without tubercle; supracloacal fold present; sheath short; tubercles scattered around and below cloaca absent. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

Color in life of the holotype: Dorsal surfaces and flanks lemon green with dark gray spots forming ocelli pattern and scattered red-brown spicules. A white ulnar fold and tarsal stripe extending to the calcar; white supra-cloacal fold. Vocal sac and ventral surfaces yellowish green and medial area with a white parietal peritoneum. Yellow mental gland with tiny brown spots; ventral surfaces of fingers, toes, webbing on hands and feet yellow-green; axillar and inguinal region light blue. Iris light gray with fine maroon reticulation. Bones white.

Color in preservative of the holotype: Dorsal surfaces creamy-brown with dark brown spots forming an ocelli pattern, flanks, fingers, toes, webbing and shanks creamy yellow with tiny brown spots. Ventral surfaces creamy white and gular region creamy white with tiny brown spots. Supratympanic stripe creamy-white; ulnar and supracloacal fold, tarsal stripe and peritoneum white.

Measurements of the holotype (in mm): SVL 36.2; HL 12.0; HW 12.5; ED 3.8; END 3.7; NSD 1.6; IND 3.2; AMD 6.9; TD 1.5; FAL 6.9; FAB 3.2; HAL 11.5; THL 18.2; TL 19.1; TAL 10.5; FL 16.3; TFD 2.1; FTD 1.7.

Variation and sexual dimorphism: Females are larger than males (males SVL 33.2–36.7, n = 18; females SVL 39.6–43.0, *n* = 6). The snout in females is truncate in profile, while in males is rounded. Females have wider head than the males. The number the red-brown spicules and gray tiny spots varies among individuals. The dorsum in some females without ocelli pattern (MHUA-A 3494, 7010). In preservation the dorsum on all females (MHUA-A 3494–95, 4307, 5022, 5033, 7010) and some males is cream (MHUA-A 5030–31), other males is brown

(MHUA-A 3496, 5018, 5029, 5032, 5035) and dark brown in (MHUA-A 3497, 4097, 5016, 5021, 5024, 5026–28). The gular region in males having dark brown pigmentation (MHUA-A 3496–97, 4097, 5016, 5018, 5021, 5024–28, 5039) and cream with little brown spots (MHUA-A 4096, 5011, 5029–5032, 5034–35, 5360, 7011) and the extension of mental gland may vary subtly. The white peritoneum is visible ventrally and the extension varies among individuals. The vomerine odontophores have a discernible gap except for (MHUA-A 4097, 5039) which has the processes abnormally divided in more than two parts. Number of vomerine teeth 5–10 / 5–10. The finger-webbing formula varies as follow I – II ($1^{2/3}$–2^{-}) – (3^{+}–3^{-}) III ($2^{1/3}$ – $2^{1/2}$) – (2^{+}–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–1) – (1^{+}–1) II (1– $1^{1/3}$) – (1^{-} – $1^{1/2}$) III (1^{+}–$1^{1/3}$) – (1^{-} –$1^{2/3}$) IV (2^{+}–2) – (1–1^{-}) V. Secondary sexual characters (mental gland, vocal slits, vocal sac) in females are absent. The ovarian oocytes in two females (MHUA-A 5022, 7010) are cream unpigmented, 2.9–3.4 mm diameter (*n* = 14).

Distribution and natural history: Hyloscirtus hydrophilus sp. nov. is known from only seven localities at elevations of 1530–1890 m, on the northeastern part of the Cordillera Central: in the vereda El Retiro (type locality), the vereda El Roble, the vereda Cañadahonda, the vereda El Nevado, all of them at municipality of Anorí, in the vereda Guayabito at municipality of Amalfi, in the vereda San Fermín at municipality of Valdivia, and in the vereda Santa Rita at municipality of Guatapé all municipalities in the department of Antioquia, Colombia. The distance between the type locality and the vereda Guayabito (municipality of Amalfi) is approximately 12.8 km, separated by the Porce river valley; the distance between the type locality and the vereda San Fermín (municipality of Valdivia) is approximately 39.2 km and between the type locality and the vereda Santa Rita (municipality of Guatapé) is approximately 74.0 km. The type locality and the two other localities (veredas El Roble and Cañadahonda) from municipality of Anorí are distanced approximately to each other 1-2 km. *Hyloscirtus hydrophilus* sp. nov. inhabit permanent streams with abundant herbaceous and shrubby vegetation inside Premontane Wet Forest. Most individuals (females and calling males) were found on herbaceous vegetation and bushes along the streams at night. Other individuals perch on tree branches. Some individuals have been found active at the end of the afternoon (between 1730–1800 h), but their activity peak is between 2100 and 0200 h. A stomach analysis of 40 individuals revealed that none of them had any food item in your diet, which could suggest an alimentation restriction during the reproductive activity or the season of year where they were collected. In the stream of the type localiy, *H. hydrophilus* sp. nov. is syntopic with *Centrolene* aff *savagei*, *Espadarana* aff *andina*, *Gastrotheca dendronastes*, *Nymphargus rosada*, *Pristimantis paisa*, *P. penelopus*, *P. suetus*, *P. viejas*, *Rhinella macrorhina*, *R. margaritifera*, and *Rulyrana susatamai*.

Advertisement call: Two calling males of *H. hydrophilus* were recorded at 20:20 h and 21:10 on large leaf of shrub two meters high approximately in the margin of a stream. The advertisement call (calls = 26) is always composed of two acoustic and semi-pulsed notes with shorter mean call length 0.280 ± 0.020 s (0.224–0.230) repeated at a low call rate (44–56 calls/min). The dominant (peak) frequency coincides with the fundamental frequency (2411.7–2584.0 Hz), and the call may have at least two additional harmonically related frequencies with mean to (7321–11913 Hz), respectively.

The highest energy input was on both notes, specifically on first semi-pulse each. The amplitude modulation of each note has a pattern consisting of ascending intensities from the beginning up to a one-quarter of the note and decreasing rapidly to the middle of the note and rises again to one-third of note and decreasing towards the end. (Fig. 25)

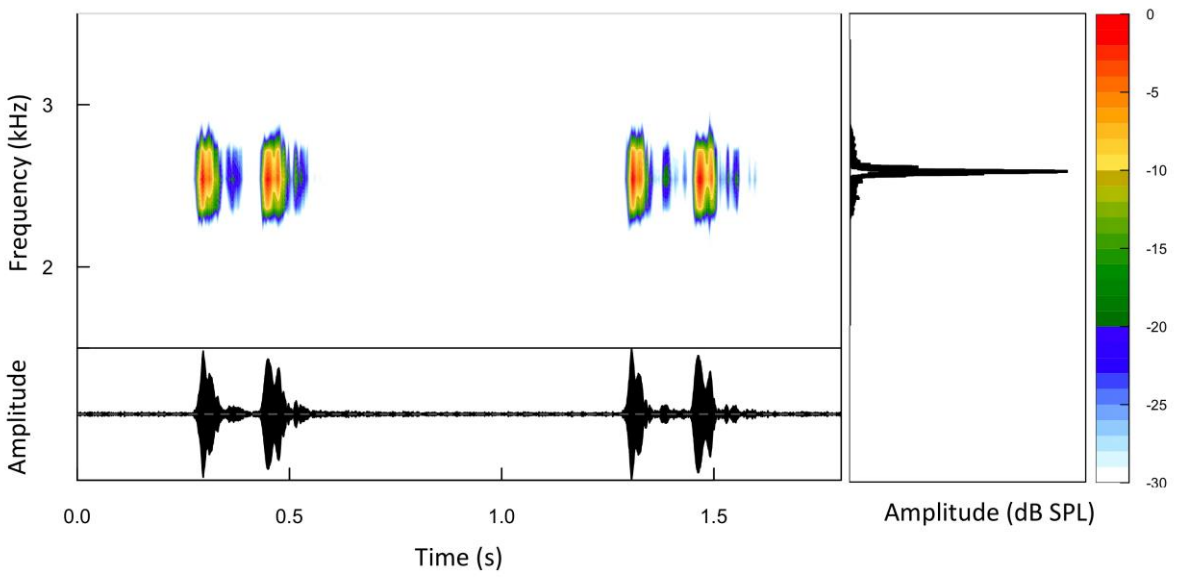

FIGURE 25. Spectrogram, oscillogram and power spectrum showing two advertisement call of *Hyloscirtus hydrophilus* sp. nov. (MHUA-A 5042, holotype) from Anorí, Departamento de Antioquia, Colombia.

Etymology: The specific name is from combination of the Greek word *hydrias*, which means "from water" and the Greek word *philios*, which means, "loving, friendly". *Hyloscirtus hydrophilus* is named in reference of the natural history of the species associated with moist habitats and streams flowing. The name is used here as a noun in apposition.

HYLOSCIRTUS MACONDO SP. NOV.

Hyla palmeri. – Acosta-Galvis et al. (2006:299).

Holotype: MHUA-A 4813, adult male, Colombia, Antioquia, Municipio de Maceo, Vereda Las Brisas, Hacienda Santa Bárbara (6º32′49.2″ N, 74º38′37.6″ W, 550m, datum = WGS84), collected 18 October 2006 by Mauricio Rivera.

Paratypes: Twelve adult males. MHUA-A 5875, Colombia, Caldas, Municipio de Norcasia, Bosque El Tigre (5º33.0′12.0″ N, 74º52′23.0″ W; 730 m), collected on 10 June

2008 by Vivian Páez. MHUA-A 7062, Colombia, Antioquia, Municipio de San Carlos, Vereda Juanes, Bosque La Villa (6º13.0′16.1″ N, 74º50′51.4″ W; 750 m), collected on June 2006 by Sandra Gallo and Eliana Muñoz. LBV 412–13, Colombia, Antioquia, Municipio de Cocorná: Vereda El Porvenir, 730 m, collected on 22 July 2009 by Laura Bravo. MHUA-A 7663, Colombia, Antioquia, Municipio de San Carlos: Corregimiento Puerto Garza, Vereda Limones, (6º12′47″ N, 74º45′36″ W; 750 m), collected on 30 September 2010 by Juan Pablo Hurtado. MHUA-A 7673–74, Colombia, Antioquia, Municipio de San Francisco, Santo Domingo River (5º58′17″ N, 75º7′28″ W; 800 m), collected between 30 January and February 2013 by Esteban Alzate. MHUA-A 7846, Colombia, Antioquia, Municipio de Nariño, Vereda Puente Linda, Quebrada Espíritu Santo (5º33′37″ N, 75º7′21″ W; 650 m) collected on 21 May 2011 by Juan Pablo Hurtado. MHUA-A 7847, Colombia, Antioquia, Municipio de Segovia, Vereda Cuturú (7º14′16″ N, 74º45′19″ W; 450 m) collected on 2 December 2011 by Juan Pablo Hurtado. MHUA-A 7848, Colombia, Antioquia, Municipio de San Roque, Corregimiento San José del Nus: Vereda La Chinca (6º29′10″ N, 74º51′30″ W; 1100 m) collected on 16 April 2013 by Felipe Duarte. MHUA-A 7849, Colombia, Antioquia, Municipio de Zaragoza, Rio Nechí above Dos Bocas (7º25′48″ N, 74º54′59″ W; 150 m) collected on 9 April 2013 by Alejandro Montoya. MHUA-A 7850, Colombia, Caldas, Municipio de Norcasia: Rio Manso (5º36′32″ N, 74º57′9″ W; 650 m) collected on 8 March 2013 by Alejandro Montoya. One adult female. MHUA-A 6977, Colombia, Antioquia, Municipio de San Rafael, Vereda El Bizcocho, Quebrada Guadualito (6º18.0′1.0″ N, 75º4′1.4″ W; 1050 m), collected on 28 March 2011 by Carlos Jiménez.

Genetype: MHUA-A 4813 (16S rRNA)

Paragenetype: MHUA-A 5250 (16S rRNA)

Referred specimens: MHUA-A 7073, male, Colombia, Antioquia, Municipio de San Carlos, Vereda Juanes, Caño Borboyones (6º13.5′51.9″ N, 74º50′50.4″ W; 850 m). MHUA-A

6303, female, Colombia, Caldas, Municipio de Samana, Vereda Samana, Quebrada La Mulata (5º37’23” N, 74º36’3.7” W; 520 m).

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 34.2–38.0 mm in males, 42.9–43.8 in females); (2) body robust, head wider than body; (3) snout truncate in dorsal view and lateral view; (4) dorsal and flanks reddish green with scattered blotches gray and white, gular region yellow, yellowish green or dirty green, ventral surfaces yellowish white to dirty green (Fig. 26); (5) axillar region light blue and inguinal region pale orange; (6) large mental gland in males present; (7) upper lip flared white and black nostril margin only in females; (8) parietal peritoneum white present; (9) iris light brown with abundant maroon reticulation, especially in superior area; (11) nuptial pad absent; (13) tympanum rounded and upper edge of tympanic annulus covered by a weak supratympanic fold; (14) canthal striped and supratympanic pale stripe absent; (15) ulnar fold and tarsal fold white above and brown below present; (16) calcar tubercle present; (17) supracloacal fold white above and brown below present (18) white warts around and below cloaca, ventrally extending up to 2/4 of the length of the thigh in females; (19) green bones in life; (20) elliptical large prepollex, not modified as a projecting spine; (21) small dentigerous processes of vomers, slightly curved, narrowly separated, with 5–11 teeth each; (22) the advertisement call consists of a single multi-pulsed note with 0,177–0,206 s of duration emitted of 70 to 140 calls per minute and a dominant frequency of 2411.9 to 2584.0 Hz. and a fundamental frequency of 1205.4 to 1292.1 Hz.

Comparison with other species: At the molecular level, *H. macondo* sp. nov. differs from *H. palmeri*, its sister species by 59 to 77 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 11.7 and 15.2% of genetic divergence (uncorrected p-distances) between both species. *Hyloscirtus macondo* is differentiable by dorsum from green to dark reddish green with circulatory system maroon partially visible; snout truncate in dorsal and lateral view; having fold ulnar, tarsal and supracloacal, white above and brown below; calcar tubercle developed and by lack of canthal and supratympanic pale stripe. This combination of characters distinguishes the new species from the other species of the *H. bogotensis* group, except of *H. palmeri* to which previously has been confused, but differs from the latter by size. *Hyloscirtus macondo* sp. nov is smaller, SVL 34.2–38.0 in males and 42.9–43.8 in females (*vs* SVL 40.8–45.0 in males, Duellman, 1970; 53.8–54.4 in females *in H. palmeri*) and mental gland relatively less developed. The new species is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having calcar tubercle (absent in *H. alytolylax, H. baios* sp. nov, *H. bogotensis*, *H. callipeza, H. colymba, H. hydrophilus* sp. nov., *H. jahni*, *H. lynchi*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi, H. torrenticola*), white labial stripe present (absent in *H. alytolylax*, *H. baios* sp. nov, *H. bogotensis*, *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. lynchi*, *H. jahni*, *H. oro* sp. nov, *H. platydactylus* and *H. simmonsi*), supracloacal fold present (absent in *H. baios* sp. nov., *H. bogotensis*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*). *Hyloscirtus alytolylax, H. baios* sp. nov., *H. callipeza, H. colymba, H. hydrophilus* sp. nov, *H. jahni, H. oro* sp. nov and *H. simmonsi* also differ by having supratympanic pale stripe (absent in *H. hyalinus* sp. nov.). *Hyloscitus hyalinus* is most similar to *H. chlorophyllus* sp. nov but differs from them by having white warts ventrally extending only to 3/4 of the length of the thigh (1/4 in *H. chlorophyllus* sp. nov.). Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

Description of holotype: An adult male of 36.1 mm SVL (Fig. 27). Body robust. Head barely wider than long; head as wide as body; head width 36% SVL; head length 32% SVL. Snout truncate in dorsal view and in lateral view (Fig. 28); *canthus rostralis* rounded, indistinct; loreal region concave; lips rounded, not flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, slightly posterior to anterior margin of lower jaw. Internarial region concave and top of head flat. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum and tympanic annulus barely visible rounded and slightly inclined medially toward dorsum, such that almost the complete tympanum is visible from above; its diameter equals 40% eye diameter. Supratympanic fold weakly developed, starting in the anterior region of tympanum, slightly covering the dorsal margin of this and extending to the posterior margin of the insertion of the arm; supratympanic pale stripe absent. Region between head and suprascapulae quite depressed. Mental gland present, large, elliptic, covering entire of the gular area and extending about the half-length of the throat.

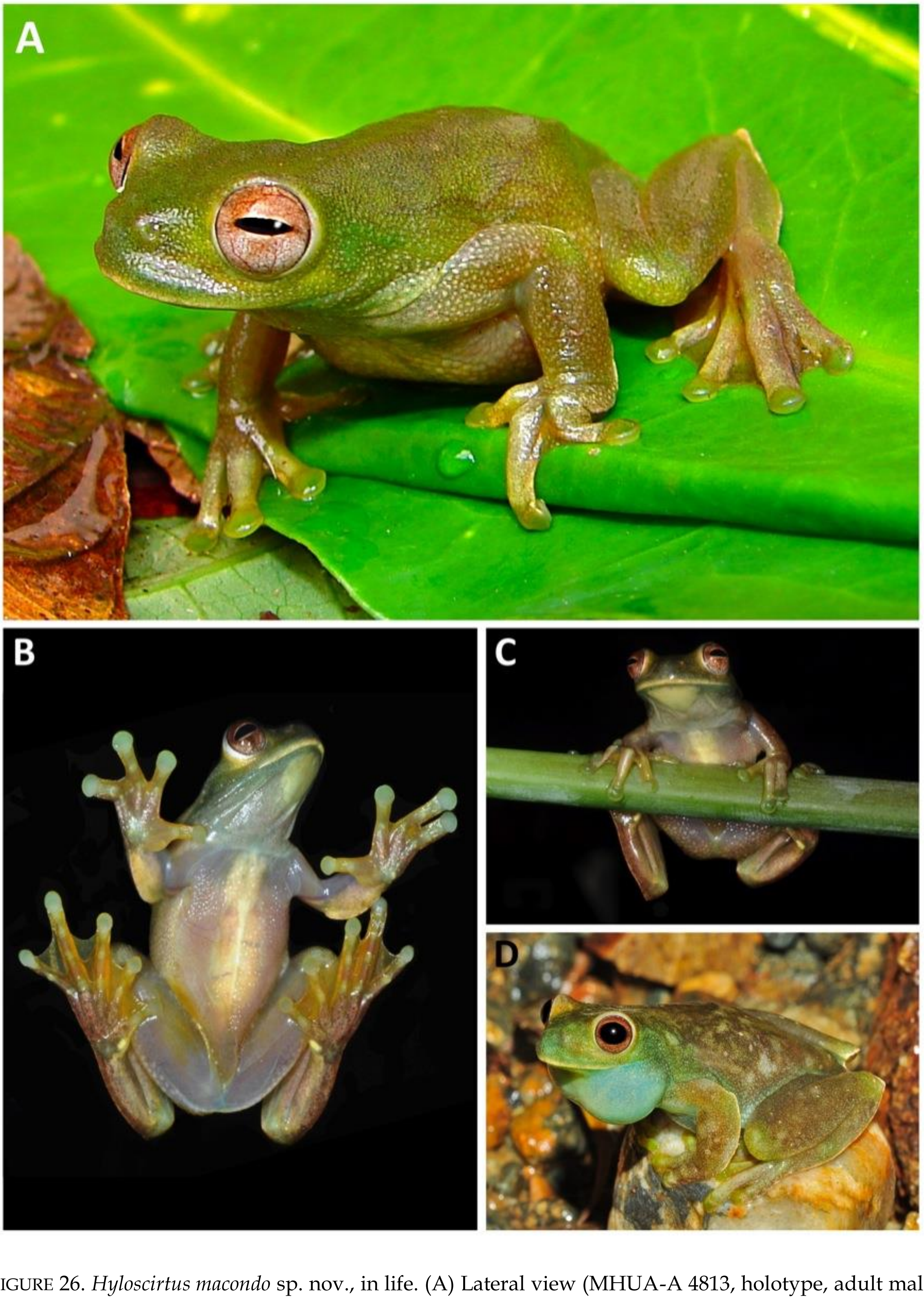

FIGURE 26. *Hyloscirtus macondo* sp. nov., in life. (A) Lateral view (MHUA-A 4813, holotype, adult male, SVL 36.1 mm,); (B and C) ventral and frontal view (MHUA-A 7848, paratype, adult male, SVL 36.0); (D) Lateral view (specimen not collected). Photos: M. Rivera-Correa (A-C); E. Alzate (D).

Dentigerous processes of vomers slightly curved, narrowly separated and posterior to large and ovoid choanae; each series bears 7 and 8 teeth. Choanae separated by a distance larger than two times their maximum diameter, almost the same size of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

FIGURE 27. *Hyloscirtus macondo* sp. nov., dorsal and ventral view. Holotype, adult male, MHUA-A 4813, SVL 36.1 mm (top); paratype, adult female, MHUA-A 6977, SVL 43.8 mm (down). Photos: M. Rivera-Correa.

Forearm robust; axillary membrane present, poorly developed extending one fifth of the length of the forearm. Outer ulnar fold present. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is slightly larger than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers with dermal fringes; webbing formula I – II $1^{2/3}$–3^{-} III 2^{+}–2 IV. Subarticular distal tubercles large and bifid. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine. Nuptial pad absent. Hind limbs robust; tibia length 50% SVL; foot length 42% SVL. Outer tarsal fold and small pyramidal calcar tubercle present; inner tarsal fold indistinct. Inner metatarsal tubercle elongate, elliptical, visible from above. Toes short, with lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 = 5 < 4; extensive toe webbing, formula I 1^{-}–1 II 1^{-}–1 III 1–$1^{2/3}$ IV $1^{2/3}$–1 V. Subarticular tubercles small, round; outer metatarsal tubercle small, round and indistinct; single rows of smaller supernumerary tubercles along the axis of every toe and absent on palm. Cloacal opening directed posteroventrally, at midlevel of thighs; supracloacal fold present; margins of vent with numerous small tubercle; cloacal sheath short; white warts around and below cloaca, ventrally extending 2/4 of the length of the thigh. Dorsal skin weakly granular; gular region smooth; flanks, pectoral region, belly and proximal ventral region of thighs granular. A parietal peritoneum present and pectoral fold absent.

Color in life of the holotype: Dorsal surfaces reddish green with a sparse grayish white flecking. Circulatory system maroon, dorsal and laterally visible. A narrow yellowish white labial stripe; white supracloacal fold; white ulnar and white tarsal fold extending to the calcar with inferior border brown. Vocal sac and ventral surfaces dirty yellowish green and a parietal peritoneum is white; yellow mental gland; ventral surfaces of fingers, toes, webbing on hands and feet, and inguinal region pale orange; axillar region light blue; proximal ventral region of the thighs and pericloacal region with tiny white spots. Iris light brown with abundant lines reticulated maroon, especially in superior area. Bones green.

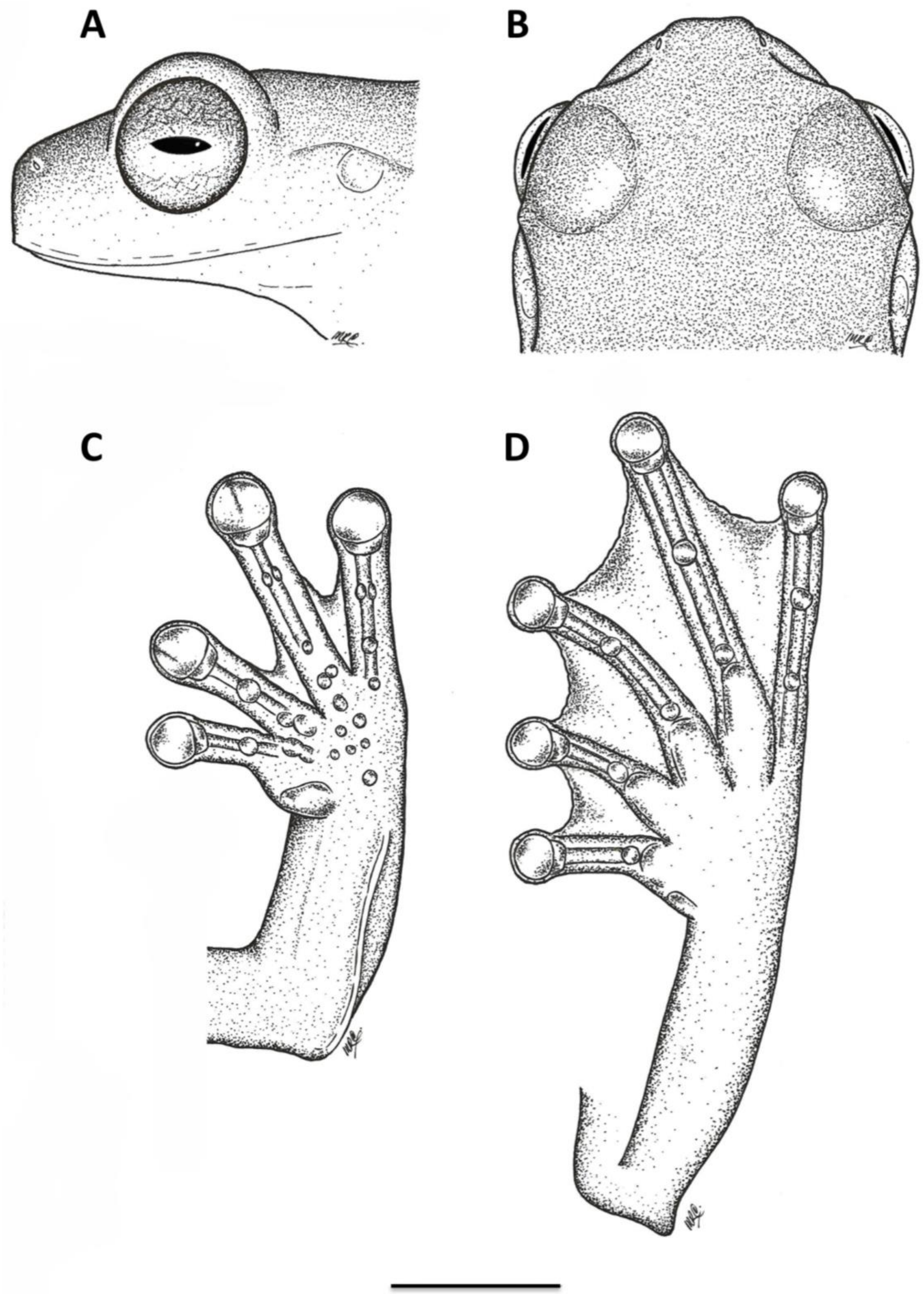

FIGURE 28. *Hyloscirtus macondo* sp. nov., holotype, MHUA-A 4813. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Color in preservative of the holotype: Dorsal surfaces cream with scattered gray blotches and white markings, flanks cream with tiny white spots. Ventral surfaces and gular region cream except fingers, toes, webbing (mainly on the palms, soles, finger IV and Toe V) and shanks having minuscule brown spots. Labial stripe, supracloacal fold, tarsal fold and calcar is white.

Measurements of the holotype (in mm): SVL 36.1; HL 11.0; HW 13.1; ED 4.1; END 2.8; NSD 1.7; IND 3.6; AMD 7.5; TD 1.7; FAL 8.0; FAB 3.5; HAL 11.2; THL 18.3; TL 18.2; TAL 10.3; FL 15.1; TFD 1.8; FTD 1.6.

Variation and sexual dimorphism: Females are larger than males (male SVL 34.7–39.0, n = 8; female SVL 43.2–44.2, n = 2). The number, size, and shape of dorsal blotches varies among individuals. Irregular brown marks and blotches more evident in female (MHUA-A 6977). In life, some individuals have green reddish more intense in dorsum (MHUA-A 5250). In preservation, various specimens have dirty gray dorsum (MHUA-A 7062, 7663, 7847) and dark gray gular region with partial pigmentation of the mental gland (MHUA-A 7062, 7663). Ventrally, dark gray coloration is more evident in some individuals (MHUA-A 7062, 7847) principally in hand, tarsus and foot. The iris of the females has fewer maroon reticulations. The vomerine odontophores contact medially (MHUA-A 6303, 6977, 7026, 7073, 7663, 7673, 7847) or have a discernible gap (MHUA-A 4813, 5875, 7674, 7846, 7848). Number of vomerine teeth 5–9 / 5–10. The finger-webbing formula varies as follow I – II ($1^{1/3}$–$1^{1/2}$) – (3^{-} –3^{+}) III (2–2^{+}) – (2^{-}–2^{+}) IV and, toe-webbing formula varies as follow I (1^{-}–1) – (1^{-}–1^{+}) II (1^{-}–1) – (1^{-}–1^{+}) III (1^{-}–1) – ($1^{1/3}$ –$1^{2/3}$) IV ($1^{1/2}$ –$1^{2/3}$) – (1^{-}–1) V. Secondary sexual characters in females (mental gland, vocal slits, vocal sac) are absent. The ovarian oocytes in a female (MHUA-A 6977) are unpigmented, 2.9–3.0 mm diameter (*n* = 10).

Distribution and natural history: The new species is known from some localities in the Andean valley of the Magdalena River in Colombia. *Hyloscirtus macondo* occupies riparian habitat in lowland to midlands between 400–1200 m a.s.l., approximately. This species occurs on wet rocks or sporadically is found in vegetation on streams. Individuals have been observed perched less than 2.5 meters high and four individuals were observed on rocks with part of the body under watercourse. Males begin their acoustic activity at the beginning of the night, and extend to dawn. Reproductive aspects remain unknown, although there a photographic record of a clutch of eggs that was found near an adult *H. macondo* and possibly belong to this species (does not belong to sympatric glassfrogs, M. Rada pers. comm.). The clutch was found suspended in raceme in the underside of a leaf lanceolate, 28 visibles eggs, transparent, diameters between 5.0–6.0 mm, and larvae with yellow pigments in state 23–24 (Gosner´s state). However, the clutch was not collected and no other data was taken limiting the confirmation.

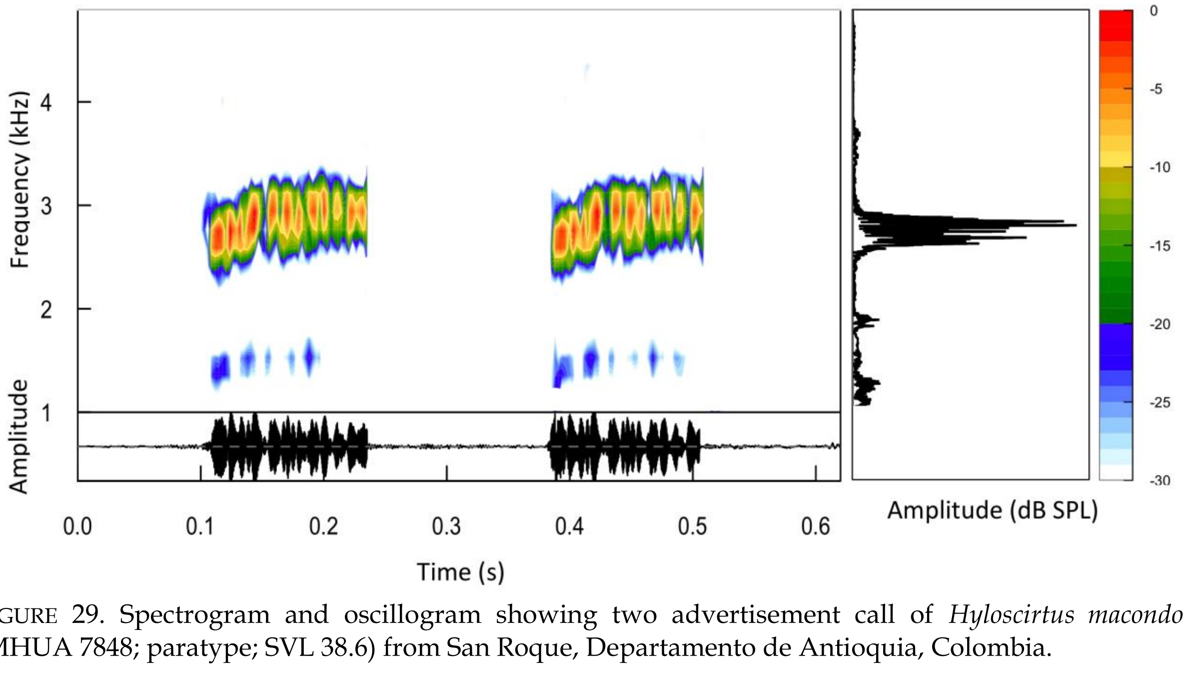

FIGURE 29. Spectrogram and oscillogram showing two advertisement call of *Hyloscirtus macondo* (MHUA 7848; paratype; SVL 38.6) from San Roque, Departamento de Antioquia, Colombia.

Etymology: The specific epithet is an indeclinable word in allusion to Macondo, a imaginary town described in the fantastic novel "Cien Años de Soledad" (One Hundred

Years of Solitude) and is used here noun in apposition. This new species is tribute to Colombian writer Gabriel García Márquez, one of the great exponents of literary magical realism.

HYLOSCIRTUS MURRI SP. NOV.

Holotype: VR 3265 adult male, Colombia, Antioquia, Municipio de Frontino, Corregimiento de Murrí (6º45′47″ N, 76º21′03″ W, 1080 m a.s.l.), collected on 27 February 1992 by José Vicente Rueda and Fabio Quevedo.

Paratypes: All adults. VR 3266, male and VR 3292, female collected with the holotype. ICN 16250-16253, Colombia, Antioquia, Municipio de Frontino, Corregimiento Murri, Km 27 road Nutibará – La Blanquita (6º45′14″ N, 76º19′33″ W, 1000 m a.s.l.), collected on 19 July 1987 by José Vicente Rueda and Alberto Pinilla.

Paragenetype: ICN 16252–53 (JMR 2815 and PR11068; 16S rRNA)

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 39.7–40.7 mm in males, n = 2; 46.1 in female, n = 1); (2) body robust, head wider than body; (3) snout truncate in lateral view and rounded dorsal view; (4) dorsum yellow-green with scattered tiny brown spots, gular region yellowish green and ventral surfaces and flanks yellowish in life (Fig. 30); (5) axillar and inguinal region light blue; (6) mental gland in males present; (7) upper lip without white stripe, not flared and immaculate nostril margin; (8) parietal peritoneum white present; (9) iris copper with abundant maroon reticulation; (11) nuptial pad absent; (13) tympanum rounded and upper edge of tympanic annulus covered by a weak supratympanic fold; (14) canthal striped absent and supratympanic pale stripe present; (15) ulnar fold and tarsal fold white present; (16) calcar tubercle absent; (17) supracloacal fold white present (18) tubercles scattered around and below cloaca absent; (19) white bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) medium dentigerous processes of vomers, slightly curved, separated, with 7–9 teeth each

FIGURE 30. *Hyloscirtus murri* sp. nov., in life (MAR voucher, adult male, SVL 40.1 mm) Photo: M. Rada

Comparison with other species: At the molecular level, *H. murri* sp. nov. differs from *Hyloscirtus* sp. "caldas", and *H. hydrophilus* sp. nov its sister clade by 62 to 74 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 12.0– 14.6 of genetic divergence (uncorrected p-distances) between both species. *Hyloscirtus murri* is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having supratympanic pale stripe (absent in *H. albopunctulatus*, *H. bogotensis*, *H. chlorophyllus* sp. nov., *H. hyalinus* sp. nov., *H. lascinius*, *H. lynchi*, *H. macondo* sp. nov., *H. palmeri*, *H. platydactylus*, *H. piceigularis*, *H. phyllognathus*, *H. simmonsi* and *H. torrenticola*), ulnar fold present (absent in *H. bogotensis*, *H. callipeza*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*), supracloacal fold present (absent in *H. bogotensis*, *H. jahni*, *H. lynchi*, *H. platydactylus* and *H. simmonsi*). *Hyloscirtus chlorophyllus* sp. nov, *H. hyalinus* sp. nov., *H. lascinius*, *H. macondo* sp. nov, *H.*

palmeri, *H. piceigularis*, and *H. phyllognathus*, also differ by having calcar tubercle and white labial stripe (absent in *H. murri* sp. nov.). *Hyloscirtus murri* is most similar to *H. hydrophilus* but differs from it by the larger size of the adult males (SVL 40.2 ± 0.71 in *H. murri* vs 34.5 ± 1.27 in *H. hydrophilus*). Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

Description of holotype: An adult male of 40.7 mm SVL (Fig. 31). Body robust. Head barely wider than long; head as wide as body; head width 34% SVL; head length 33% SVL. Snout rounded in dorsal view and truncate in profile (Fig. 32); *canthus rostralis* rounded, slightly distinct; loreal region concave; lips rounded, not flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, posterior to anterior margin of lower jaw. Internarial region and top of head flat. Interorbital distance longer than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum and tympanic annulus barely visible, rounded and no discernible from above; its diameter equals 40% eye diameter. Supratympanic fold weakly developed, starting in the anterior region of tympanum, slightly covering the dorsal margin of this and without reaching the posterior margin of the insertion of the arm; supratympanic stripe present. Mental gland present, medium, semicircular, covering partially of the gular area and extending about the third length of the throat.

Dentigerous processes of vomers slightly curved, separated and posterior ovoid choanae; each series bears eight (right) and seven (left) teeth. Choanae separated by a distance larger than three times their maximum diameter, smaller regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

Forearm moderately robust; axillary membrane absent. Outer ulnar fold present.

Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is equal than the tympanum diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers without dermal fringes; webbing formula I – II 2^{-}–3^{-} III $2^{1/2}$ –2^{+} IV . Subarticular distal tubercles large and elliptical. A small supernumerary tubercle, high and round at the base of the palm. Outer metacarpal tubercle poorly differentiated, bifid. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine; nuptial excrescencies absent.

FIGURE 31. *Hyloscirtus murri* sp. nov., dorsal and ventral view. Holotype, adult male, VR3265, SVL 40.7 mm (top); paratype, adult female, VR3292, SVL 46.1 mm (down). Photos: M. Rivera-Correa.

Hind limbs moderately robust; tibia length 51% SVL; foot length 44% SVL. Outer tarsal fold and calcar tubercle absent, but pale tarsal stripe evident; inner tarsal fold indistinct. Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes 1 < 2 < 3 = 5 < 4; extensive toe webbing, formula I 1–1^{+} II 1 – $1^{1/2}$ III 1–$1^{2/3}$ IV 2 –1 V. Inner metatarsal tubercle elongate, elliptical, visible from above; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive. Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent without tubercle; supracloacal fold present; sheath short; tubercles scattered around and below cloaca absent. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

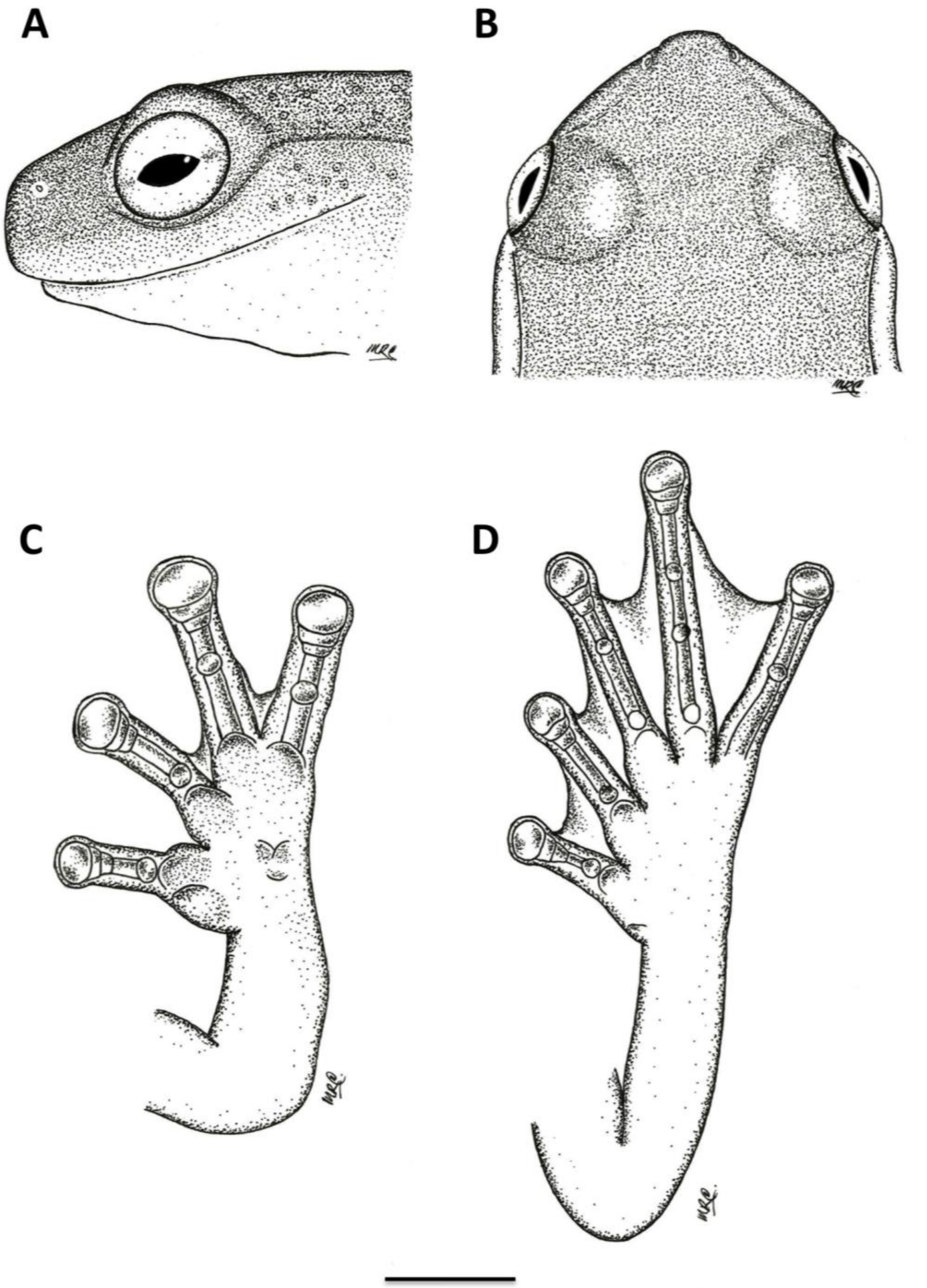

FIGURE 32. *Hyloscirtus murri* sp. nov., holotype, VR 3265 (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Color in life of the holotype: Dorsal surfaces yellow-green with scattered tiny brown spots and flanks yellowish. Supratympanic stripe, ulnar fold and tarsal stripe creamy-white; white supra-cloacal fold. Vocal sac yellowish green and ventral surfaces cream with a white parietal peritoneum. Yellow mental gland with tiny brown spots; ventral surfaces of fingers, toes, webbing on hands and feet yellow; axillar and inguinal region light blue. Iris copper with abundant maroon reticulation. Bones white.

Color in preservative of the holotype: Dorsal surfaces and flanks creamy-brown with dark brown spots, flanks, fingers, toes, webbing and shanks creamy yellow with tiny brown spots. Ventral surfaces creamy white and gular region cream with tiny brown spots. Supratympanic stripe creamy-white; ulnar and supracloacal fold, tarsal stripe and peritoneum white.

Measurements of the holotype (in mm): SVL 40.7; HL 13.3; HW 14.0; ED 3.9; END 2.8; NSD 1.5; IND 3.8; AMD 8.0; TD 1.2; FAL 8.1; FAB 3.5; HAL 13.0; THL 20.0; TL 20.8; TAL 12.9; FL 17.9; TFD 1.9; FTD 1.7.

Variation and sexual dimorphism: Female are larger than males (males SVL 39.7–40.7, n = 2; females SVL 46.1, *n* = 1). The female and the tympanum and supratympanic stripe is more visible than the males. In preservative, the dorsum is cream in female and creamy-brown in males. Number of vomerine teeth 8/ 8–7. The finger-webbing formula varies as follow I – II ($1^{2/3}$–2^{-}) – (3^{+}–3^{-}) III ($2^{1/3}$ – $2^{1/2}$) – (2^{+}–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–1) – (1^{+}–1) II (1– $1^{1/3}$) – (1^{-} – $1^{1/2}$) III (1^{+}–$1^{1/3}$) – (1^{-} –$1^{2/3}$) IV (2^{+}–2) – (1–1^{-}) V. Secondary sexual characters (mental gland, vocal slits, vocal sac) in females are absent.

Distribution and natural history: Hyloscirtus murri sp. nov. is known from proximal localities, Corregimiento de Murri, Municipio de Frontino, departamento de Antioquia, at elevations of 1000–2000 m on the northeastern of the Cordillera Occidental from Colombia. This new species inhabit permanent streams and some individuals were observed from calling on bushes.

Etymology: The specific epithet, *murri*, is used as a noun in apposition and refers to Corregimiento de Murrí, Municipio de Frontino, Antioquia, Colombia, where the type locality of *Hyloscirtus murri* is situated.

Holotype: QCAZ 37250, adult male, Ecuador, Provincia El Oro, Bella María, cerca de Valle Hermoso, collected on 14 October 2004 by SAA

Paratypes: All adults. QCAZ 37246, male, QCAZ 37234, female, collected with the holotype by SAA

Genetype: QCAZ 37250 (16S rDNA)

Characterization: The new species is characterized by the following combination of characters: (1) medium size (SVL 35.7–36.9 mm in males, n = 2; 45.9 in females, n = 1); (2) body slender, head wider than body; (3) snout rounded and truncate in dorsal and lateral view in males and females respectively; (4) dorsal and flanks light yellow with scattered maroon spicules in life (known only in female (Fig. 33), gular region and ventral surfaces yellowish in life; (5) axillar and inguinal region light green; (6) mental gland in males present, pigmented with brown spots; (7) upper lip without white stripe, not flared and immaculate nostril margin; (8) parietal peritoneum white present; (9) iris light gray with abundant tiny maroon reticulation; (11) nuptial pad absent; (13) tympanum rounded upper edge of covered by a supratympanic fold and tympanic annulus only visible in female; (14) supratympanic pale stripe present and canthal stripe present only female; (15) ulnar fold and tarsal stripe white present; (16) calcar tubercle absent; (17) supracloacal fold white present (18) tubercles scattered around and below cloaca absent; (19) white bones in life; (20) elliptical prepollex, not modified as a projecting spine; (21) dentigerous processes of vomers small, slightly curved, with discernible gap, with 5–11 teeth each; (22) the advertisement call unknown.

FIGURE 33. *Hyloscirtus oro* sp. nov. in life (QCAZ 37234, paratype, adult female, SVL 45.9 mm). Photo: S. Ron

Comparison with other species: At the molecular level, *H. oro* sp. nov. differs from *H. alytolylax* its sister species by 26 to 27 fixed nucleotide differences in 505 bp of 16S rDNA, this represents 5.1-5.3% of genetic divergence (uncorrected p-distances) between both species. The new species is morphologically distinguishable from other species of the *Hyloscirtus bogotensis* group in having supratympanic pale stripe (absent in *H. albopunctulatus*, *H. bogotensis*, *H. chlorophyllus* sp. nov., *H. hyalinus* sp. nov., *H. lascinius*, *H. lynchi*, *H. macondo* sp. nov., *H. palmeri*, *H. platydactylus*, *H. piceigularis*, *H. phyllognathus*, *H. simmonsi* and *H. torrenticola*), ulnar fold present (present in *H. albopunctulatus*, *H. alytolylax*, *callipeza, H. colymba, H. hyalinus* sp. nov., *H. hydrophilus* sp. nov., *H. jahni, H. lascinius, H. macondo* sp. nov., *H. palmeri, H. phyllognathus, H. piceigularis, H. oro* sp. nov., *H. torrenticola*), supracloacal fold present (absent in *H. baios* sp. nov., *H. bogotensis, H. jahni, H. lynchi, H. platydactylus* and *H. simmonsi*). *Hyloscirtus chlorophyllus* sp. nov., *H. hyalinus* sp. nov., *H. lascinius*, *H. macondo* sp.

nov., *H. palmeri*, *H. piceigularis*, and *H. phyllognathus*, also differ by having calcar tubercle and white labial stripe (absent in *H. oro* sp. nov.). *Hyloscirtus oro* sp. nov. is most similar to *H. alytolylax, H. hydrophilus* sp nov. and *H. colymba* but differs from them by having head wider than long (longer than wide or equal proportion in *H. alytolylax, H. hydrophilus* sp. nov and *H. colymba*), and color pattern. Other diagnostic characters of species of the *Hyloscirtus bogotensis* group are summarized in the Appendix 6.

Description of holotype: An adult male of 36.9 mm SVL (Fig. 34). Body slender. Head barely wider than long; head wider than body; head width 35% SVL; head length 32% SVL. Snout rounded in dorsal view and profile (Fig. 35); *canthus rostralis* angular, distinct; loreal region concave; lips rounded, not flared. Canthal stripe absent. Nostrils not protuberant, directed anterolaterally, at the level of the anterior margin of the lower jaw. Internarial region and top of head flat. Interorbital distance equal than upper eyelid. Eye prominent, its diameter larger than eye–nostril distance. Tympanum visible, tympanic annulus inconspicuous, rounded and subtly discernible from above; its diameter equals 30% eye diameter. Supratympanic fold developed, starting in the anterior region of tympanum, slightly covering the dorsal margin of this and reaching the posterior margin of the insertion of the arm; supratympanic stripe present. Mental gland present, medium, with diamond-shaped, covering partially of the gular area and extending about the third length of the throat.

Dentigerous processes of vomers straight, separated and posterior ovoid choanae; each series bears six (right) and five (left) teeth. Choanae separated by a distance larger than two times their maximum diameter, equal regarding of the dentigerous process. Tongue cordiform, attached overall (narrowly free around lateral and posterior margin); vocal slits present, longitudinal, originating on the sides of the tongue and extending to the corner of the mouth. Vocal sac moderately distensible, evident externally, single, median and subgular.

FIGURE 34. *Hyloscirtus oro* sp. nov., dorsal and ventral view. Holotype, adult male, QCAZ 37250, SVL 36.9 mm (top); paratype, adult female, QCAZ 37234, SVL 45.9 (down) Photos: M. Rivera-Correa.

Forearm moderately robust; axillary membrane absent. Outer ulnar fold present. Fingers short, thick and bearing small, ovoid discs, with circumferential groove of each disc defined by the size difference between the disc and the smaller pad; width of disc on Finger III is greater than the tympanum diameter. Relative lengths of fingers I < II < IV < III. Fingers without dermal fringes; webbing formula I – II $2^{1/4}$–3 III 2^{+}–2 IV (Fig. 3C). Subarticular distal tubercles large and elliptical. Few supernumerary tubercle, flat at the base of the palm. Outer metacarpal tubercle poorly differentiated. Inner metacarpal tubercle large, thick, elliptical; broad elliptical prepollex, not modified as a spine. Nuptial excrescencies absent.

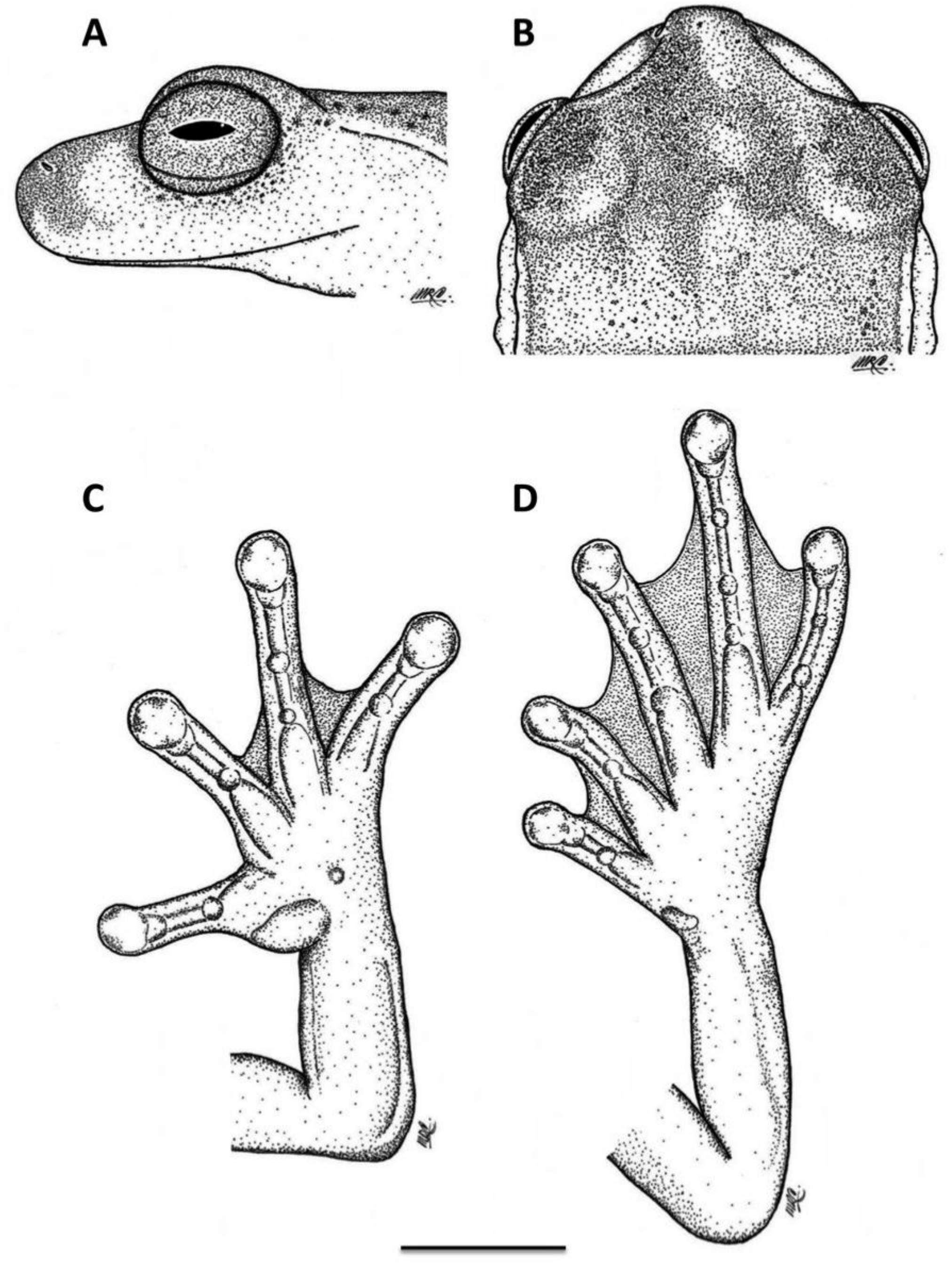

FIGURE 35. *Hyloscirtus oro* sp. nov., holotype, QCAZ 37250. (A) Head in lateral view, (B) head in dorsal view, (C) left hand in ventral view, (D) left foot in ventral view. Scale bar = 5 mm. Drawings: M. Rivera-Correa

Hind limbs moderately robust; tibia length 55% SVL; foot length 46% SVL. Outer tarsal fold and calcar tubercle absent, but pale tarsal stripe evident; inner tarsal fold indistinct.

Toes short, without lateral fringes, bearing discs smaller than those on fingers. Relative length of toes I < II < III < V < IV; extensive toe webbing, formula I 1–$1^{1/2}$ II $1^{1/3}$ – $1^{1/2}$ III 1^{+}–$1^{2/3}$ IV 2–1 V (Fig. 3D). Inner metatarsal tubercle elongate, elliptical, flat; subarticular tubercles small, round; outer metatarsal tubercle absent; supernumerary tubercles not distinctive. Cloacal opening directed posteroventrally, at midlevel of thighs; margins of vent with tubercle; supracloacal fold present; sheath short; tubercles scattered around and below cloaca absent. Dorsal skin, gular and pectoral region, flanks and ventral region of thighs smooth; belly granular. A parietal peritoneum present and pectoral fold absent.

Color in life of the holotype — No notes were taken at the time of capture.

Color in preservative of the holotype: Dorsal surfaces and flanks creamy with irregular dark brown blotches with scattered brown spots; fingers, toes, webbing and shanks creamy yellow with tiny brown spots. Ventral surfaces creamy white and gular region creamy white with tiny brown spots. Supratympanic stripe creamy-white; ulnar and supracloacal fold, tarsal stripe and peritoneum white.

Measurements of the holotype (in mm): SVL 36.9; HL 11.7; HW 13.0; ED 4.7; END 3.5; NSD 1.6; IND 3.3; AMD 7.0; TD 1.4; FAL 7.7; FAB 3.2; HAL 11.5; THL 18.3; TL 20.3; TAL 11.8; FL 17.0; TFD 2.0; FTD 1.8.

Variation and sexual dimorphism: Females are larger than males (males SVL 35.7–36.9, n = 2; female SVL 45.9, n = 1). The snout in females is truncate in profile, while in males is rounded. Females have wider head wider head than the males. In preservation the dorsum is yellow cream in female and brown cream with irregular blotches in males. Internarial area concave and canthal stripe visible in female, absent in males. Mental gland in males having dark brown pigmentation, immaculate in female. The white peritoneum is visible ventrally and the extension varies among individuals. Number of vomerine teeth 10/11 in female, dentigerous processes of vomers more next and concave shape than the males, and vomerine teeth 5 to 6 in males. The finger-webbing formula varies as follow I – II ($1^{2/3}$–2^{-}) – (3^{+}–3^{-}) III ($2^{1/2}$ – $2^{1/2}$) – (2^{+}–$2^{1/3}$) IV and, toe-webbing formula varies as follow I (1^{+}–1) – (1^{+}–1) II (1– $1^{1/3}$) – (1^{-} – $1^{1/2}$) III (1^{+}–$1^{1/3}$) – (1^{-} –$1^{2/3}$) IV (2^{+}–2) – (1–1^{-}) V. Secondary sexual characters (mental gland, vocal slits, vocal sac) in females are absent.

Distribution and natural history: Hyloscirtus oro is currently known only from the type locality. The geographic distribution of this species represents the most southern locality on the western flank of the Andes, for a species of the genus *Hyloscirtus*. The natural history data are very limited, except that individuals were collected in outside creek on bush. The reproductive aspects, advertisement call and tadpoles are still unknown.

Etymology: The specific epithet *oro* is a Spanish word, meaning "gold", is used here as a noun in apposition. The species name is in allusion to Provincia El Oro, Ecuador, and locality of origin of the species.

DISCUSSION

I provide an overview of the current knowledge of evolution and diversity of Hyloscirtus in the Andes and mesoamerican regions. Given the geographical scope I attempted to cover, however some populations and many topics could not be included. Nonetheless, this is, by far, the most comprehensive sampling for *Hylosocirtus* systematics throughout its distribution range.

PHYLOGENETIC RELATIONSHIP

Outgroup relationships. The topology among species of outgroup is relatively congruent with previous hypotheses (Faivovich et al. 2005; Wiens et al. 2010; Pyron and Wiens, 2011; Faivovich et al., 2013). The sampling was not designed to resolve the external relationships of *Hyloscirtus*, however, there are some interesting points that deserve to be commented. As described earlier, the main difference with previous works is related to the non-monophyly of *Mayersiohyla.* Faivovich et al. (2013) recently described two new species of this genus and conducted a test of its monophyly, which had been challenged in recent studies (Wiens et al. 2010; Pyron and Wiens, 2011). At the time of my analysis, Faivovich´s study had not yet been published, so I have not included their new sequences. The inclusion of new sequences by Faivovich et al. (2013) results in a monophyletic *Myersiohyla* and better supported than in earlier analyses, including the one presented here. So my results are not inconclusive regarding relations of *Myersiohyla.* Some terminals species of the genus *Hypsiboas* were not considered in this analysis, so it should not be view as a test of the monophyly of taxonomic group recovered by Faivovich et al. (2005, 2013), which presented a broader sampling of species to this genus. Herein the phylogenetic position of *Hypsiboas pugnax* is inferred for the first time and recovering as a member of the *Hypsiboas faber* group. This results in accordance with the inclusion in this group previously suggested by Faivovich et al. 2005.

Ingroup relationships. Hyloscirtus genus is monophyletic and strongly supported, as was previously inferred with less taxon available (Faivovich et al., 2005; Wiens et al., 2006; Wiens et al., 2010; Pyron and Wiens, 2011; Coloma et al., 2012; Faivovich et al., 2013). *Hyloscirtus armatus* was retrieved as non-monophyletic with *H. charazani* nested within this and further this clade was predominantly recovered with polytomies. This could be due to under-representation of sequences for most of the terminals of the group (i.e. missind data), which leads me to believe that increasing the sampling in this species can recover reciprocally monophyletic. *Hyloscirtus armatus* and *H. charazani* are markedly different morphologically, for example, in the degree of hypertrophy of the arms, directly associated with the degree of development of the processes of the humerus, much more developed in the former. Given this, the results here are not conclusive. Add new molecular evidence will help better understand the phylogenetic relationship between these two species, especially to resolve these polytomies.

The monophyly of *H. bogotensis* group as had been previously conceived by Faivovich et al. (2005) not was recovered, because the phylogenetic position of *H. jahni* (species not included in previous analyzes) as sister species to all other species in the genus. Although this result was obtained only in the dynamic homology topology. The analysis with static alignments in TNT considering gaps as fifth data recovered the different topology as POY, placing *H. jahni* as sister species to all other species of the *H. bogotensis* group but with extremely low support (GB support = 1). The inclusion of molecular evidence for this species, particularly of nuclear origin and decreasing missing data, possibly provide more stability and will prevent inconsistencies between the two topologies.

The topologies recovered here are similar to those found by Pyron and Wiens (2011), Coloma et al. (2012) (except in ML topology), and Faivovich et al. (2013) who recovered *H. bogotensis* group as sister of the clade constituted by *H. armatus* and *H. larinopygion*. Specifically, Coloma et al. 2012 significantly increased sampling for species of the *H. larinopygion* group, data set recently re-analyzed by Faivovich et al. (2013). The results here are consistent with the inferences drawn from Coloma et al. (2012) in his analysis maximum likelihood, and different from the results obtained by them and Faivovich et al. (2013) in their analysis of maximum parsimony. Differences with their topologies only involve the position the *H. larinopygion*, recovering them as sister taxon of a clade containing all species of *Hyloscirtus* excluding *H. lindae* + *H. pantostictus* clade and *H. tapichalaca* on their analyses. My analysis has recovered the former species as part of *H. sarampiona* (*H. larinopygion* + *H. lindae*) clade, and closely related to *H. lindae* + *H. pantostictus*. This difference could be related to the fact sequences for these terminal species was limited only mitochondrial origin.

HYLOSCIRTUS DIVERSITY

The present tesis, which included analysis of 542 specimens (including DNA voucher and specimens for evaluation of phenotypic characters) and 106 localities encompassing seven countries, is of the most extensive sampling efforts to understand the diversity and relationships of the Andean stream-breeding frogs of the genus *Hyloscirtus*.

The results presented here reveal a conclusive pattern where two widespread nominal species (i.e. *H. palmeri, H. phyllognathus*) are composed by at least two new species confirmed, relatively wide distributions and high genetic variability. The analysis of molecular evidence, morphology and calls uncovered exceptionally high levels of undescribed diversity, increased species richness significantly. Thus the results support an increase in the species diversity of *Hyloscirtus* from 33 recognized species, taking into account one species synonymized here, to 42 (27.3% more species), including only confirmed species, or 48 more species (45.5%), if is included all candidate species.

Candidate species within *Hyloscirtus* are not randomly distributed across the species groups. The *H. bogotensis* group alone contributes with thirteen candidate species (seven of them formally described here) and accounts for 82% of the previously unrecognized species. While here describe two species for the *H. tapichalaca* group, increasing to 50% of its previously diversity known.

Nonetheless, it is possible that the level of diversity found here in *Hyloscirtus* is atypically high. Even so, intensive sampling in northern Andes and integrative systematic analyses are necessary to estimate total *Hyloscirtus* species richness in the region, specifically in unexplored geographical areas such as the Western Andes of Colombia and in northern Peru. The results also suggest that *Hyloscirtus* diversity in the Andes is geographically partitioned differently. *Hyloscirtus bogotensis* group is remarkably rich in Colombia in the Amazonian slopes of the Andes of Colombia, Ecuador and northern Peru. *Hyloscirtus larinopygion* group is specially diversified in southern Colombia and northern central Andes of Ecuador. *Hyloscirtus tapichalaca* group is endemic from Andean southern Ecuador and northern Peru. *Hyloscirtus armatus* is restricted to southern Peru and northern to central Andes of Bolivia, while *H. jahni* the unique representative of the group, is endemic to the Cordillera de Merida in western Venezuela.

A genetic distance of 3% in the mitochondrial 16S rRNA gene has been proposed as an operational threshold to establish independent evolutionary lineages in neotropical amphibians (Fouquet et al., 2007). However, using a threshold value is arbitrary because it violates predictions under the evolutionary species concept (Padial et al., 2009). The magnitude of differences in intraspecific genetic divergences varies strongly between lineages because of the different factors involved in the divergences at the population level (Whitlock 2003). Levels of molecular diversity are not necessarily linked to phenotipical distinction among species (e.g. Chek et al. 2001; Vences et al. 2011; Jungfer 2013) or conversely, rapid adaptive radiations can result in morphologically divergent species with low levels of genetic differentiation (Cunha et al. 2005; Padial et al. 2009). This seems to be the case to some species of the group *H. larinopygion***.** For example *H. pacha and H. staufferorum* have relatively low genetic distances (1.9%) while it is clearly distinguishable in acoustical attributes or between *H. princecharlesi* and *H. ptychodactylus* with a difference of 1.7% between them despite morphological distinction.

In contrast, the genetic differentiation and morphologically cryptic species are being commonly revealed in many taxa by either large genetic distances or by the non-monophyly of populations previously considered a single one species. Many epecies of the bogotensis group show this pattern, with a marked morphological inertia and high rates of change in nucleotides mitochondrials. *Hyloscirtus phyllognathus* and *H. chlorophyllus* are morphologically very similar and were recovered not-monophyletic with genetic distances between these, remarkably high.

CHARACTER EVOLUTION

The implications of the evolution of several characters, variation and diversity of hand morphology, hypertrophied forearms, vomerine odontophores process, mental gland, and nuptial excrescence in the phylogeny of *Hyloscirtus* are discussed. Optimization of phenotypic characters in the phylogeny of *Hyloscirtus* suggests that all characters evaluated have complex histories, with multiple transformations involved. Finally, some problems involving the definition of a putative synapomorphy of *Hyloscirtus*, specifically related to tadpole external morphology are mentioned.

HAND MORPHOLOGY

A morphological synapomorphy suggested for genus *Hyloscirtus* is the wide dermal fringes on fingers and toes (Faivovich et al., 2005). The authors also mentioned that the wide dermal fringes in fingers and toes are present in the three species groups traditionally recognized; in the *H. armatus* group they are more obvious in the first manual digit. In the *H. bogotensis* and *H. larinopygion* groups the fringes look even wider, apparently due to a combination of proportionally smaller discs and wider fringe, which gives the finger or toe the appearance of being almost as wide as the disc (Faivovich et al., 2005). Lateral dermal expansions (i.e. fringes) of the digits are usually described in the *Hyloscirtus* literature, but explicit delimitations of character generally is lacking, which has led to substantial misinterpretation. For example, in the *H. larinopygion* group, different authors have referred to as dermal fringes (Duellman and Altig, 1978; Ruiz-Carranza and Lynch, 1982; Ardila-Robayo et al., 1993; Mueses-Cisneros and Angonoy-Criollo, 2008), others have avoided mentioning (Duellman, 1973; Duellman and Berger, 1982; Duellman and Hillis, 1990) or clearly mentioned their absence (Duellman and Coloma, 1993; Coloma et al., 2012). Kizirian et al., (2003), described the fingers of *H. tapichalaca* as lacking lateral fringes but being broad, is the same appearance observed for *H. arcanus* and *H. diabolus* and *H. condor* (S. Ron, personal communication), which fingers are very broad with thickened edge but apparently no fringes was observed. Rivera-Correa and Faivovich (2013) considered that these differences to *Hyloscirtus* could be more from diverse perceptions on how fringes should be defined or different mechanical styles of descriptions, than from actual morphological variation. Nevertheless, due to the different perceptions more detailed analyses on morphological variability of the hand and foot (i.e. histological studies) are needed to establish the level of variation in genus *Hyloscirtus* and tribe Cophomantini.

A notable difference between *H. tapichalaca* species group with respect to remaining species of the *Hyloscirtus* is the morphology of the prepollex. *Hyloscirtus* species have an enlarged, broad and elliptical prepollex (Duellman and Hillis, 1990; Faivovich et al., 2005; trapezoidal according Kizirian et al., (2003), except to *H. armatus* group, which having keratin-covered spines on the expanded distal element of the prepollex and on the first metacarpal (Duellman et al., 1997; Faivovich and de la Riva, 2006; and to *H. tapichalaca* species group, which prepollex that is modified into an enlarged protruding spine (Kizirian et al., 2003; Almendáriz et al. 2014). Evolutionary history of the prepollex with protruding spine is complex and could not be unambiguously optimized. The optimization of this character requires at least three steps and two equally parsimonious alternative scenarios were found (Fig. 36). These alternative scenarios are as diverse as the postuation of a single origin with three independent appearances and subsequent losses of prepollex with protruding spine. Regarding *Hylocirtus* this independent appearance independently in the *H. tapichalaca* group.

The prepollical spine to frogs has been associated with fighting behaviour, including *Hypsiboas faber*, *H. pardalis*, and *H. rosenbergi* (Lutz, 1960; Lutz, 1973; Kluge, 1981; Martins and Haddad, 1988; Martins et al., 1998). In these species, males use the spine to slash opponents during prolonged wrestling bouts (Wells, 2007), males are frequently injured in fights, sometimes fatally (Kluge, 1981), showing skin scarring. In *Hyloscirtus* the function of the prepollical spine is not known. Two specimens from *H. tapichalaca* (both paratypes), has scars on its body suggests that they may have been incurred as a result of combat between males (Kizirian et al., 2003). In the specimens from *H. aracanus* and *diabolus* currently known, I did not observe scars that reveal some sparring between males; this does not mean that this species has no struggle events. In the context of results here presented, the prepollical spine is a morphological synapomorphy to the species constituting the *H. tapichalaca* group (i.e. *H. arcanus, H. condor, H. diabolus, H. tapichalaca*). Further field observations from are needed to understand what is the relationship of this morphological structure with the behavior in the *genus.*

MENTAL GLAND

The mental glands is a cluster of individual glands in the gular region of adult males, occur widely in Plethodontidae [Caudata] (Sever, 1976, 2003; González-León and Ramírez-

Pinilla, 2009). These are cutaneous secretory structures involved for chemical communication (Wells 2007; and references therein). This structure is present in some species of anurans, thus could be strictly involved in reproductive activities such as courtship and mating. The mental glands have been observed in members of the tribe Cophomantini, such as the *Hypsiboas benitezi* species group (Faivovich et al., 2006), *H. cinerascens* and *H. punctatus* of the *H. punctatus* species group (Hoogmoed, 1979; Brunetti et al., 2012), *H. heilprini,* a species of the *H. albopunctatus* group (Trueb and Tyler, 1974), and *Bokermannohyla* (Faivovich et al., 2009). In more recently in at four species of *Myersiohyla* (*M. chamaeleo, M. kanaima, M. loveridgei, and M. neblinaria*; Faivovich et al. 2013).

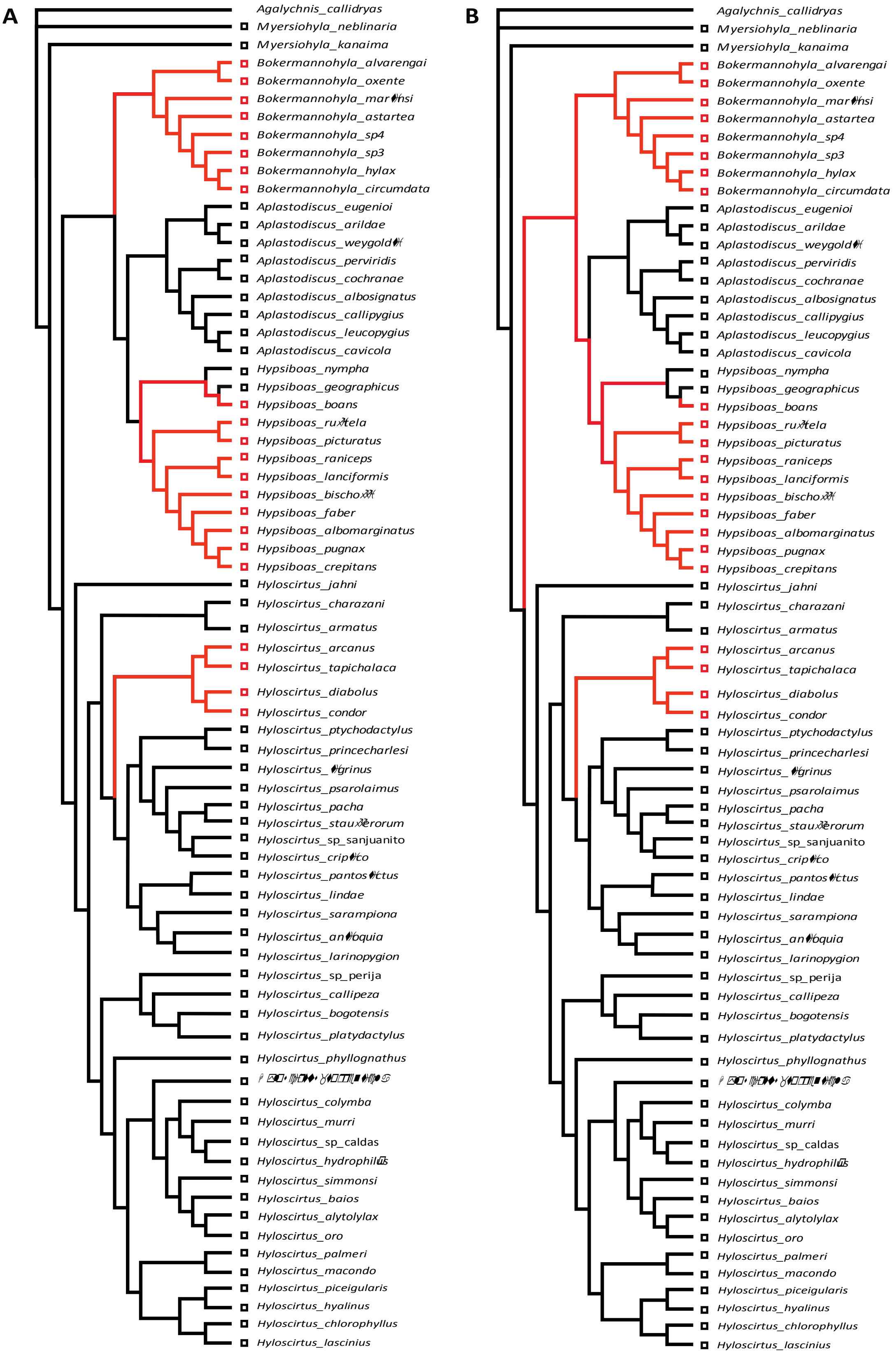

FIGURE 36. Ambiguous optimization of prepollex with protruding spine in Cophomantini, absence (black) and presence (red). Optimizations of character required three steps. Absence of protuding spine is the plesiomorphic state, and they were appears three times independently. Taxonomy updated to the one proposed in the present work.

In *Hyloscirtus* have been previously reported to *Hyloscirtus bogotensis* and *H. armatus* groups (Duellman, 1972; Faivovich and De la Riva, 2006). However, mental glands are not present in species of the *H. larinopygion* group, suggesting that this character state might have reversed in this clade (Rivera-Correa and Faivovich et al., 2013). Nonetheless, apparently the condition of the gland in *H. bogotensis* group and *H. jahni* (pers. obs.), is different from the state in the *H. armatus* group. In the former group and *H. jahni*, the mental gland is subscribed as a quadrangular area and externally visible (except *Hyloscirtus lynchi* which is detectable only with a cut of the skin) whereas in *H. armatus* group the glandular units appear to be more densely distributed towards the mental area more scattered posteriorly (Faivovich and de la Riva 2006).

Faivovich et al. (2013) indicates that mental glands are more frequent than previously reported to Cophomantini, for instance, the results found by them in the genus *Myersiohyla.* These structures sometimes are evident only through superficial dissections (Faivovich et al., 2013; personal observation) suggests that they might be more widely distributed and unnoticed. Thus, the taxonomic distribution and the macroscopic variation observed in members of the tribe Cophomantini in previous contributions suggested a complex scenario with respect to its evolution; accordingly, comparative histological studies are needed to better understand these observations and will corroborate whether these two morphologies could be considered as states of the same transformation series. However, in light of current evidence and in the context of the phylogenetic hypothesis of *Hyloscirtus* relationships inferred herein, the mental gland in Cophomantini were unambiguous and needed four steps four, represent instances of independent origin in *Aplastodiscus perviridis, Bokermannohyla martinsi, Hypsiboas nympha, Myersiohyla kanaima, M. neblinaria* and *Hyloscirtus.* Added to the above in the context of evolution of *Hyloscirtus*, the optimization of mental gland is unambiguous, is apparently plesiomorphic, present in *H. armatus, H. bogotensis* and *H. jahni* and was lost one time in the clade formed by *H. larinopygion* and *H. tapichalaca* groups (Fig. 37).

The reproductive biology of *Hyloscirtus* is largely unknown, and only a few observations are available for *H. platydactylus* (La Marca, 1985) and an ex-situ observation of clutch in captivity for *H. criptico* (S. Ron personal communication). When more field observations or in laboratory are available, can evaluate the direct association of the mental gland with reproductive behavior in the genus *Hyloscirtus*.

NUPTIAL EXCRESCENCES

The nuptial excrescences are secondary sexual characters present in adult males of several species of anuran amphibians (Lynch, 1971; Luna et al., 2012). They are modified epidermal and dermal tissues typically located in the first digit of the hand (Noble, 1931), sometimes second finger (Lynch, 1971) and facilitate the grip of the female during the amplexus (Lataste, 1876; Boulenger, 1897; Noble, 1931; Liu, 1936; personal observation). It has been suggested that nuptial pads are an adaptation to aquatic amplexus, being more developed in species that have amplexus on streams than in those that reproduce in calm water, and less developed or absent in species with terrestrial amplexus (Parker, 1940; Duellman and Trueb, 1986).). Presumably this is because females would be more difficult to hold in water, particularly running water (Wells, 2007). The pads usually consist of alveolar glands having secretory cells with well-defined cellular boundaries (Fujikura et al., 1988; Thomas et al., 1993; Epstein and Blackburn, 1997).

In several species, the epidermis of the pad is distinctively thick, heavily keratinized, and dark colored; in some species, the glands are hypertrophied and form external protrusions of the skin (Fujikura et al., 1988); in other species, the pad is devoid of a thick epidermis and is mostly glandular. Nuptial pads exhibit different morphological and physiological properties during the reproductive cycle (Luna et al. 2012).

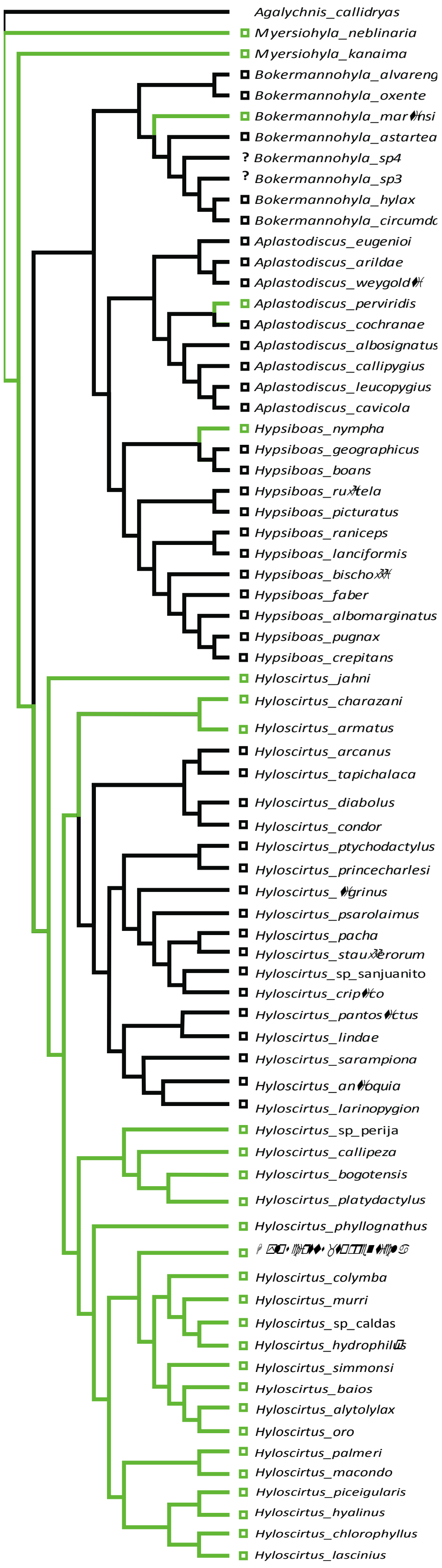

FIGURE 37. Unambiguous optimization of mental gland in Cophomantini, absence (black) and presence (green). Optimizations of character required four steps. Mental gland is the plesiomorphic state, and they were lost two times independently and appear three times independently. Taxonomy updated to the one proposed in the present tesis.

In *Hyloscirtus*, the nuptial pads have been reported in *H. sarampiona* (Ruiz-Carranza and Lynch, 1982), *H. lynchi* (Ruiz-Carranza and Ardila-Robayo, 1991), *H. caucanus, H. larinopygion, H. lindae* (Ardila-Robayo et al., 1993), *H. staufferorum* (Duellman and Coloma, 1993), *H. tigrinus* (Mueses-Cisneros and Anganoy-Criollo, 2008; Mueses-Cisneros and Perdomo-Castillo, 2011), *H. princescharlesi* (Coloma et al., 2012), *H. pantostictus* (personal observation), and *H. antioquia* (Rivera-Correa and Faivovich, 2013). As was suggested by Rivera-Correa and Faivovich (2013) to *H. tigrinus* and after the review of the type series of *H. lynchi* and *H. staufferorum*, the nuptial pads in were not found, contrary to suggest by previous authors (i.e. Ruiz-Carranza and Ardila-Robayo, 1991; Duellman and Coloma, 1993). This may be because there is a clear area in the thumb (without evidence of thickening or keratinization process) of many specimens and contrasts with the color pattern of the rest of the finger, which could lead to misinterpretation.

In *Hyloscirtus*, the shape of the nuptial pad is a medial component on the prepollex that expands distally and covers dorsally a portion of the digit, this may be covered with dark-brown epidermal projections (i.e. *H. antioquia, H. lindae, H. pantostictus, H. sarampiona*) or white-cream epidermal projections as in *H. princecharlesi* (Coloma et al., 2012) and *H. larinopygion* (Rivera-Correa and Faivovich, 2013). Also this character has been reported in some species of the tribe Cophomantini. For instance, in *Myersiohyla* [*M. aromatica* Ayarzagüena and Señaris, (1994) *M. loveridgei* (Rivero, 1961), *M. chamaeleo, M. neblinaria* (Faivovich et al., 2013); *Bokermanohyla* [*B. caramaschii* (Napoli, 2005), *B. gouveai* (Peixoto and Cruz, 1992), *B. hylax* (Heyer, 1985), *B. itapoty* (Lugli and Haddad, 2006), *B. luctuosa* (Pombal and Haddad, 1993), and *B. ravida* (Caramaschi et al., 2001)] and *Hypsiboas* [*H. calcarata*, *H. fasciatus* e *H. geographicus* (Duellman, 1973)]. The unambiguously Optimization of this phenotypic character on the optimal hypothesis here provided, suggests that the nuptial pad has complex scenario of the evolutionary history in Cophomantini with multiple apparitions and lost. The hypotheses presented here demonstrate that the nuptial pad arose independently in *H. armatus* group, *H. princecharlesi*, and the clade formed by ((*H. lindae* + *H. pantostictus*) *H. sarampiona* (*H. antioquia* + *H. larinopygion*)) (Fig. 38).

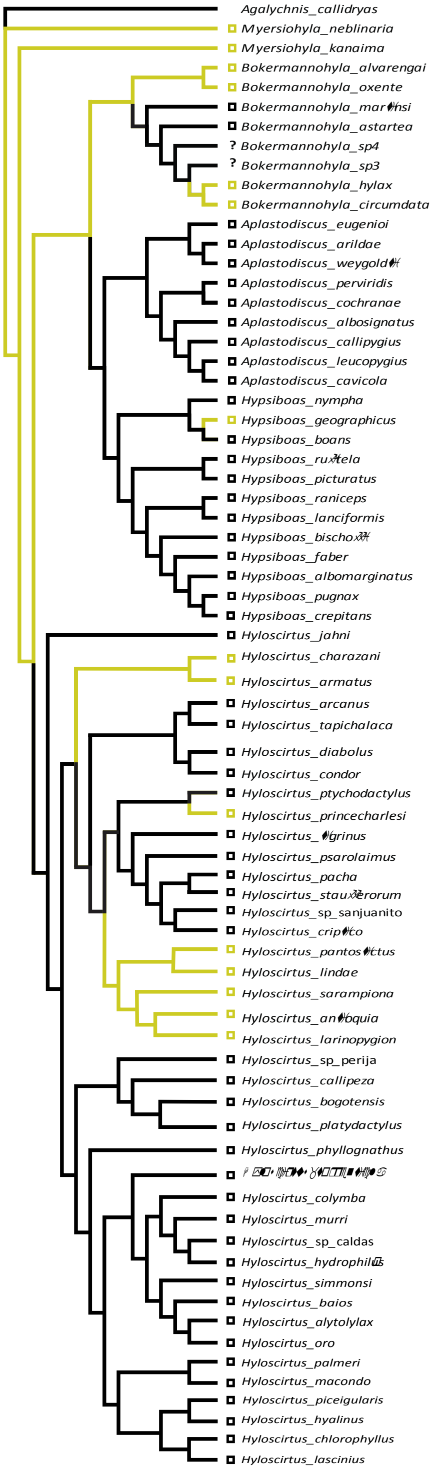

FIGURE 38. Unambiguous optimization of nuptial pad in Cophomantini, absence (black) and presence (yellow). Optimization of character required five steps. Nuptial pad is the plesiomorphic state, and they were lost two times independently and appear three times independently. Taxonomy updated to the one proposed in the present tesis.

TADPOLES

Sánchez (2010) proposed as unambiguous morphological synapomorphy a saccular structure (the bag over the vent tube) that covers developing hindlimbs in larvae of *Hyloscirtus*. In addition also delimited two phenotypic species groups within *Hyloscirtus* based on some morphological attributes of larvae (i.e. tooth row formulae, upper jaw sheath morphology, and shape of the nostril). As Coloma et al. (2012), the results presented here are inconsistent with the Groups A and B of *Hyloscirtus* proposed by Sánchez (2010). The allocation of *H. pantostictus* in Group B (mislabeling as Group A) was based on the number of upper and lower tooth rows (4/6) provided in the description of by Duellman and Hillis (1990). Coloma et al. (2012) stated that the larvae of *H. pantostictus* LTRF varied from 4(4)/5(1) to 6(4–6)/7(1) and has one simple triangular fleshy projection on the inner margin of naris, the anterior jaw sheath is narrow and has small serrations. The anterior morphological condition is present in most tadpoles of *Hyloscirtus* currently known, except to *H. bogotensis, H. callipeza*, and *H. platydactylus* which exhibit an ornamentation around naris with crown shape, large size of upper jaw sheath serration and absence of shelf on the anterior upper jaw sheath. The latter character states are recovered in this analysis as unambiguous synapomorphies for the clade formed by (*H. callipeza* (*H. platydactylus* + *H. bogotensis*)) pending which is the condition in H. sp "Perija", which even do not know their larva. According Sanchez´s these character states is present in *H. lynchi*, but posteriorly to material revision found that these specimens are misidentified which corresponds to *H. callipeza*.

SYSTEMATIC IMPLICATIONS

On the basis in phylogenetic inferences and results presented here I provide a new a taxonomic rearrangement of the species groups of *Hyloscirtus.* Although the change is not necessarily a result of paraphyletic relationships, whether are based on topology and phenotypic synapomorphy proposed to facilitate the recognition of groups.

Hyloscirtus Peters 1882

Type species*: Hyloscirtus bogotensis* Peters 1882.

Hylonomus Peters 1882. Type species: *Hylonomus bogotensis* Peters 1882, by monotypy. Primary homonym of *Hylonomus* Dawson 1860.

Diagnosis. This genus is diagnosed here by 42 transformations in nuclear and mitochondrial and ribosomal genes. Complete list of these molecular synapomorphies (Appendix 9). The phenotypic synapomorphies of *Hyloscirtus* are: (1) The wide dermal fringes on fingers and toes (Faivovich et al., 2005); (2) a saccular structure associated with the vent tube covers developing hindlimbs in the tadpoles (Sánchez 2010); (3) adults inhabit alongside streams; (4) Tadpoles develop in lotic waters.

Content. This genus contains forty-three species placed in four species groups. *Hyloscirtus armatus* group (Duellman et al., 1997), *H. bogotensis* group (Duellman, 1972), *H. larinopygion* group (Duellman and Hillis, 1990), *Hyloscirtus tapichalaca* group and *Hyloscirtus jahni,* currently not assigned to any group.

Remarks. Faivovich et al. (2005) resurrected *Hyloscirtus* from the synonymy of *Hyla* where it was placed by Duellman (1970), for all species formerly placed in the *Hyla armata*, *Hyla bogotensis*, and *Hyla larinopygion* species group. These authors placed the genus in the tribe Cophomantini together with the genera *Aplastodiscus, Bokermannohyla, Hypsiboas* and *Myersiohyla*. Subsequently, Sánchez (2010) delimited two species groups to *Hyloscirtus* based on larval morphology, inference molecularly no recovered by Coloma et al. (2012).

Distribution. From wet forest Central Costa Rica to eastern and western slopes of the Andes in Colombia and Ecuador; Colombian inter-Andean valleys and Amazonian basin of Venezuela, Colombia, Ecuador, Peru, and Bolivia (Frost, 2014), from 300 to *ca*. 3500 m a.s.l.

Hyloscirtus armatus Species Group (Duellman et al., 1997)

Diagnosis. This species group is diagnosed here by 106 transformations in nuclear and mitochondrial and ribosomal genes. Complete list of these molecular synapomorphies (Appendix 9). The phenotypic synapomorphies of *Hyloscirtus armatus* group are: (1) The keratin-covered bony spines on the proximal ventral surface of the humerus, (2) on the expanded distal element of the prepollex, and on the first metacarpal, (3) tadpole tail long with low fins and bluntly rounded tip (Duellman et al., 1997).

Content. Three species. *Hyloscirtus armatus* (Boulenger, 1902); *Hyloscirtus charazani* (Vellard, 1970). *Hyloscirtus chlorosteus* (Reynolds and Foster, 1992)

Remarks. Hyloscirtus armatus (*Hyla armata* group) species group is constituted by Duellman et al. (1997) to include *H. armatus* as a unique species. De la Riva et al. (2000) suggested that the species is composed of more than one independent taxonomic entity. *Hyloscirtus charazani* is allocated in *Hyla albopunctata* group according to the original description and *Hyloscirtus chlorosteus* in *Hyla chlorostea* group according to Duellman et al. (1997). *Hyloscirtus armatus* and *H. charazani* are included in the *Hyloscirtus armatus* group by Faivovich et al. (2005), and then *H. chlorosteus* also by Faivovich and De la Riva (2006). Posteriorly, *H. armatus* and *H. charazani* are allocated in *Hyloscirtus* species group B according to Sánchez (2010).

Distribution. Cloud forests on the eastern slopes of the Andes from central Peru to central Bolivia at elevations of 1700-3200 m (Frost, 2014).

Hyloscirtus bogotensis Species Group (Duellman, 1972)

Diagnosis. This species group is diagnosed here by 65 transformations in nuclear, mitochondrial and ribosomal genes. Complete list of these molecular synapomorphies (Appendix 9). The phenotypic synapomorphies of *Hyloscirtus bogotensis* group are: (1) distal process of sacral diapophyses broadly dilated, relative to the proximal region.

Content. Twenty-one species. *Hyloscirtus albopunctulatus* (Boulenger, 1882); *Hyloscirtus alytolylax* (Duellman, 1972); *Hyloscirtus baios* sp. nov; *Hyloscirtus bogotensis* Peters 1882; *Hyloscirtus callipeza* (Duellman, 1989); *Hyloscirtus chlorophyllus* sp. nov.; *Hyloscirtus colymba* (Dunn, 1931); *Hyloscirtus estevesi* (Rivero, 1968); *Hyloscirtus hyalinus* sp. nov.; *Hyloscirtus hydrophilus* sp. nov.; *Hyloscirtus lascinius* (Rivero, 1969); *Hyloscirtus lynchi* (Ruiz-Carranza and Ardila-Robayo, 1991); *Hyloscirtus macondo* sp. nov.; *Hyloscirtus murri* sp. nov.; *Hyloscirtus oro* sp. nov.; *Hyloscirtus palmeri* (Boulenger, 1908); *Hyloscirtus phyllognathus* (Melin, 1941); *Hyloscirtus piceigularis* (Ruiz-Carranza and Lynch, 1982); *Hyloscirtus platydactylus* (Boulenger, 1905); *Hyloscirtus simmonsi* (Duellman, 1989); *Hyloscirtus torrenticola* (Duellman and Altig, 1978).

Remarks. Hyloscirtus bogotensis (as *Hyla bogotensis* group) species group is constituted by Duellman (1972) and reviewed by Faivovich et al. (2005). Faivovich and De la Riva (2006) suggested that the *Hyloscirtus armatus* group might render paraphyletic the *Hyloscirtus bogotensis* group.

Distribution. Widely distributed in the neotropics; occurs from Costa Rica along western, central and eastern slopes of the Andes in Colombia; along the length of the western slopes of the Andes in Ecuador. In addition, on the Amazonian slopes of the western Andes in Colombia, Ecuador and to southern Peru. Distributed at elevations of 300-2,200 m asl.

Hyloscirtus larinopygion Species Group (Duellman and Hillis, 1990)

Diagnosis. This species group is diagnosed here by 99 transformations in nuclear and mitochondrial and ribosomal genes. Complete list of these molecular synapomorphies (Appendix 9). Morphological synapomorphies: (1) Vomerine odontophores large slightly S-shaped and bear eight to 25 small teeth each; (2) distal process of sacral diapophyses no dilated, distal process of sacral diapophyses broadly dilated, relative to the proximal region.

Content. Thirteen species. *Hyloscirtus antioquia* Rivera-Correa and Faivovich 2013; *Hyloscirtus caucanus* (Ardila-Robayo, Ruiz-Carranza, and Roa-Trujillo, 1993); *Hyloscirtus criptico* Coloma, Carvajal-Endara, Dueñas, Paredes-Recalde, Morales-Mite, Almeida-Reinoso, Tapia, Hutter, Toral-Contreras, and Guayasamin 2012; *Hyloscirtus larinopygion* (Duellman, 1973); *Hyloscirtus lindae* (Duellman and Altig, 1978); *Hyloscirtus pacha* (Duellman and Hillis, 1990); *Hyloscirtus pantostictus* (Duellman and Berger, 1982); *Hyloscirtus princecharlesi* Coloma, Carvajal-Endara, Dueñas, Paredes-Recalde, Morales-Mite, Almeida-Reinoso, Tapia, Hutter, Toral-Contreras, and Guayasamin 2012; *Hyloscirtus psarolaimus* (Duellman and Hillis 1990); *Hyloscirtus ptychodactylus* (Duellman and Hillis, 1990); *Hyloscirtus sarampiona* (Ruiz-Carranza and Lynch 1982) new comb.; *Hyloscirtus staufferorum* (Duellman and Coloma, 1993); *Hyloscirtus tigrinus* Mueses-Cisneros and Anganoy-Criollo, 2008

Remarks. Hyloscirtus larinopygion (as *Hyla larinopygion* group) species group is constituted by Duellman and Hillis (1990) and reviewed by Duellman and Coloma (1993). Kizirian et al. (2003) noted that the *Hyla larinopygion* group couldn't be diagnosed as monophyletic without including *Hyla armata*. Subsequently, is updated by Faivovich et al. (2005) including *Hyla tapichalaca,* which its phylogenetic position was uncertain in his original description. Recently, Coloma et al. (2012) proposes most comprehensive phylogenetic analysis regarding taxon sampling for the group.

Distribution. The range of this species group includes eastern slopes of Cordillera Occidental, along Cordillera Central and eastern slopes of Cordillera Oriental of Colombia to western and eastern Andes in Ecuador. Distributed at elevations of 2000-3500 m asl.

Hyloscirtus tapichalaca Species Group

Diagnosis. This species group is diagnosed here by 104 transformations in nuclear and mitochondrial and ribosomal genes. Complete list of these molecular synapomorphies (Appendix 9). The putative phenotypic synapomorphies of *Hyloscirtus tapichalaca* group are: (1) prepollex modified into an enlarged, curved and protruding spine; (2) Vomerine odontophores small, bulky shape and bears four to six four prominent teeth each; (3) prominent maxilar teeth, projecting to the outside as much as the length of root; (4) hypertrophied muscles of the forearm in males.

Content. Four species. *Hyloscirtus arcanus*; *Hyloscirtus condor* Almendáriz, Brito, Batallas and Ron 2014; *Hyloscirtus diabolus*; *Hyloscirtus tapichalaca* (Kizirian, Coloma, and Paredes-Recalde, 2003)

Remarks. The previous association of *H. tapichalaca* with *H. larinopygion* group was because this was recovered as sister species of the clade formed by *H. pantostictus* and *H. pacha* (Faivovich et al., 2005). A convenient choice because the phylogenetic position of *H. tapichalaca* was unclear in his original description (Kizirian et al, 2003). Subsequently, *H. tapichalaca* is recovered as sister species *H. lindae* (KU 202728; Wiens et al., 2006, Wiens et al., 2010, Pyron and Wiens, 2011), however, the latter species remained misidentified and here is described as *H. arcanus* sp nov. (this study)

Distribution. The range of this species group from eastern Andean slopes southern Ecuador to northwestern Andes of Peru, specifically, Cordillera del Condor of Ecuador to departamento de Amazonas of Perú, at elevetaions of 2000 to 2700 m a.s.l.

Hyloscirtus jahni

Remarks. Hyla jahni was removed from the synonymy of *Hyla platydactyla* by La Marca (1985) where it had been placed by Duellman (1972). Faivovich et al. (2005) allocated in the *Hyloscirtus bogotensis* group. The creation of this group is supported by the phylogenetic position of *H. jahni* in the dynamic alignment analysis, despite of the phylogenetic position with low support within *H. bogotensis* group, suggested by the static alignment. I consider this to be a viable option given that the analysis presented herein represents the most effort, considering taxon sampling to test explicitly the hypotheses of relationships within *Hyloscirtus.*

Distribution. Cloud forests of the Mérida Andes in the states of Mérida and Trujillo, Venezuela (Frost, 2014). It has been recorded at elevations of 1800-3000 m a.s.l. (Stuart, 2008).

FUTURE WORKS

Until recently, very little was known about the phylogenetic affinities of the many species of *Hyloscirtus*. This began to change with the work specifically by Faivovich et al. 2005, Coloma et al. 2012 and Faivovich et al. 2013, and continued with the present Tesis. Despite this recent advance, is still lacking some species that could not be included (i.e. *H. albopunctulatus, H. chlorosteus, H. caucanus* and *H. estevesi*). We also envision benefits from the inclusion of phenotypic characters into the phylogenetic estimation, in a context of total evidence. Being *Hyloscirtus* a widespread genus in the Andes and occurring in several biomes at different altitudes, has the potential to be of great relevance for biogeographic studies and this should be further explored.

The phenotypic diversity that exists within *Hyloscirtus* can also be more deeply explored once a more complete ecological knowledge of the group increases. Thus, It is extremely important that future work on *Hyloscirtus* account for variation among populations and that effort is directed to the collection of tadpoles, the recording of advertisement calls, documentation of reproductive behavior, and the collection of tissue samples to improve coverage of populations in phylogenetic studies. These data are essential for further exploration of systematics, geographical variation, and character evolution in the group. Particularly in some lineages with a very conservative morphology, constituting classic example cases of cryptic diversity. Many species of *Hyloscirtus* are in any endangered categories (see IUCN 2013) while others are Deficient Data and, naturally the new species not have been evaluated. Evaluate the IUCN criteria from the knowledge generated here will be essential to understand the actual status of threatened of this fascinating Andean clade.

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APPENDIX

APPENDIX 1. Sequences obtained from GenBank, species identification, acronym voucher or tissue identification number and GenBank accession number for each locus. Locus abbreviations: 12S (12S rDNA), 16S (16S rDNA), cytb (cytochrome b), RHODOP (rhodopsin exon 1), RAG1 (recombination activating gene 1), SIAH 1 (seven in absentia homolog 1), 28S (large nuclear ribosomal subunit), COI (cytochrome c oxidase I), POMC (proopiomelanocortin A gene) and H3 (histone H3)

SPECIES	**ACRONYM**	**12S**	**16S**	**CYTB**	**RHODOP**	**RAG 1**	**Tyrosinase**	**SIAH 1**	**28S**	**COI**	**POMC**	**H3**
OUTGROUP												
A. callidryas	RdS537	AY843563	AY843563	EF125028	AY844537	EF174312	DQ283018	AY844765	–	FJ766570	EF158395	DQ284401
A. perviridis	MACN 37791	AY843569	AY843569	AY843791	AY844543	AY844366	AY844025	AY844771	AY844201	_	_	DQ284044
A. leucopygius	USNM303038	AY843638	AY843638	AY843873	AY844622	AY844425	AY844084	AY844840	AY844261	_	_	_
A. callipygius	CFBH3909	AY843614	AY843614	AY843840	AY844592	AY844402	AY844058	AY844813	AY844236	_	_	_
A. arildae	USNM303022	AY843604	AY843604	AY843825	AY844578	AY844392	AY844049	AY844803	AY844223	_	_	_
A. albosignatus	CFBH3184	AY843596	AY843596	AY843817	AY844570	AY844385	AY844042	AY844796	AY844219	_	_	_
A. cochranae	CFBH3001	AY843568	AY843568	AY843790	AY844542	AY844365	_	AY844770	AY844200	_	_	_
A. cavicola	AF0070	AY843617	AY843617	AY843843	AY844594	AY844405	–	AY844814	–	_	_	_
A. weygoldti	AF0068	AY843685	AY843685	AY843931	AY844678	AY844467	–	AY844887	–	_	_	_
A. eugenioi	CFBH5915	AY843669	AY843669	AY843913	AY844660	AY844456	–	AY844875	–	_	_	_
B. hylax	USNM303036	AY549338	AY549338	AY549391	AY844614	AY844419	AY844077	AY844832	AY844254	_	_	_
B. martinsi	AF414	AY843641	AY843641	AY843878	AY844626	–	AY844086	AY844844	AY844264	_	_	_
B. astartea	USNM303032	AY549322	AY549322	AY549375	AY844580	–	–	–	AY844225	_	AY819113	_
B. alvarengai	CFBH5652	AY843677	AY843677	AY843922	AY844669	AY844461	–	AY844881	AY844294	_	_	_
B. oxentes	CFBH5642	AY843676	AY843676	AY843919	AY844667	AY844460	AY844101	AY844879	AY844292	_	_	_
B. circumdata	CFBH3621	AY549328	AY549328	AY549381	AY844598	AY844409	AY844064	AY844817	AY844242	_	_	_
B. sp3	CFBH 5766	AY843673	AY843673	AY843916	AY844664	–	AY844115	–	–	_	_	_
B. sp4	CFBH 5917	AY843674	AY843674	AY843917	AY844665	AY844458	AY844116	AY844877	–	_	_	_
H. nympha	NMP6V712022	AY843670	AY843670	AY843914	AY844661	AY844457	AY844112	–	AY844289	_	_	_
H. faber	MACN37000	AY549334	AY549334	AY549387	AY844607	–	–	AY844825	–	JQ627303	–	–
H. geographicus	AMNHA141054	AY843628	AY843628	–	–	–	EF376122	–	–	_	_	_
H. picturatus	KU202737	AY326055	AY326055	–	–	–	–	–	–	_	_	_
H. raniceps	MACN37795	AY843657	AY843657	AY843900	AY844646	–	AY844103	AY844863	–	_	AY819125	–

H. bischoffi	CFBH3356	AY549324	AY549324	AY549377	AY844586	AY844398	–	–	–	–	–	–
H. albomarginatus	USNM284519	AY549316	AY549316	AY549369	AY844568	AY844384	–	AY844794	AY844218	–	–	–
H. rufitela	KRL798	AY843662	AY843662	AY843905	AY844652	–	AY844105	AY844867	AY844282	–	–	–
H. crepitans	CFBH2966	AY843621	AY843621	AY843850	AY844601	AY844412	AY844067	–	–	–	JN970904	–
H. pugnax	MRC514	MRC514	MRC514	–	–	–	–	–	–	–	–	–
H. boans	RWM17746	AY843610	AY843610	AY843835	AY844588	–	AY844055	AY844809	AY844231	–	AY819114	DQ284086
H. lanciformis	MJH564	AY843636	AY843636	AY843870	AY844619	–	AY844081	AY844837	AY844258	–	JN970900	–
M. neblinaria	RWM17688	AY843672	AY843672	–	AY844663	–	AY844114	AY844876	AY844291	–	–	–
M. kanaima	ROM39582	AY843634	AY843634	AY843868	AY844617	AY844422	AY844079	AY844835	–	–	–	–
INGROUP												
H. armatus	AMNHA165163	AY549321	AY549321	AY843826	AY844804	AY844393	AY844050	AY844804	AY844224	–	–	DQ284070
H. armatus	KU173222	AY819423	–	–	–	–	–	–	–	–	–	–
H. alytolylax	QCZA24376	JX155799	JX155826	–	–	–	–	–	–	–	–	–
H. alytolylax	QCZA24377	JX155798	JX155825	–	–	–	–	–	–	–	–	–
H. charazani	AMNHA165132	AY843618	AY843618	AY843844	AY844595	AY844406	AY844061	–	AY844239	–	–	–
H. colymba	SIUCH6926	DQ380353	–	–	–	–	–	–	–	–	AY819157	–
H. colymba	SIUCH7079	AY843620	AY843620	AY843848	AY844599	AY844410	AY844065	–	AY844243	FJ766722	–	–
H. criptico	QCAZ45466	JX155840	JX155813	–	–	–	–	–	–	–	–	–
H. criptico	QCAZ43422	JX155841	JX155814	–	–	–	–	–	–	–	–	–
H. criptico	QCAZ43421	JX155839	JX155839	–	–	–	–	–	–	–	–	–
H. larinopygion	QCAZ45462	JX155818	JX155845	–	–	–	–	–	–	–	–	–
H. larinopygion	QCAZ41826	JX155817	JX155844	–	–	–	–	–	–	–	–	–
H. lascinius	KU181086	DQ380359	–	–	–	–	–	–	–	–	–	–
H. lindae	KU202728	DQ380361	–	–	–	–	–	–	–	–	–	–
H. lindae	QCAZ45463	JX155823	JX155850	–	–	–	–	–	–	–	–	–
H. lindae	QCAZ41232	JX155821	JX155821	–	–	–	–	–	–	–	–	–
H. lindae	QCAZ45342	JX155824	JX155824	–	–	–	–	–	–	–	–	–
H. lindae	QCAZ45346	JX155822	JX155849	–	–	–	–	–	–	–	–	–
H. pacha	KU202760	AY326057	AY326057	–	–	–	–	–	–	–	–	–

H. pantostictus	KU202732	AY326052	AY326052	_	_	_	_	_	_	_	_	_
H. pantostictus	QCAZ45438	JX155819	JX155819	_	_	_	_	_	_	_	_	_
H. pantostictus	QCAZ45435	JX155820	_	_	_	_	_	_	_	_	_	_
H. palmeri	SIUCH6924	AY843650	AY843650	AY843890	AY844636	AY844439	AY844095	_	AY844273	FJ766723	AY819158	DQ284088
H. psarolaimus	QCAZ46095	JX155809	JX155836	_	_	_	_	_	_	_	_	_
H. psarolaimus	QCAZ27049	JX155808	JX155835	_	_	_	_	_	_	_	_	_
H. ptychodactylus	QCAZ46030	JX155804	JX155804	_	_	_	_	_	_	_	_	_
H. ptychodactylus	QCAZ46031	JX155805	JX155804	_	_	_	_	_	_	_	_	_
H. simmonsi	KU181167	DQ380376	AY819555	_	_	_	_	_	_	_	AY819159	_
H. staufferorum	QCAZ45967	JX155815	JX155842	_	_	_	_	_	_	_	_	_
H. staufferorum	QCAZ45962	JX155816	JX155843	_	_	_	_	_	_	_	_	_
H. princecharlesi	QCAZ43654	JX155807	JX155834	_	_	_	_	_	_	_	_	_
H. princecharlesi	QCAZ42165	JX155806	JX155833	_	_	_	_	_	_	_	_	_
H. tigrinus	QCAZ31550	JX155811	JX155838	_	_	_	_	_	_	_	_	_
H. tigrinus	QCAZ41351	JX155810	JX155837	_	_	_	_	_	_	_	_	_
H. tapichalaca	QCAZ 15083	JX155803	JX155830	_	_	_	_	_	_	_	_	_
H. tapichalaca	QCAZ 16704	AY563625	AY563625	AY843925	AY844672	_	AY844121	_	AY844297	_	_	_
H. phyllognathus	QCAZ41032	JX155801	JX155828	_	_	_	_	_	_	_	_	_
H. phyllognathus	QCAZ32271	JX155802	JX155829	_	_	_	_	_	_	_	_	_
H. phyllognathus	QCAZ23938	JX155800	JX155827	_	_	_	_	_	_	_	_	_

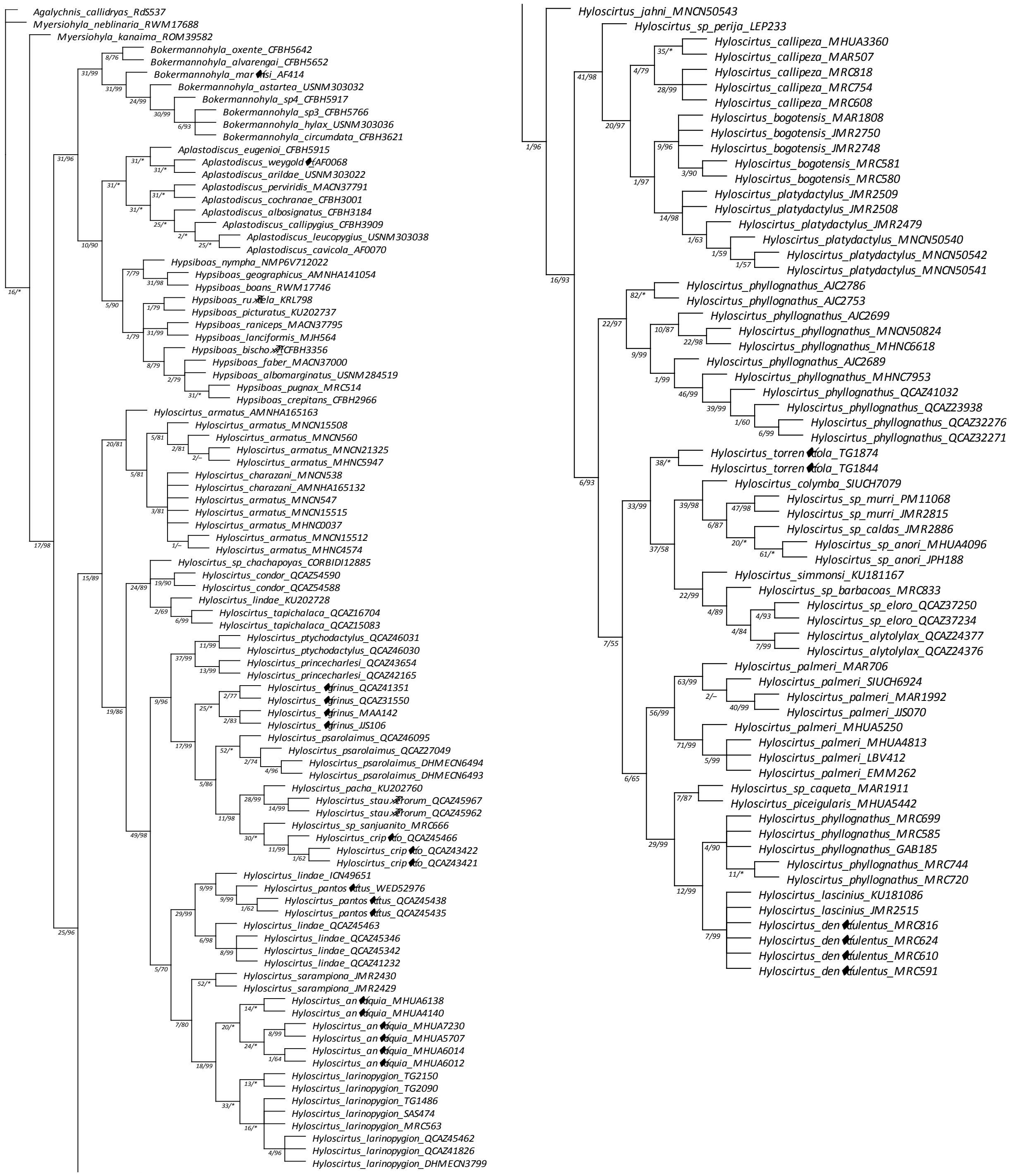
APPENDIX 2. Sequences generated in this work, species identification, acronym voucher or tissue identification number and and length for each locus. Locus abbreviations: 12S (12S rDNA), 16S (16S rDNA), cytb (cytochrome b), RHODOP (rhodopsin exon 1), COI (cytochrome c oxidase I), POMC (proopiomelanocortin A gene)

Specie	**Voucher**	**T. length**	**12s16s**	**COI**	**Cyt b**	**POMC**	**RHODOP**
H. alytolylax	QCAZ24376	2474 bp	2474	–	–	–	–
H. alytolylax	QCAZ24377	4439 bp	2474	658	386	603	318
H. antioquia	MHUA4140	2474 bp	2474	–	–	–	–
H. antioquia	MHUA5707	3836 bp	2474	658	386	–	318
H. antioquia	MHUA6012	3178 bp	2474	–	386	–	318
H. antioquia	MHUA6014	3450 bp	2474	658	–	–	318
H. antioquia	MHUA6138	3518 bp	2474	658	386	–	–
H. antioquia	MHUA7230	3836 bp	2474	658	386	–	318
H. armatus	MHNC0037	2383 bp	1997	–	386	–	–
H. armatus	MHNC4574	3178 bp	2474	–	386	–	318
H. armatus	MHNC5947	3518 bp	2474	658	386	–	–
H. armatus	MNCN15508	1526 bp	550	658	–	–	318
H. armatus	MNCN15512	1526 bp	550	658	–	–	318
H. armatus	MNCN15515	1526 bp	550	658	–	–	318
H. armatus	MNCN21325	1484 bp	550	658	–	–	276
H. armatus	MNCN547	1526 bp	550	658	–	–	318
H. armatus	MNCN560	1208 bp	550	658	–	–	–
H. bogotensis	JMR2748	1903 bp	1517	–	386	–	–
H. bogotensis	JMR2750	1133 bp	1133	–	–	–	–
H. bogotensis	MAR1808	3836 bp	2474	658	386	–	318
H. bogotensis	MRC580	2701 bp	1997	–	386	–	318
H. bogotensis	MRC581	1903 bp	1517	–	386	–	–
H. callipeza	MAR507	3473 bp	2474	613	386	–	–
H. callipeza	MHUA3360	3791 bp	2474	613	386	–	318
H. callipeza	MRC608	2701 bp	1997	–	386	–	318
H. callipeza	MRC754	3359 bp	1997	658	386	–	318
H. callipeza	MRC818	2383 bp	1997	–	386	–	–
H. charazani	MNCN538	658 bp	–	658	–	–	–
H. condor	QCAZ54588	2473 bp	2474	–	–	–	–
H. condor	QCAZ54590	2474 bp	2474	–	–	–	–
H. criptico	QCAZ43421	2436 bp	2436	–	–	–	–
H. criptico	QCAZ43422	4401 bp	2436	658	386	603	318
H. criptico	QCAZ45466	4401 bp	2436	658	386	603	318
H. denticulentus	MRC591	3836 bp	2474	658	386	–	318
H. denticulentus	MRC610	2879 bp	1517	658	386	–	318
H. denticulentus	MRC624	3836 bp	2474	658	386	–	318
H. denticulentus	MRC816	704 bp	–	–	386	–	318
H. jahni	MNCN50543	2493 bp	1517	658	–	–	318
H. larinopygion	DHMECN3799	3450 bp	2474	658	–	–	318
H. larinopygion	MRC563	3450 bp	2474	658	–	–	318
H. larinopygion	QCAZ41826	4439 bp	2474	658	386	603	318
H. larinopygion	QCAZ45462	2474 bp	2474	–	–	–	–
H. larinopygion	SAS474	3836 bp	2474	658	386	–	318
H. larinopygion	TG1486	3836 bp	2474	658	386	–	318
H. larinopygion	TG2090	3178 bp	2474	–	386	–	318
H. larinopygion	TG2150	3836 bp	2474	658	386	–	318

H. lascinius	JMR2515	2474 bp	2474	–	–	–	–
H. lindae	ICN49651	3132 bp	2474	658	–	–	–
H. lindae	QCAZ48240	885 bp	885	–	–	–	–
H. lindae	QCAZ41232	4121 bp	2474	658	386	603	–
H. lindae	QCAZ45342	4121 bp	2474	658	386	603	–
H. lindae	QCAZ45346	2474 bp	2474	–	–	–	–
H. lindae	QCAZ45463	2436 bp	2436	–	–	–	–
H. palmeri	EMM262	3178 bp	2474	–	386	–	318
H. palmeri	JJS070	2860 bp	2474	–	386	–	–
H. palmeri	LBV412	3178 bp	2474	–	386	–	318
H. palmeri	MAR,1992	3518 bp	2474	658	386	–	–
H. palmeri	MAR706	3835 bp	2474	657	386	–	318
H. palmeri	MHUA4813	3836 bp	2474	658	386	–	318
H. palmeri	MHUA5250	2860 bp	2474	–	386	–	–
H. pantostictus	QCAZ45435	2474 bp	2474	–	–	–	–
H. pantostictus	QCAZ45438	4121 bp	2474	658	386	603	–
H. pantostictus	WED52976	2474 bp	2474	–	–	–	–
H. phyllognathus	AJC2689	2879 bp	1517	658	386	–	318
H. phyllognathus	AJC2699	3450 bp	2474	658	–	–	318
H. phyllognathus	AJC2753	3836 bp	2474	658	386	–	318
H. phyllognathus	AJC2786	3836 bp	2474	658	386	–	318
H. phyllognathus	GAB185	1133 bp	1133	–	–	–	–
H. phyllognathus	MHNC6618	3836 bp	2474	658	386	–	318
H. phyllognathus	MHNC7953	3836 bp	2474	658	386	–	318
H. phyllognathus	MNCN50824	1517 bp	1517	–	–	–	–
H. phyllognathus	MRC585	3836 bp	2474	658	386	–	318
H. phyllognathus	MRC699	3745 bp	2474	658	386	–	227
H. phyllognathus	MRC720	3178 bp	2474	–	386	–	318
H. phyllognathus	MRC744	3178 bp	2474	–	386	–	318
H. phyllognathus	QCAZ23938	2303 bp	2303	–	–	–	–
H. phyllognathus	QCAZ32271	2303 bp	2303	–	–	–	–
H. phyllognathus	QCAZ32276	3610 bp	2303	–	386	603	318
H. phyllognathus	QCAZ41032	3016 bp	2312	–	386	–	318
H. piceigularis	MHUA5442	3186 bp	1902	580	386	–	318
H. platydactylus	JMR2479	1423 bp	1423	–	–	–	–
H. platydactylus	JMR2508	1133 bp	1133	–	–	–	–
H. platydactylus	JMR2509	1865 bp	1479	–	386	–	–
H. platydactylus	MNCN50540	2221 bp	1517	–	386	–	318
H. platydactylus	MNCN50541	2175 bp	1517	658	–	–	–
H. platydactylus	MNCN50542	1517 bp	1517	–	–	–	–
H. princecharlesi	QCAZ42165	4340 bp	2375	658	386	603	318
H. princecharlesi	QCAZ43654	2375 bp	2375	–	–	–	–
H. psarolaimus	DHMECN6493	3178 bp	2474	–	386	–	318
H. psarolaimus	DHMECN6494	3836 bp	2474	658	386	–	318
H. psarolaimus	QCAZ27049	4439 bp	2474	658	386	603	318
H. psarolaimus	QCAZ46095	4359 bp	2474	658	386	523	318
H. ptychodactylus	QCAZ46030	4340 bp	2375	658	386	603	318
H. ptychodactylus	QCAZ46031	2375 bp	2375	–	–	–	–
H. pugnax	MRC514	3178 bp	2474	–	386	–	318
H. sarampiona	JMR2429	3836 bp	2474	658	386	–	318
H. sarampiona	JMR2430	3836 bp	2474	658	386	–	318
H. sp anori	JPH188	3518 bp	2474	658	386	–	–

H. sp anori	MHUA4096	3836 bp	2474	658	386	–	318
H. sp barbacoas	MRC833	3836 bp	2474	658	386	–	318
H. sp caldas	JMR2886	3518 bp	2474	658	386	–	–
H. sp caqueta	MAR,1911	3745 bp	2474	658	386	–	227
H. sp chachapoyas	CORBIDI12885	2527 bp	1997	530	–	–	–
H. sp eloro	QCAZ37234	2854 bp	889	658	386	603	318
H. sp eloro	QCAZ37250	1307 bp	–	–	386	603	318
H. sp murri	JMR2815	1517 bp	1517	–	–	–	–
H. sp murri	PM11068	1903 bp	1517	–	386	–	–
H. sp perija	LEP233	2963 bp	1997	580	386	–	–
H. sp sanjuanito	MRC666	3836 bp	2474	658	386	–	318
H. staufferorum	QCAZ45967	4277 bp	2312	658	386	603	318
H. tapichalaca	QCAZ15083	4401 bp	2436	658	386	603	318
H. tapichalaca	QCAZ16704	3178 bp	2474	–	386	–	318
H. tigrinus	JJS106	3132 bp	2474	658	–	–	–
H. tigrinus	MAA142	3518 bp	2474	658	386	–	–
H. tigrinus	QCAZ31550	4401 bp	2436	658	386	603	318
H. tigrinus	QCAZ41351	2436 bp	2436	–	–	–	–
H. torrenticola	TG1844	3359 bp	1997	658	386	–	318
H. torrenticola	TG1874	3041 bp	1997	658	386	–	–

APPENDIX 3. Phylogenetic relationships of *Hyloscirtus* inferred from maximum parsimony analysis under static homology. The topology reflects consensus tree (length 16783 steps). Numbers on branches are Goodman-Bremer support and parsimony jackknife absolute frequencies respectively and calculated for the static parsimony analysis. Asterisks indicate parsimony jackknife frequencies of 100%; dashes indicate frequencies ≤ 50%. The "*x*" indicate that the relationship was not recovered in the analysis to establish jackknife support.



APPENDIX 4. Intraspecific and interspecific uncorrected p-distances based on a 505 base pair fragment of the 16S rRNA (range are given in %) for members of *Hyloscirtus bogotensis* group. Sample size in parentheses follows species names.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1. H. alytolylax (2)	0.6									
2. H. hydrophilus (2)	16.5-16.7	0.0								
3. H. baios (1)	8.5–8.7	19.3	-							
4. H. bogotensis (5)	19.2-19.8	19.4-19.9	20.9-21.3	0.0-0.4						
5. H. chlorophyllus (4)	18.9-19.5	16.1-19.1	21.1-21-7	16.2-17.2	0.0-4.4					
6. H. “caldas” (1)	18.1-18.7	6.6	19.8	20.6-20.8	19.2-20.6	-				
7. H. callipeza (5)	18.7-19.9	18.7-19.7	20.6-21.2	3.8-4.6	16.6-18.2	19.6-20.7	0.0-3.4			
8. H. hyalinus (1)	19.6-19.8	19.5	22.0-23.9	16.0-16.4	8.0-8.4	20.9	17.1	-		
9. H. colymba (1)	19.9–20.1	14.7	21.8	23.0-23.2	21.4-22.2	16.9	22.9-23.5	22.4	-	
10. H. denticulentus (4)	17.5-17.7	17.0	19.6	16.4-16-6	4.4-5.7	19.4	15.8-17.1	7.4	21.3	0.0
11. H. oro (1)	5.1–5.3	17.4	8.5	19.2-19.6	18.6-23.6	19.0	19.1-20.1	19.4	20.3	18.1
12. H. jahni (1)	24.4-24.6	25.0	26.8	20.8-20.9	22.1-23.8	26.6	21.6-22.1	20.8	26.5	22.5
13. H. lascinius (2)	17.3-17.5	16.8	19.4	16.2-16.4	4.2-5.5	19.2	15.6-16.9	7.1	21.1	0.2-0.6
14. H. macondo (4)	20.7-21.4	22.1-22.5	23.3-23.6	19.5-20.1	18.6-20.3	23.7-23.9	19.5-20.8	19.4-19.6	23.3-23.7	17.2-18.6
15. H. murri (2)	18.6-19.0	12.0		18.4-19.0	18.8-20.1	14.6	18.7-19.6	18.4-18.8	17.0	18.4-18.8
16. H. palmeri (4)	19.7-21.0	21.7-22.3	21.9-22.9	18.5-20.3	18.3-20.1	22.1-22.5	17.9-19.6	19.3-20.3	23.6-24.1	18.6-19.2
17. H. “perija” (1)	20.9-21.4	21.2	21.0	9.5-9.9	18.2-19.5	21.4	10.6-11.1	19.0	24.4	17.6
18. H. platydactylus (6)	20.7-21.3	19.7-20.3	22.4-22.9	3.8-4.6	17.0-18.5	20.6-21.1	4.8-5.3	17.2-17.7	23.5-23.9	17.2-17.7
19. H. phyllognathus (11)	18.2-24.4	16.1-23.1	18.2-26.4	15.9-20.8	16.5-21.4	18.4-22.0	15.8-19.7	18.3-20.1	20.3-22.3	16.5-18.8
20. H. piceigularis (1)	18.1-18.3	17.4	20.3	15.6-15.8	6.1-6.5	19.8	15.8-16.3	6.1	21.3	5.0
21. H. torrenticola (2)	16.5-16.7	16.1-16.3	18.9-19.1	19.3-19.9	17.5-18.1	17.5-17.7	18.7-20.4	18.1-18.4	17.7-17.9	17.9-18.2

	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
11. H. oro (1)	-										
12. H. jahni (1)	24.7	-									
13. H. lascinius (2)	18.0	22.3	0.0								
14. H. macondo (4)	22.0-22.4	23.2-24.1	18.4-19.0	0.0-1.1							
15. H. murri (2)	18.0-18.2	23.7	18.2-18.6	21.4-21.6	0.0-0.4						
16. H. palmeri (4)	21.0-22.3	23.8-24.4	17.4-18.4	11.7-15.2	21.6-22.6	5.7-7.7					
17. H. “perija” (1)	20.6	22.1	17.4	22.0-22.7	19.1-19.3	22.0-22.6	-				
18. H. platydactylus (6)	20.7-21.1	22.1-22.7	17.0-17.5	20.7-21.2	19.2-20.1	19.0-20.8	10.4-10.8	0.0-0.8			
19. H. phyllognathus (11)	18.6-20.2	22.7-25.2	16.3-21.4	19.1-23.1	16.7-20.4	16.4-22.2	18.7-22.2	16.3-18.6	0.0-15.4		
20. H. piceigularis (1)	17.7	21.2	4.8	19.4-19.6	18.0-18.4	18.3-19.9	17.6	16.4-16.8	16.9-18.8	-	
21. H. torrenticola (2)	17.6-17.8	23.1-23.3	17.7-18.0	20.6-21.3	17.0-17.2	18.3-20.2	20.9-21.1	20.1-20.6	16.0-19.2	17.3-17.5	0.2

APPENDIX 5. Intraspecific and interspecific uncorrected p-distances based on a 505 base pair fragment of the 16S rRNA (range are given in %) for members of *Hyloscirtus larinopygion* and *H. tapichalaca* (bold) groups. Sample size in parentheses follows species names.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. H. antioquia (6)	0.0-2.1							
2. ***H. diabolus*** *(1)*	17.2-17.4	-						
3. ***H. condor*** *(2)*	14.6-14.9	8.2	0.0					
4. H. criptico (3)	7.4-8.5	19.1-19.5	18.0-18.3	0.2-0.6				
5. H. larinopygion (8)	3.6-4.2	16.6-17.2	15.0-15.5	7.4-8.0	0.0-2.3			
6. H. lindae (5)	4.7-5.9	16.8-17.2	14.4-15.5	6.5-7.8	4.6-5.5	0.0-1.7		
7. H. pacha (1)	7.0-7.2	19.5	17.6	4.6-5.0	7.2-7.6	7.0-7.8	-	
8. H. pantostictus (3)	4.9-5.5	17.6-17.8	15.5-15.7	6.8-7.4	5.1-5.7	0.6-1.9	7.2-7.4	0.0-0.2
9. H. princecharlesi (2)	4.9-5.3	17.2-17.4	15.7-15.9	4.6-5.3	4.4-5.1	4.8-5.3	5.5-5.7	5.3-5.7
10. H. psarolaimus (4)	6.0-7.0	18.0	16.5-16.6	6.1-6.6	7.0-7.6	5.9-6.8	6.5-6.8	5.7-6.1
11. H. ptychodactylus (2)	4.7-4.9	17.0	15.7	5.1-5.5	4.2-4.6	4.0-4.6	4.6	4.4-4.6
12. H. "sanjuanito" (1)	7.0-7.4	19.1	18.0-18.1	1.3-1.7	6.8-7.0	6.3-7.2	4.2	6.5-6.8
13. H. sarampiona (2)	3.6-4.0	18.0	16.1	5.9-6.1	3.6-3.8	3.8-4.9	5.1	4.0-4.2
14. H. staufferorum (2)	5.5-6.1	18.7	16.9-17.0	4.6-5.0	6.5-7.0	6.1-7.0	1.9	6.3-6.5
15. ***H. tapichalaca*** *(2)*	15.3-15.9	7.4-8.0	7.0-7.4	18.0-18.7	15.5-15.9	15.4-16.1	18.2-18.5	15.8-16.2
16. H. tigrinus (4)	6.1-6.6	17.0-17.2	15.7-15.9	5.1-5.7	5.5-6.1	5.1-5.3	5.1-5.3	6.3-6.7

	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
9. H. princecharlesi (2)	0.2							
10. H. psarolaimus (4)	4.2-4.6	0.0-0.2						
11. H. ptychodactylus (2)	1.7	4.6-4.9	0.0					
12. H. "sanjuanito" (1)	4.0-4.2	5.5-5.7	4.4	-				
13. H. sarampiona (2)	3.8-4.0	5.1-5.3	3.2	5.3	0.0			
14. H. staufferorum (2)	4.8	5.9-6.1	4.0	3.8	4.6	0.0		
15. H. tapichalaca (2)	16.4-16.5	16.8-16.9	15.7-15.9	18.5-18.7	16.4-16.5	17.6-17.7	0.8	
16. H. tigrinus (4)	3.6-3.8	4.6-5.3	3.8-4.0	4.2-4.4	4.4-4.6	5.3-5.5	16.2-16.7	0.2-0.4

APPENDIX 6. Comparison of some characters in species of the *Hyloscirtus bogotensis* and *H. jahni* groups. Sources: (1) Melin, 1941; (2) Cochran and Goin, 1970; (3) Duellman, 1972; (4) Lutz and Ruiz, 1977; (5) Duellman, 1989; (6) Dunn, 1930; (7) Rivero, 1969; (8) Ruiz-Carranza and Ardila-Robayo, 1991; (9) La Marca, 1985; (10) Myers and Duellman, 1982; (11) Duellman, 2001; (12) Ruiz-Carranza and Lynch, 1982; (13) Duellman and Altig, 1982; (*) this work. (M) Males. Characters of *Hyloscirtus estevesi* no were included because the holotype is a juvenile (only known specimen).

Species	**Snout (profile)**	**Labial stripe**	**Ulnar fold**	**Tarsal ornamentation**	**Supratympanic stripe**	**Supracloacal ornamentation**	**Pericloacal spots**	**Calcar tubercle**	**Additional diagnostic characters**	**Source**
H. albopunctulatus	Rounded	Present	Present	White stripe present	Absent	White fold present	Absent	Absent	Dorsal white spot present,	1, 2, *
H. alytolylax	Truncate to rounded	Absent	Present	White stripe present	Present	White stripe present	Absent	Absent	Pale canthal stripe; prepollex small; snout acuminate in dorsal view; mental gland small	3, *
H. baios	Slightly protruding	Absent	Absent	Absent	Present	Absent	Absent	Absent	Prepollex small; snout acuminate in dorsal view; mental gland small	*
H. bogotensis	Truncate	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Prepollex large; mental gland large	3, 4, *
H. callipeza	Truncate	Absent	Absent	Absent	Present	White stripe present	Present	Absent	Brown canthal stripe; prepollex large; mental gland small	5, *
H. chlorophyllus	Truncate or slightly sloping	Present	Present	White and brown fold present	Absent	White and brown fold present	Present	Present	Prepollex small; snout truncate in dorsal view (M); nostrils pigmented; mental gland large	12, *
H. colymba	Rounded	Absent	Present	White fold present	Present	White stripe present	Absent	Absent	Prepollex small; snout rounded in dorsal view (M); mental gland small	3, 6, 11, *
H. hyalinus	Truncate or slightly sloping	Present	Present	White and brown fold present	Absent	White and brown fold present	Present	Present	Prepollex large; nostrils pigmented; mental gland large	
H. hydrophilus	Truncate to rounded	Absent	Present	White stripe present	Present	White fold present	Absent	Absent	Prepollex small; mental gland small	*
H. jahni	Protruding	Absent	Absent	White stripe present	Present	Absent	Present	Absent	Pale canthal stripe, snout acuminate in dorsal view; prepollex small, spicules on skin (M)	9, *
H. lascinius	Sloping	Present	Present, white and brown	White and brown fold present	Absent	White and brown fold present	Present	Present	Prepollex large; nostrils pigmented; mental gland large	7, *

H. lynchi	Truncate	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Veneer on snout; mental gland no visible externally, small; prepollex large, lavender brown dorsum	8, *
H. macondo	Truncate	Present	Present, white and brown	White and brown fold present	Absent	White and brown fold present	Present	Present	Skin weakly granular; prepollex large; snout truncate in dorsal view; mental gland large	*
H. murri	Truncate to rounded	Absent	Present	White stripe present	Present	White fold present	Absent	Absent	Prepollex small; mental gland small	*
H. oro	Rounded or truncate	Absent	Present	White stripe present	Present	Present	Absent	Absent		
H. palmeri	Truncate	Present	Present, white and brown	White and brown fold present	Absent	White and brown fold present	Present	Present	Skin weakly granular; prepollex large; snout truncate in dorsal view; mental gland large	2, 10, 11, *
H. platydactylus	Truncate	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Skin smooth; prepollex large; snout rounded in dorsal view	3, 9, *
H. phyllognathus	Sloping to rounded	Present	Present	White and brown fold present	Absent	White and brown fold present	Present	Present	Dorsal white spot present; prepollex small; snout rounded in dorsal view; mental gland large	3, 11, *
H. piceigularis	Sloping to rounded	Present	Present	White and brown fold present	Absent	White and brown fold present	Present	Present	Skin smooth; prepollex small; snout rounded in dorsal view; mental gland large; brown gular region	12, *
H. simmonsi	Protruding	Absent	Absent	Absent	Absent or present	Absent	Absent	Absent	Skin smooth; snout acuminate in dorsal view, prepollex small; mental gland small	4, *
H. torrenticola	Truncate	Present	Present	White stripe present	Absent	White stripe present	Absent	Absent	Skin smooth; prepollex small; snout rounded in dorsal view; mental gland small	13, *

APPENDIX 7. Measurements and proportions of the new species of *Hyloscirtus* redescribed herein (mean ± standard deviation follow of ranges). Mesaur. = Measurement. See text for other abbreviations

	H. baios		*H. chlorophyllus*		*H. hyalinus*				*Hyloscirtus hydrophilus*			
	Males (n = 5)		Males (n = 6)		Males (n = 7)		Females (n = 2)		Males (n = 18)		Females (n = 6)	
Measur.	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max
SVL	30.1 ± 0.68	29.2–31.1	34.9 ± 1.46	32.9–36.6	34.3 ± 1.39	31.9–36.2	41.6 ±1.13	40.8–42.4	34.5 ± 1.27	33.0–36.7	40.8 ± 1.36	39.6–42.4
HL	10.0 ± 0.29	9.5–10.2	10.9 ± 0.53	9.8–11.2	10.7 ± 0.55	10.1–11.6	12.9 ±0.21	12.7–13.0	11.4 ± 0.46	10.8–12.0	13.1 ± 1.09	12.1–14.8
HW	10.4 ± 0.34	10.0–10.9	11.1 ± 0.66	10.4-12	11.2 ± 0.43	10.5–11.9	13.6 ±0.57	13.2–14.0	12.4 ± 0.64	11.4–13.7	14.4 ± 0.76	13.3–15.4
ED	3.2 ± 0.16	3.1–3.5	3.7 ± 0.21	3.4–3.9	3.5 ± 0.34	3.0–4.0	3.9 ± 0.07	3.8–3.9	3.6 ± 0.33	3.3–4.4	4.4 ± 0.45	3.8–4.9
END	2.5 ± 0.45	2.1–3.2	2.3 ± 0.23	1.9–2.5	2.2 ± 0.31	1.7–2.6	3.0 ± 0.35	2.7–3.2	3.2 ± 0.36	2.7–3.8	3.6 ± 0.46	3.1–4.1
NSD	1.4 ± 0.18	1.2–1.7	1.4 ± 0.20	1.3–1.8	1.6 ± 0.35	1.2–2.1	1.6 ± 0.14	1.5–1.7	1.6 ± 0.10	1.5–1.8	1.9 ± 0.22	1.8–2.3
IND	2.4 ± 0.25	2.0–2.6	2.7 ± 0.22	2.4–3.0	2.8 ± 0.30	2.5–3.2	3.2 ± 0.28	3.0–3.4	2.9 ± 0.27	2.5–3.3	3.5 ± 0.25	3.2–3.7
AMD	6.0 ± 0.48	5.5–6.7	6.7 ± 0.26	6.3–7.0	6.3 ± 0.36	5.8–6.7	7.2 ± 0.14	7.1–7.3	7.1 ± 0.40	6.4–7.7	7.9 ± 0.51	7.5–8.6
TD	1.2 ± 0.15	1.0–1.3	1.3 ± 0.27	0.9–1.6	1.2 ± 0.11	1.2–1.5	2.0 ± 0.28	1.8–2.2	1.5 ± 0.11	1.4–1.7	2.0 ± 0.25	1.7–2.4
FAL	5.9 ± 0.30	5.5–6.2	6.8 ± 0.37	6.2–7.1	7.0 ± 0.30	6.7–7.5	8.8 ± 0.85	8.2–9.4	6.5 ± 0.61	5.8–7.7	7.8 ± 0.58	7.1–8.4
FAB	2.7 ± 0.37	2.3–3.2	3.2 ± 0.25	2.9–3.5	2.8 ± 0.29	2.3–3.2	3.3 ± 0.49	2.9–3.6	2.9 ± 0.19	2.5–3.2	3.2 ± 0.11	3.1–3.4
HAL	9.4 ± 0.36	9.0–9.9	10.4 ± 0.92	9.5–11.7	10.5 ± 0.30	10.1–11.0	12.8 ± 0.21	12.6–12.9	10.9 ± 0.41	10.2–11.5	12.2 ± 0.43	11.6–12.7
THL	14.7 ± 0.72	13.8–15.4	16.0 ± 0.84	14.6–16.9	16.1 ± 0.70	15–16.9	20.9 ± 0.85	20.3–21.5	17.9 ± 0.58	16.9–18.8	22.2 ± 0.69	21.0–22.8
TL	15.1 ± 0.44	14.6–15.7	16.6 ± 1.03	15.1–18.1	17.0 ± 0.50	16.3–17.6	21.5 ± 0.49	21.1–21.8	17.9 ± 0.72	16.8–19.1	21.4 ± 0.46	20.8–22.0
TAL	8.9 ± 0.66	8.2–9.6	10.0 ± 0.61	9.0–10.6	10.1 ± 0.38	9.6–10.7	13.1 ± 0.01	13.1–13.2	10.1 ± 0.56	9.0–10.6	12.2 ± 0.54	11.6–12.9
FL	12.8 ± 0.38	12.5–13.3	14.4 ± 1.47	12.6–16.3	14.1 ± 0.48	13.6–15.0	18.0 ± 1.48	16.9–19.0	15.1 ± 0.65	14.3–16.3	17.9 ± 0.52	17.3–18.7
TFD	1.5 ± 0.07	1.4–1.6	1.7 ± 0.23	1.4–2.1	1.6 ± 0.13	1.5–1.8	2.1 ± 0.14	2.0–2.2	1.8 ± 0.15	1.6–2.1	2.2 ± 0.36	1.8–2.5
FTD	1.4 ± 0.07	1.3–1.5	1.5 ± 0.20	1.3–1.9	1.5 ± 0.15	1.3–1.7	1.9 ± 0.07	1.8–1.9	1.6 ± 0.11	1.4–1.7	1.8 ± 0.36	1.2–2.0

APPENDIX 7 (continued). Measurements and proportions of the new species of *Hyloscirtus* redescribed herein (mean ± standard deviation follow of ranges). Mesaur. = Measurement. See text for other abbreviations.

	Hyloscirtus macondo				*Hyloscirtus murri*			*Hyloscirtus oro*			*Hyloscirtus arcanus*		
	Males (n = 12)		Females (n = 2)		Males (n = 2)		Female (n = 1)	Males (n=3)		Female (n = 1)	Males (n = 3)		Female (n = 1)
Measur.	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max		Mean ± SD	Min–Max		Mean ± SD	Min–Max	
SVL	36.8 ± 1.25	34.2–38.0	43.4 ± 0.64	42.9-43.8	40.2 ± 0.71	39.7-40.7	46.1	39.5 ± 5.55	35.7-45.9	36.9	66.5 ± 1.5	64.9-67.8	70.8
HL	11.6 ± 0.52	10.9–12.2	13.0 ± 0.14	12.9-13.1	13.7 ± 0.49	13.3-	15.3	12.6 ± 1.53	11.3-14.3	11.7	18.6 ± 0.8	17.7-19.3	19.6
HW	12.9 ± 1.27	12.3–14.5	15.1 ± 0.14	15.0-15.2	13.8 ± 0.28	13.6-14.0	15.8	12.6 ± 1.89	11.0-14.7	13.0	21.6 ± 0.7	21.0-22.4	22.7
ED	4.0 ± 0.33	3.4–4.3	3.6 ± 0.07	3.5-3.6	3.8 ± 0.21	3.6-3.9	4.5	4.1 ± 0.40	3.6-4.3	4.7	7.4 ± 0.2	7.2-7.5	7.6
END	2.7 ± 0.21	2.4–3.0	4.2 ± 0.14	4.1-4.3	3.1 ± 0.42	2.8-3.4	3.6	3.2 ± 0.58	2.9-3.9	3.5	4.2 ± 0.2	4.0-4.3	3.8
NSD	1.8 ± 0.18	1.5–2.1	2.1 ± 0.07	2.0-2.1	1.8 ± 0.35	1.5-2.0	2.0	1.2 ± 0.12	1.1-1.3	1.6	3.3 ± 0.3	3.1-3.6	3.6
IND	3.4 ± 0.17	3.1–3.6	3.5 ± 0.21	3.3-3.6	3.8 ± 0.07	3.7-3.8	3.6	3.0 ± 0.15	2.8-3.1	3.3	5.1 ± 0.5	4.7-5.7	5.1
AMD	7.3 ± 0.34	6.7–7.8	7.9 ± 0.14	7.8-8.0	7.9 ± 0.21	7.7-8.0	8.5	7.1 ± 0.75	6.4-7.9	7.0	11.2 ± 0.8	10.5-12.1	11.6
TD	1.8 ± 0.15	1.6–2.0	2.1 ± 0.07	2.0-2.1	1.3 ± 0.07	1.2-1.3	1.5	1.3 ± 0.25	1.0-1.5	1.4	–	–	3.4
FAL	7.7 ± 0.55	7.2–9.0	8.2 ± 0.14	8.1-8.3	8.3 ± 0.21	8.1-8.4	9.0	8.1 ± 1.01	7.0-9.0	7.7	10.9 ± 0.5	10.5-11.6	11.4
FAB	3.2 ± 0.22	2.9–3.5	3.4 ± 0.07	3.3-3.4	3.6 ± 0.14	3.5-3.7	3.9	3.1 ± 0.42	2.6-3.4	3.2	9.8 ± 0.2	9.6-10.0	6.0
HAL	11.0 ± 0.52	10.3–12.0	11.4 ± 0.14	11.3-11.5	12.7 ± 0.49	12.3-13.0	13.8	11.4 ± 1.85	9.6-13.3	11.5	22.4 ± 1.0	21.4-23.4	22.9
THL	19.2 ± 0.92	17.4–20.2	22.8 ± 0.14	22.7-22.9	19.5 ± 0.71	19.0-20.0	22.9	18.7 ± 2.63	16.7-21.7	18.3	31.1 ± 0.2	31.0-31.3	34.8
TL	19.1 ± 0.82	18.2-20.5	22.1 ± 0.14	22.0-22.2	20.5 ± 0.49	20.1-20.8	23.6	20.2 ± 2.78	17.7-23.2	20.3	30.7 ± 0.7	30.1-31.4	34.0
TAL	10.7 ± 0.62	10.0–11.5	11.8 ± 0.14	11.7-11.9	12.4 ± 0.71	11.9-12.9	13.7	12.4 ± 2.25	10.6-14.9	11.8	16.6 ± 0.1	16.5-16.7	20.0
FL	16.2 ± 0.91	15.1–17.5	17.5 ± 0.07	17.4-17.5	17.8 ± 0.14	17.7-17.9	19.9	16.8 ± 1.85	15.0-18.7	17.0	28.6 ± 1.1	27.4-29.4	32.3
TFD	1.9 ± 0.10	1.7–2.0	1.8 ± 0.07	1.8-1.9	2.0 ± 0.14	1.9-2.1	2.6	1.8 ± 0.15	1.7-2.0	2.0	3.6 ± 0.5	3.1-4.0	3.4
FTD	1.6 ± 0.09	1.4–1.7	1.7 ± 0.07	1.7-1.8	1.6 ± 0.14	1.5-1.7	2.0	1.6 ± 0.06	1.6-1.7	1.8	3.5 ± 0.1	3.4-3.6	3.2

APPENDIX 8. List of molecular transformations common to all most parsimonious trees of the parsimony analysis, supporting the monophyly of genus and for all taxonomic groups of *Hyloscirtus.* Codification character-states from molecular characters are as follows: 0=A, 1=C, 2=G, 3=T

Hyloscirtus genus
Char. 1173: 1 --> 3
Char. 1188: 2 --> 1
Char. 1480: 1 --> 3
Char. 1523: 1 --> 0
Char. 1615: 0 --> 2
Char. 1618: 3 --> 0
Char. 1624: 3 --> 1
Char. 1642: 1 --> 4
Char. 1661: 4 --> 2
Char. 1671: 3 --> 1
Char. 1822: 0 --> 3
Char. 1853: 0 --> 3
Char. 1860: 3 --> 4
Char. 1895: 1 --> 0
Char. 1947: 1 --> 4
Char. 2082: 0 --> 3
Char. 2105: 4 --> 0
Char. 2120: 1 --> 3
Char. 2131: 4 --> 0
Char. 2148: 3 --> 4
Char. 2153: 1 --> 3
Char. 2196: 0 --> 3
Char. 2460: 0 --> 3
Char. 2613: 1 --> 3
Char. 2709: 0 --> 4
Char. 2805: 4 --> 0
Char. 2823: 0 --> 3
Char. 2877: 4 --> 3
Char. 2945: 1 --> 3
Char. 3994: 1 --> 0
Char. 4044: 1 --> 3
Char. 4089: 0 --> 2
Char. 4208: 1 --> 0
Char. 4232: 0 --> 1
Char. 4286: 0 --> 1
Char. 4339: 3 --> 1
Char. 4413: 2 --> 3
Char. 4514: 0 --> 3
Char. 4557: 0 --> 2
Char. 6416: 1 --> 3
Char. 6548: 2 --> 0
Char. 6603: 1 --> 3
Hyloscirtus armatus group
Char. 7: 2 --> 0
Char. 92: 0 --> 3
Char. 103: 3 --> 1
Char. 119: 3 --> 1
Char. 261: 3 --> 1
Char. 264: 1 --> 3
Char. 292: 1 --> 0
Char. 295: 1 --> 3
Char. 322: 3 --> 1
Char. 332: 3 --> 0
Char. 339: 0 --> 3
Char. 341: 01 --> 3
Char. 475: 3 --> 1
Char. 504: 3 --> 1
Char. 520: 1 --> 3
Char. 610: 3 --> 0
Char. 650: 1 --> 3
Char. 683: 3 --> 1
Char. 694: 1 --> 3
Char. 699: 2 --> 0
Char. 700: 1 --> 3
Char. 719: 1 --> 3
Char. 724: 3 --> 2
Char. 730: 3 --> 0
Char. 741: 3 --> 1
Char. 759: 3 --> 0
Char. 761: 0 --> 1
Char. 830: 0 --> 3
Char. 832: 0 --> 3
Char. 885: 1 --> 3
Char. 935: 0 --> 1
Char. 988: 1 --> 3
Char. 996: 4 --> 0
Char. 997: 4 --> 3
Char. 1026: 0 --> 3
Char. 1030: 1 --> 0
Char. 1035: 0 --> 1
Char. 1043: 1 --> 3
Char. 1173: 3 --> 1
Char. 1232: 1 --> 3
Char. 1315: 3 --> 0
Char. 1326: 3 --> 0
Char. 1376: 0 --> 3
Char. 1377: 1 --> 3
Char. 1380: 1 --> 3
Char. 1403: 0 --> 4
Char. 1448: 0 --> 3
Char. 1478: 1 --> 3
Char. 1603: 0 --> 3
Char. 1618: 0 --> 3
Char. 1663: 0 --> 3
Char. 1671: 1 --> 0
Char. 1678: 4 --> 0
Char. 1755: 1 --> 0
Char. 1806: 0 --> 3
Char. 1808: 1 --> 0
Char. 1822: 3 --> 1
Char. 1851: 3 --> 0
Char. 1877: 13 --> 0
Char. 1892: 1 --> 0
Char. 1929: 3 --> 1
Char. 1936: 1 --> 3
Char. 1963: 3 --> 0
Char. 1981: 3 --> 0
Char. 1984: 3 --> 1
Char. 2015: 3 --> 0
Char. 2028: 0 --> 2
Char. 2270: 3 --> 0
Char. 2323: 1 --> 2
Char. 2354: 0 --> 2
Char. 2388: 1 --> 3
Char. 2607: 1 --> 3
Char. 2616: 4 --> 1
Char. 2634: 0 --> 3
Char. 2732: 0 --> 3
Char. 2786: 0 --> 3
Char. 2837: 0 --> 1
Char. 2838: 0 --> 3
Char. 2845: 3 --> 1
Char. 2895: 3 --> 0
Char. 2955: 1 --> 3
Char. 3271: 0 --> 2
Char. 3500: 2 --> 4
Char. 4604: 3 --> 1
Char. 4695: 1 --> 3
Char. 4734: 1 --> 3
Char. 4815: 1 --> 0
Char. 4857: 1 --> 0
Char. 4882: 3 --> 1
Char. 4897: 0 --> 2
Char. 4924: 3 --> 2
Char. 6409: 2 --> 0
Char. 7111: 0 --> 2
Char. 7116: 1 --> 3
Char. 7120: 0 --> 1
Char. 7174: 1 --> 3
Char. 7232: 2 --> 3
Char. 7235: 0 --> 1
Char. 7252: 2 --> 0
Char. 7322: 3 --> 0
Char. 7336: 1 --> 2
Char. 7342: 1 --> 3
Char. 7360: 1 --> 3
Char. 7407: 2 --> 0
Char. 7408: 3 --> 1
Char. 7591: 1 --> 3
Hyloscirtus bogotensis group
Char. 10: 0 --> 2
Char. 73: 0 --> 4
Char. 166: 0 --> 1
Char. 167: 1 --> 3
Char. 242: 1 --> 0
Char. 290: 4 --> 1
Char. 295: 1 --> 0
Char. 328: 4 --> 1
Char. 342: 3 --> 1
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Char. 612: 4 --> 0
Char. 693: 0 --> 2
Char. 795: 0 --> 3
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Char. 991: 1 --> 0
Char. 1024: 0 --> 1
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Char. 1446: 3 --> 1
Char. 1506: 2 --> 0
Char. 1556: 1 --> 3
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Char. 1606: 1 --> 3
Char. 1696: 0 --> 3
Char. 1882: 3 --> 4
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Char. 1949: 3 --> 4
Char. 1969: 3 --> 1
Char. 1978: 2 --> 0
Char. 1999: 1 --> 0
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Char. 2056: 1 --> 0
Char. 2068: 0 --> 3
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Char. 2184: 3 --> 1
Char. 2274: 1 --> 0
Char. 2277: 1 --> 3
Char. 2457: 2 --> 0
Char. 2564: 0 --> 3
Char. 2568: 1 --> 2
Char. 2687: 3 --> 0
Char. 2971: 3 --> 1
Char. 3957: 0 --> 3
Char. 3974: 2 --> 0
Char. 3995: 3 --> 0
Char. 4008: 2 --> 0
Char. 4053: 0 --> 3
Char. 4065: 3 --> 1
Char. 4098: 0 --> 1
Char. 4238: 1 --> 2
Char. 4260: 2 --> 3
Char. 4278: 2 --> 0
Char. 4450: 3 --> 1
Char. 4451: 3 --> 1
Char. 4508: 3 --> 1
Char. 4572: 3 --> 4
Char. 4580: 4 --> 1
Char. 4725: 1 --> 0
Char. 4924: 3 --> 0
Char. 4963: 1 --> 0
Char. 4972: 3 --> 0
Hyloscirtus larinopygion group
Char. 17: 0 --> 2
Char. 76: 0 --> 2
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Char. 89: 2 --> 3
Char. 167: 1 --> 3
Char. 213: 0 --> 2
Char. 242: 1 --> 0
Char. 405: 1 --> 3
Char. 456: 1 --> 3
Char. 461: 0 --> 3
Char. 510: 1 --> 3
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Char. 757: 0 --> 3
Char. 849: 0 --> 2
Char. 906: 2 --> 0
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Char. 1005: 0 --> 1
Char. 1035: 0 --> 2
Char. 1057: 1 --> 3
Char. 1217: 0 --> 2
Char. 1239: 3 --> 1
Char. 1241: 0 --> 2
Char. 1303: 1 --> 3
Char. 1310: 0 --> 2
Char. 1311: 3 --> 1
Char. 1390: 1 --> 0
Char. 1558: 1 --> 3
Char. 1671: 1 --> 4
Char. 1741: 1 --> 0
Char. 1801: 3 --> 1
Char. 1822: 3 --> 0
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Char. 1970: 0 --> 3
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Char. 2187: 3 --> 0
Char. 2328: 3 --> 0
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Char. 2577: 3 --> 1
Char. 2630: 3 --> 1
Char. 2648: 3 --> 1
Char. 2717: 0 --> 4
Char. 2740: 1 --> 3
Char. 2762: 0 --> 3
Char. 2795: 0 --> 3
Char. 2817: 4 --> 0
Char. 2843: 1 --> 0
Char. 3002: 1 --> 3
Char. 3909: 1 --> 3

Char. 3960: 1 --> 3
Char. 3963: 1 --> 3
Char. 4164: 1 --> 3
Char. 4171: 1 --> 3
Char. 4191: 3 --> 2
Char. 4192: 1 --> 3
Char. 4197: 4 --> 0
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Char. 4366: 0 --> 3
Char. 4381: 1 --> 0
Char. 4401: 1 --> 3
Char. 4407: 1 --> 3
Char. 4426: 3 --> 0
Char. 4429: 13 --> 0
Char. 4451: 3 --> 1
Char. 4471: 13 --> 0
Char. 4540: 0 --> 3
Char. 4572: 3 --> 1
Char. 4573: 1 --> 3
Char. 4579: 3 --> 1
Char. 4626: 1 --> 3
Char. 4627: 1 --> 3
Char. 4640: 2 --> 0
Char. 4672: 0 --> 1
Char. 4684: 0 --> 3
Char. 4731: 1 --> 3
Char. 4735: 0 --> 2
Char. 4737: 3 --> 1
Char. 4776: 1 --> 3
Char. 4798: 1 --> 0
Char. 4808: 1 --> 3
Char. 4829: 0 --> 1
Char. 4838: 3 --> 1
Char. 4841: 3 --> 1
Char. 4850: 3 --> 1
Char. 4933: 1 --> 3
Char. 5657: 0 --> 2
Char. 5693: 2 --> 3
Char. 6446: 3 --> 2
Char. 6634: 0 --> 3
Hyloscirtus tapichalaca group
Char. 77: 3 --> 1
Char. 84: 0 --> 2
Char. 192: 3 --> 1
Char. 272: 1 --> 3
Char. 296: 4 --> 0
Char. 330: 0 --> 3
Char. 339: 0 --> 1
Char. 340: 3 --> 1
Char. 351: 3 --> 1
Char. 377: 3 --> 1
Char. 383: 3 --> 1
Char. 390: 3 --> 2
Char. 391: 3 --> 1
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Char. 759: 3 --> 1
Char. 831: 3 --> 2
Char. 930: 0 --> 1
Char. 993: 3 --> 1
Char. 998: 0 --> 1
Char. 1006: 3 --> 1
Char. 1031: 0 --> 3
Char. 1188: 3 --> 0
Char. 1190: 0 --> 3
Char. 1269: 1 --> 3
Char. 1282: 0 --> 3
Char. 1316: 0 --> 4
Char. 1328: 3 --> 1
Char. 1336: 0 --> 1
Char. 1379: 0 --> 2
Char. 1398: 3 --> 1
Char. 1477: 0 --> 1
Char. 1491: 2 --> 0
Char. 1514: 0 --> 1
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Char. 1537: 3 --> 1
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Char. 1651: 3 --> 1
Char. 1663: 0 --> 1
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Char. 1685: 4 --> 2
Char. 1692: 3 --> 4
Char. 1696: 0 --> 1
Char. 1795: 3 --> 1
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Char. 1930: 3 --> 1
Char. 1945: 3 --> 0
Char. 1980: 2 --> 0
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Char. 2094: 0 --> 1
Char. 2096: 0 --> 1
Char. 2110: 0 --> 2
Char. 2122: 3 --> 1
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Char. 2184: 3 --> 1
Char. 2196: 3 --> 1
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Char. 2444: 3 --> 1
Char. 2458: 3 --> 1
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Char. 2646: 3 --> 2
Char. 2663: 3 --> 1
Char. 2741: 0 --> 2
Char. 2770: 0 --> 3
Char. 2775: 0 --> 1
Char. 2785: 0 --> 2
Char. 2791: 0 --> 2
Char. 2837: 0 --> 3
Char. 2877: 3 --> 1
Char. 2881: 3 --> 1
Char. 2902: 3 --> 1
Char. 2940: 1 --> 3
Char. 4044: 3 --> 1
Char. 4047: 3 --> 1
Char. 4071: 3 --> 2
Char. 4074: 0 --> 2
Char. 4119: 0 --> 2
Char. 4125: 0 --> 2
Char. 4137: 0 --> 2
Char. 4170: 0 --> 2
Char. 4214: 0 --> 2
Char. 4217: 3 --> 0
Char. 4265: 0 --> 2
Char. 4296: 3 --> 1
Char. 4317: 3 --> 1
Char. 4365: 0 --> 1
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Char. 4413: 3 --> 1
Char. 4430: 3 --> 1
Char. 4432: 0 --> 3
Char. 4438: 3 --> 0
Char. 4457: 1 --> 3
Char. 4495: 3 --> 1
Char. 4505: 3 --> 1
Char. 4508: 3 --> 1
Char. 4559: 3 --> 2
Char. 4560: 1 --> 3
Hyloscirtus jahni group
Char. 1175: 3 --> 1
Char. 1185: 0 --> 2
Char. 1190: 0 --> 1
Char. 1195: 2 --> 0
Char. 1204: 1 --> 3
Char. 1212: 0 --> 1
Char. 1230: 1 --> 2
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Char. 1270: 0 --> 2
Char. 1277: 3 --> 1
Char. 1282: 0 --> 3
Char. 1283: 0 --> 2
Char. 1287: 0 --> 1
Char. 1289: 3 --> 0
Char. 1305: 0 --> 2
Char. 1309: 0 --> 3
Char. 1315: 3 --> 0
Char. 1319: 3 --> 1
Char. 1374: 0 --> 4
Char. 1383: 3 --> 0
Char. 1397: 2 --> 0
Char. 1400: 2 --> 0
Char. 1403: 0 --> 3
Char. 1409: 0 --> 2
Char. 1430: 1 --> 3
Char. 1461: 3 --> 0
Char. 1507: 2 --> 0
Char. 1537: 3 --> 1
Char. 1555: 1 --> 3
Char. 1635: 2 --> 0
Char. 1651: 3 --> 0
Char. 1655: 03 --> 1
Char. 1658: 3 --> 1
Char. 1663: 0 --> 3
Char. 1692: 3 --> 1
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Char. 1705: 4 --> 2
Char. 1731: 1 --> 3
Char. 1733: 0 --> 2
Char. 1757: 1 --> 3
Char. 1758: 3 --> 1
Char. 1808: 1 --> 4
Char. 1847: 4 --> 0
Char. 1876: 0 --> 3
Char. 1877: 13 --> 0
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Char. 1907: 3 --> 4
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Char. 1937: 0 --> 1
Char. 1952: 4 --> 0
Char. 1982: 0 --> 2
Char. 2002: 1 --> 0
Char. 2016: 3 --> 1
Char. 2039: 0 --> 3
Char. 2052: 0 --> 1
Char. 2058: 1 --> 0
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Char. 2164: 0 --> 3
Char. 2181: 0 --> 3
Char. 2202: 3 --> 1
Char. 2243: 0 --> 3
Char. 2305: 1 --> 3
Char. 2314: 4 --> 3
Char. 2316: 0 --> 3
Char. 2336: 2 --> 0
Char. 2423: 3 --> 1
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Char. 2514: 0 --> 2
Char. 2521: 0 --> 2
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Char. 2545: 0 --> 3
Char. 2626: 1 --> 0
Char. 2633: 0 --> 3
Char. 2640: 3 --> 1
Char. 2731: 1 --> 0
Char. 2748: 1 --> 3
Char. 2785: 0 --> 2
Char. 2802: 2 --> 4
Char. 2816: 3 --> 2
Char. 2824: 0 --> 3
Char. 2828: 1 --> 3
Char. 2829: 1 --> 3
Char. 2837: 0 --> 3
Char. 2842: 2 --> 1
Char. 2897: 4 --> 0
Char. 3015: 1 --> 3
Char. 3027: 3 --> 1
Char. 3924: 3 --> 1
Char. 3942: 0 --> 2
Char. 3953: 1 --> 2
Char. 3960: 1 --> 3
Char. 3963: 1 --> 0
Char. 3973: 0 --> 3
Char. 3982: 0 --> 2
Char. 3995: 3 --> 2
Char. 3997: 0 --> 1
Char. 4007: 0 --> 3
Char. 4028: 3 --> 2
Char. 4031: 3 --> 1
Char. 4038: 3 --> 2
Char. 4041: 1 --> 0
Char. 4083: 0 --> 3
Char. 4092: 0 --> 1
Char. 4107: 3 --> 1
Char. 4116: 0 --> 3
Char. 4158: 3 --> 1
Char. 4191: 3 --> 0
Char. 4217: 3 --> 2
Char. 4226: 0 --> 2
Char. 4229: 13 --> 0
Char. 4253: 1 --> 3
Char. 4271: 0 --> 3
Char. 4274: 1 --> 0
Char. 4296: 3 --> 0
Char. 4307: 3 --> 1
Char. 4335: 3 --> 0
Char. 4341: 0 --> 2
Char. 4348: 0 --> 2
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Char. 4374: 3 --> 1
Char. 4380: 0 --> 2
Char. 4389: 0 --> 3
Char. 4401: 1 --> 3
Char. 4410: 1 --> 3
Char. 4426: 3 --> 1

Char. 4430: 3 --> 1
Char. 4432: 0 --> 1
Char. 4462: 3 --> 1
Char. 4468: 0 --> 3
Char. 4474: 0 --> 2
Char. 4489: 3 --> 1
Char. 4505: 3 --> 1
Char. 4508: 3 --> 0
Char. 4547: 0 --> 3
Char. 4550: 0 --> 2
Char. 4560: 1 --> 3
Char. 6394: 0 --> 3
Char. 6447: 1 --> 2
Char. 6451: 2 --> 0
Char. 6508: 3 --> 1
Char. 6511: 1 --> 3
Char. 6620: 1 --> 3
Char. 6656: 3 --> 2

APPENDIX 9. Specimens revised of the genus *Hyloscirtus* and some species of outgroup

Hyloscirtus alytolyax: ECUADOR, Cotopaxi, Las Pampas, Quebrada de Santa Elena. QCAZ 24374, 24383.

Hyloscirtus antioquia*:* COLOMBIA, Antioquia, Bello, San Félix, Serranía Las Baldías, Corregimiento de San Félix, Vereda Las Huertas, Quebrada El Hato, 2660 m, Km. 5 San Félix road – Truchera San Félix., MHUA-A 7227 (holotype), 7228–29 (paratypes)

Hyloscirtus armatus: BOLIVIA, La Paz, Franz Tomayo, Arroyo Bilunto, cerca de Santa Cruz de Valle Ameno, MNCN/ADN 546; La Paz, Sud Yungas, Afluente del rio Unduavi em Chaco; MNCN/ADN 560; La Paz, Senda entre San Jose y Apolo, Arroyo Huacataya, MNCN/ADN 6008, 15512; La Paz, Larecaja, Serrania Bella Vista, MNCN/ADN 15515; Cochabamba, Ayopaya, Altamachi, Valle de Pampa Grande, MNCN/ADN 9535, 9536; Cochabamba, Carrasco, Kharahuasi, MNCN/ADN 15508, 15509. PERU, Puno, Minas Dos de Mayo, Santo Domingo, MNCN/ADN 21325.

Hyloscirtus baios: COLOMBIA, Nariño, Barbacoas, Corregimiento de Altaquer, Inspeccion de Policia, Quebrada La Carbonera, PR 16161, 16275, 16276, 16278, 16280.

Hyloscirtus bogotensis*:* COLOMBIA, Casanare, El Arenal, cerca al Km. 108 carretera Socha - La Punta, La Salina, quebrada, ICN 05851, 05854; Virolin, orillas del rio Virolin y quebradas aledanas, ICN 04416, 04421.

Hyloscirtus callipeza: COLOMBIA, Santander, Tona, PR 16315, VR 5980, 5993, 6159, 6160.

Hyloscirtus caucanus*:* COLOMBIA, Cauca, Páez, Hacienda Montenegro, Km. 34.5 Belalcazar–Tacueyo, 2400 m., ICN 7071 (holotype), 7002, 7056, 7072–76, 7238, 7241–48, 7250–52, 7055, 7239, 7253.

HYloscirtus charazani: BOLIVIA, La Paz, Saavedra, Rio Wasahuaco, Charazani. MNCN/ADN 538, 539.

Hyloscirtus colymba: COLOMBIA, Chocó, Tacarcuna, MAR 1466, 1472, 1529, 1629, 1676, 1697.

Hyloscirtus condor**:** ECUADOR, Zamora Chinchipe, Cantón Nangaritza, Parroquia Nuevo Paraíso, Reserva Biológica Cerro Plateado, 2317 m., EPN 14758 (holotype), EPN 14754, 14755, 14756, 14757, 14759, 14760 (paratypes).

Hyloscirtus criptico*:* ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2760–2885 m., QCAZ 43516–18, 43528 (paratypes).

Hyloscirtus diabolus: PERU, Amazonas, Chachapoyas, La Jalca, 2300 m., CORBIDI 12885 (holotype).

Hyloscirtus jahni: VENEZUELA, Táchira, Q. Honda. 12′ em carro subiendo desde La grita hacia El Páramo La Negra, MNCN/ADN 50543, MHNLS 17229.

Hyloscirtus hydrophilus: COLOMBIA, Antioquia, Anorí, El Retiro, Fca. El Chaquiral, MHUA 4786, 5041, 5042; El Roble, Bosque La Forzosa, MHUA 5040; Murri, VR 3265, 3266, 3292;

Hyloscirtus larinopygion*:* COLOMBIA, Caldas, Villa Maria, Km. 7 Villa Maria–Mariquita, ICN 34433; Caldas, Vereda Montano, Bosque Central Hidroelectra de Caldas, Predio La Mesa, TG 2150, Caldas, Pensilvania, Km. 24 Pensilvania–Arboleda, 2000 m., ICN 36518–19; Cauca, Popayan, Santa Teresa stream, 2200 m., KU 144127 (holotype); Huila, Belalcazar, Parque Nacional Natural Nevado del Huila, 2900 m., ICN 41880; Quindío, Salento, Hacienda La Caleria, 2300 m., ICN 15626–27; Risaralda, Pereira, Parque Regional Ucumarí, Las Delicias stream, 2340 m., ICN 36133–38, 34970–72; Risalda, Pereira, Parque Regional Natural Ucumarí, Refúgio Ecológico La Pastora, MRC 563, 574; Tolima, Ibague, Juntas, El Silencio, 2600–2820 m., ICN 9380–82, 9670; Valle del Cauca, El Cairo, Las Amarillas, 2140–2200 m., ICN 28926, 28828–29, 42779; Valle del Cauca, Farallones de Cali, Campamento Corea, 2600 m., ICN 13595. ECUADOR, Carchi, Espejo, Morán, 2452 m., QCAZ 41826; Antioquia, Bello, Serrania Las Baldias, 6.6-8.1 km al WSW de San Felix, ICN 9384, 9387; Antioquia, Belmira, El Yerbal, km 6 al N de Belmira, INC 9389; Antioquia, Belmira, Vereda Los Patos, quebrada Los Patos, 3.7 km al N de Belmira, ICN 9390, 9392.

Hyloscirtus lascinius: COLOMBIA, Norte Santander, Chinacota, Cerca a km por carretera, sur este Chinacota, ICN 15033, 15034, 15035; Tona, Finca El Diviso, km 22 carretera Bucaramanga a Pamplona, ICN 15070, 15071; Santander, Florida Blanca, MRC760.Santander, Charta, Vereda El Centro, Quebrada La Prensa, MRC 624.

***Hyloscirtus lindae**:* ECUADOR, Napo, Papallacta, 2.660 m., KU 164402 (holotype), 155476 (paratype); Pacto Sumaco, Parque Nacional Sumaco, Refugio La Laguna, 2476–2775 m., QCAZ 41232, 41294–98, 45345–47. COLOMBIA, Caquetá, Florencia, 0.7 km E. Limite Huila-Caquetá, ICN 20795, 2796; Putumayo, Colon, ICN 49655, 49657; Putumayo, San Francisco, San Antonio Del Poroyac, ICN 49662.

Hyloscistus lynchi: COLOMBIA, Santander, Piedecuesta, km36 carretera Bucamanga a Pamplona, ICN 33504, 33521; Santa Barbara, PR 16300, 16301, 16304.

Hyloscistus oro: ECUADOR, El Oro, Bella María, Cerca de Valle Hermoso, QCAZ 37234, 37246, 37250.

***Hyloscirtus pacha**:* ECUADOR, Morona Santiago, Plan de Milagro, 2350 m., KU 202762 (holotype); Plan de Milagro, Km 8 Plan de Milagro–Cuenca, 2150–2300 m., QCAZ 48237–41.

Hyloscirtus palmeri: COLOMBIA, Chocó, Tacarcuna, MAR 1992, 66D-070, 66D-074; Dagua, Valle San Jose Del Palmar, MAR 1084, 1104, 1165, 1195.

***Hyloscirtus pantostictus**:* ECUADOR, Sucumbios, km 5 Santa Bárbara–La Bonita, 2650 m., KU 190000 (holotype); Santa Barbara, 2590–2800 m., QCAZ 06596, 10488,10671, 11660–67, 12171, 12174, 14084, 30529–31, 38421, 40331, 41393, 41412, 42350, 45434–38, 45443–44, 45446, 45450–53.

Hyloscirtus perija: COLOMBIA, Cesar, Perijá, LEP 233, 258.

Hyloscirtus piceigularis: COLOMBIA, Santander, Charala, Virolin, cabaceras rio Luisito, ICN 04696, 05441, 05291, 5304, 5307; Cundinamarca, Paramos Guerrero ICN 11079.

Hyloscirtus platydactylus: VENEZUELA, Táchira, Q. Honda. 12′ em carro subiendo desde La grita hacia El Páramo La Negra, MNCN/ADN 50540-50542, MHNLS 17226, 17228.

***Hyloscirtus princecharlesi**:* ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2720–2794 m., QCAZ 43654, 44893 (paratypes).

***Hyloscirtus psarolaimus**:* ECUADOR, Napo Papallacta, Km 11 Papallacta–Baeza, 2660 m., KU 164313 (holotype), QCAZ 13252, 23070; Carchi, Tulcán–Santa Bárbara road, QCAZ 15366; Morona Santiago, San Vicente, Parque Nacional Sangay, 15 Km to road Lagunas de Atillo, 2815 m., QCAZ 31671; Sucumbios, La Sofía, Campamento Río Verde, 2726 m., DHMECN 6493–94.

Hyloscirtus chlorophyllus: COLOMBIA, Boyacá, Chivor, Vereda Camolla, Quebrada La Mula, km 7 vía puente El Resbaloso, MRC 699, 700, 703; Miraflores, Vereda Tunjita, Quebrada Agua Blanca, MRC 720; Vereda Tunjita, km 43.5 vía Garagoa, MRC 744;

Hyloscirtus hyalinus: Caquetá, Florencia, MAR 1911, MC 9190, 9413, 9747, 9750, 9751.

Hyloscirtus phyllognathus: ECUADOR, Morona Santiago, 16 km al N de El Ideal em La via a Cuenca (desde Gualaquiza), QCAZ 23937, 23938, 23942; Gral. Leonidas Plaza Gutiérrez (Limón), Em El Rio

Napinaza, QCZA 42047, PUCE 21362; Provincia Napo, Rio Huataraco, a 70 km al Este de Hollín via Hollín-Loreto, QCAZ 07118; Zamora Chinchipe, Miazi Alto, QCAZ 41031.

Hyloscirtus ptychodactylus: ECUADOR, Cotopaxi, Pilalo, 2320 m., KU 209780 (holotype).

Hyloscirtus sarampiona: COLOMBIA, Cauca, Parque Nacional Natural Munchique, Sopladero stream, 33 Km., 2190 m., ICN 7440 (holotype), 7441 (paratype).

Hyloscirtus simmonsi: COLOMBIA, Cauca, El Tambo, Funacion Proselva, Hacienda El Tambito, ICN 32842; Cauca, Purace, Parque Natural Nacional Munchique, 46 km al NNW de La Uribe, ICN 25906; Valle, Dagua, Cabeceras de La Quebrada La Seca, 8km cerca S. Queremal, ICN 41304.

Hyloscirtus staufferorum: ECUADOR, Napo, 27 Km N Jondachi, 2040 m., KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m., QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m., QCAZ 45962–63, 45965–67; Napo, Rio Consafa, 13.4km Del puente sobre El Rio Consangua, QCAZ 11150 (topotipo).

Hyloscirtus sp.: PERU, Palcazu, MNCN/ADN 50824, MUSA 924.

Hyloscirtus tapichalaca: ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m., QCAZ 15083–85, 16704–06, 17776–77 (paratypes).

Hyloscirtus tigrinus: COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m., ICN 53804 (holotype), ICN 53805–06 (paratypes).

Hyloscirtus torrenticola: COLOMBIA, Caquetá, Corregimento El Caraño, Vereda Villaraz, Finca Villa Marcela, km 20 Florencia-Suaza, TG 1818, 1819; Florencia, Vereda La Portada, 35.2km, ICN 23917; San José de La Fragua, Vereda La Esmeralda, Finca Buena Esperanza, km 20 Florencia-Suaza TG 1844; Caquetá, MAR 1961, TG 1930; Caquetá, Escuela Tarqui km 53-54, carretera Altamira-Florencia, ICN 23614.

Hyloscirtus staufferorum: ECUADOR, Napo, 27 Km N Jondachi, 2040 m., KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m., QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m., QCAZ 45962–63, 45965–67.

Hyloscirtus tapichalaca: ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m., QCAZ 15083–85, 16704–06, 17776–77 (paratypes).

Hyloscirtus tigrinus: COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m., ICN 53804 (holotype), ICN 53805–06 (paratypes).

Hyloscirtus **sp "caldas"*:*** COLOMBIA, Caldas, Florencia, PR 14927, 14930, JMR 2815, 2883, 2887.

Aplastodiscus perviridis: BRASIL, Rio Grande do Sul, São Francisco de Paula, MCP 4053; Santa Catarina, Campo Belo do Sul, MCP 10460; Lebon Régis, MCP 8974.

Bokermannohyla hylax: Brasil, Santa Catarina, Florianópolis, MCP 3122, 6362; Joinville, MCP 8634.

Hypsiboas bischoffi: BRASIL, Rio Grande do Sul, São Francisco de Paula, MCP 10680, 10681; Santa Catarina, Campo Belo do Sul, MCP 10464; Lebon Régis, MCP 8968.

Hypsiboas faber: BRASIL, Santa Catarina, Campo Belo do Sul, MCP 10448, 10450.

Hypsiboas raniceps: BRASIL, Goiás, Minacu, MCP 6206; Paraná, Santarem, MCP 2665, 4012.

CONCLUSÕES GERAIS

Esta tese é a mais importante contribuição ao conhecimento da sistemática, evolução, taxonomia e diversidade do gênero *Hyloscirtus.*

No primer capitulo inicialmente contribuímos a resolver um problema taxonômico de velha data a partir da rediagnoses de *Hyloscirtus larinopygion*, uma espécie de ampla distribuição nos Andes de Colômbia y Equador, e descrevemos que populações do norte de Colômbia constituem uma espécie independente que descrevemos como *H. antioquia*. Além disso, discutimos a variação e diversidade das excrecencias nupciais, o tubérculo calcar e alguns aspectos relacionados com a região cloacal inchada no grupo de *H. larinopygion*. Finalmente, discutimos a biologia reprodutiva e os problemas que envolvem a determinação de sinapomorfias putativas de *Hyloscirtus.*

No segundo capitulo descrevemos e comparamos os cantos de anúncio das duas espécies acima mencionadas. A partir de dados de sete localidades no norte da Colômbia, incluindo a localidade tipo de *H. antioquia*. Os cantos consistem em sinais estereotipados de uma única nota multipulsada. Nos avaliamos desde uma perspectiva estadística se existiam diferenças entre ambas espécies em termos acústicos, encontrando evidencia de diferenças significativas em as variáveis espectrais e temporais avaliadas. No entanto, nenhuma característica acústica pode ser considerada como diagnóstica, o que sugere que processos de diversificação acústica ainda não ocorreram. De qualquer forma, as espécies podem se diferenciar por outro conjunto de caracteres fenotípicos e moleculares. A hipótese filogenética mais completa publicada do grupo de *H. larinopygion* sugere que os cantos múlti-pulsados evoluíram de forma independente em pelo menos dois eventos.

No terceiro y quarto capítulos descrevemos duas novas espécies do sudeste do Equador e nordeste do Peru pertencentes ao clado pouco diverso de *Hyloscirtus* com presença de espinho prepolíco desenvolvido e uma evidente hipertrofia dos braços entre outros caracteres morfológicos. *Hyloscirtus arcanus* estava erradamente identificada em trabalhos anteriores na literatura, e historicamente confundida com *H. lindae.* Nos discutimos alguns estados de caráteres associados à morfologia que poderiam sugerir uma relação próxima com *Hyloscirtus condor* e *Hyloscirtus tapichalaca* e alguns aspectos de historia natural das espécies que poderiam sugerir eventos de combates entre machos

Finalmente, no capitulo cinco se trata a sistemática filogenética e taxonomia do gênero *Hyloscirtus* desde uma perspectiva molecular e no contexto da tribo Cophomantini. Com a evidência de quase 7000 caracteres moleculares e mediante analises de máxima parcimônia sob diferentes estratégias se reconstrói as relações filogenéticas para um total de 157 terminais, que representam 44 linhagens. Nesta tese foram geradas aproximadamente 60% sequências de DNA para dos terminais analisados. Mediante a inclusão de toda a informação molecular disponível, a monofilia do género *Hyloscirtus* é testado assim como a relação com o seu clado irmão, conformado pelos gêneros *Aplastodiscus*, *Bokermannohyla* e *Hypsiboas*. Por isso, se apresenta uma nova hipótese sobre o relacionamento das espécies é uma nova classificação para os grupos tradicionalmente tratados na taxonomia de *Hyloscirtus,* criando o grupo *Hyloscirtus tapichalaca* suportado pelo espinho no prepolex como uma sinapomorfía putativa do clado.

Além do anterior, uma extraordinária diversidade é revelada; das 15 espécies consideradas candidatas a novas espécies, sete delas são formalmente delimitadas, diagnosticas e descritas fornecendo evidencia fenotípica. De nossa evidência alguns caracteres fenotípicos são discutidos, tais como a morfologia da mão, glândulas mentais, pads nupciais e morfologia de girinos.

Até recentemente, pouco se sabia sobre as afinidades filogenéticas de muitas espécies de *Hyloscirtus*. Isso começou a mudar com o trabalho especificamente por Faivovich et al. 2005, Coloma et al. 2012 e Faivovich et al. De 2013, e continuou com a presente Tese. Apesar deste avance recente algumas algumas espécies não poderem ser avaliadas (ou seja, *H.*

albopunctulatus, H. chlorosteus, H. caucanus e H. estevesi). Nós também prevemos benefícios da inclusão de caracteres fenotípicos na estimativa filogenética num contexto de evidência total. Sendo *Hyloscirtus* um gênero amplamente distribuído nos Andes e que ocorrem em diversos biomas e a diferentes altitudes, tem um grande potencial para estudos biogeográficos no futuro.

A diversidade fenotípica que existe dentro *Hyloscirtus* também pode ser mais profundamente explorada, uma vez o conhecimento da historia natural mais completo do grupo aumente. Assim, é extremamente importante que os trabalho futuros por conta de *Hyloscirtus* fizeram esforço e ser direcionados para a coleta de girinos, obtenção de datos acústicos, documentação do comportamento reprodutivo, bem como a recolha de amostras de tecido para melhorar a cobertura das populações em estudos filogenéticos. Estes dados são essenciais para uma maior exploração da sistemática, a variação geográfica e evolução de caracteres no grupo. Particularmente em algumas linhagens com uma morfologia muito conservadora, constituindo exemplos clássicos de diversidade críptica. Muitas espécies de *Hyloscirtus* estão atualmente em alguma categoria em perigo (ver IUCN 2013), enquanto outros são dados deficientes DD e naturalmente, as novas espécies não foram avaliadas ainda. Avaliar todos os critérios da IUCN a partir do conhecimento gerado aqui será essencial para compreender o estado atual da ameaça do gênero *Hyloscirtus.*

Os resultados aqui apresentados abrem a porta para a compreensão e melhor entendemento da história evolutiva do gênero *Hyloscirtus* e da família Hylidae, que poderá ser acrescentada com futuros estudos da morfologia, ecologia reprodutiva, biogeografia história de vida, biologia da conservação entre outros, deste clado endêmico dos riachos das montanhas dos Andes.