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Filogeografía comparada y taxonomía integrativa de seis especies de ranas arborícolas (Hylidae) ampliamente distribuidas en tierras bajas del norte de Sudamérica: en busca de nuevos enfoques que soporten decisiones de conservación

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Universidad Nacional de Colombia
Facultad de Ciencias, Posgrado en Ciencias - Biología
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Bogotá, Colombia
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Para Ellie y Sofi, quienes al convertirme en mamá me hicieron más paciente, más ambiciosa, más juiciosa y más consciente de la necesidad de buscar soluciones creativas a los problemas de este mundo que nos tocó vivir.

Para el marido, por la paciencia.

Para todos los estudiantes que he tenido a lo largo de la vida, que me han hecho querer meterme en esta vacaloca y querer salir de ella.

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Resumen

Filogeografía comparada y taxonomía integrativa de seis especies de ranas arborícolas (Hylidae) ampliamente distribuidas en tierras bajas del norte de Sudamérica: en busca de nuevos enfoques que soporten decisiones de conservación

En este trabajo se evaluaron los linajes evolutivos, las distribuciones actuales y potenciales y la evolución de seis especies nominales de hílidos neotropicales de tierras bajas pertenecientes a los géneros *Scarthyla, Scinax* y *Sphaenorhynchus,* así como su potencial uso como indicadores para la toma de decisiones relacionadas con la conservación de sus hábitats. Para ello se propusieron hipótesis filogenéticas bajo inferencia bayesiana y de máxima verosimilitud utilizando cinco marcadores genéticos (tres mitocondriales y dos nucleares) y se plantearon hipótesis datadas por coalescencia que fueron comparadas con la información geológica y geográfica disponible sobre sus áreas de distribución. Para evaluar la diversidad real y la distribución de los linajes incluidos en tales especies nominales se calcularon redes de haplotipos, se hicieron descripciones morfológicas y morfométricas detalladas de los especímenes disponibles en colecciones biológicas y se construyeron modelos de distribución de especies, que además permitieron estimar las consecuencias de cambios ambientales sobre los linajes identificados. Como resultado principal se evidenció una asociación íntima entre la morfología, la biología de las especies y su preferencia por hábitats abiertos o arbolados en las localidades que ocupan que puede informar decisiones de conservación; se establecieron las relaciones filogenéticas dentro y entre los grupos de estudio y se ordenó la taxonomía de un grupo de ranas extenso y problemático por más de 30 años en el país; se identificaron 24 especies candidatas confirmadas incluidas en los seis nombres inicialmente considerados y con ello se detallaron e incrementaron las increíbles cifras de la biodiversidad conocida para el país.

Palabras clave: conservación, diversidad críptica, Neotrópico, ranas arborícolas, biogeografía histórica, Amazonia, Colombia**.**

Abstract

Comparative phylogeography and integrative taxonomy of six tree-frog species (Hylidae) widely distributed in the lowland areas of northern South America: In search of new approaches for supporting conservation decisions

We evaluated the evolutionary lineages, current and potential distributions and the evolution of six nominal species of lowland Neotropical hylids belonging to the genera *Scarthyla*, *Scinax* and *Sphaenorhynchus*, as well as their potential use as indicators for decision-making related to the conservation of their habitats. For this purpose, phylogenetic hypotheses were proposed under Bayesian and Maximum Likelihood (ML) inference using five genetic markers (three mitochondrial and two nuclear) and hypotheses dated by coalescence were proposed and compared with the available geological and geographical information on their distribution areas. To evaluate the real diversity and distribution of the lineages included in such nominal species, haplotype networks were calculated, detailed morphological and morphometric descriptions were made of the specimens available in biological collections and species distribution models were constructed, which also allowed estimating the consequences of environmental changes on the identified lineages. As a main result, an intimate association between morphology, species biology and their preference for open or forested habitats in the localities they occupy was evidenced, which can inform conservation decisions. Phylogenetic relationships within and among the study groups were established and the taxonomy of an extensive and problematic --for more than 30 years-- group of frogs in the country was sorted out, yielding 24 confirmed candidate species that were included in the six names initially considered, thus detailing and increasing the incredible known biodiversity of this corner of South America.

Keywords: conservation, cryptic diversity, Neotropical Region, tree-frogs, historical biogeography, Amazonia, Colombia.

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Lista de Símbolos y abreviaturas

Abreviaturas

Abreviatura	Término
ºC	Grados centígrados
12S	Mitochondrial encoded 12S ribosomal ribonucleic acid gene
16S	Mitochondrial encoded 16S ribosomal ribonucleic acid gene
ABGD	Automatic Barcode Gap Discovery algorithm
ADN	Ácido desoxirribonucleico
AMP	Anti-microbial peptides
ANH	Agencia Nacional de Hidrocarburos de Colombia
ANLA	Agencia Nacional de Licencias Ambientales de Colombia
ANSP	Academy of Natural Sciences of Drexel University, U.S.A.
ASM	Ángela Suárez Mayorga – acrónimo de colección
BI	Bayesian inference
bp	Base pairs
BPP	Bayesian posterior probability
BTBC	Banco de ADN y Tejidos de la Biodiversidad Colombiana
ca.	*Circa* - Aproximadamente
CAN	Comunidad Andina de Naciones
CCS	Confirmed candidate species
COI	Mitochondrial cytochrome oxidase subunit I gene
CPZ-UV	Colección Zoológica de Prácticas de Docencia of Universidad del Valle
CR	En peligro crítico, categoría de la IUCN
DD	Datos deficientes
DNA	Deoxyribonucleic acid
EIA	Evaluación de Impacto Ambiental/ Environmental Impact Asessment
ED	Eye diameter
EN	Amenazado, categoría de la IUCN
E-N	Distancia ojo-narina/eye-nostril
E-S	Eye-snout distance

Abreviatura	**Término**
ESS	Estimated sample size
FC	Filogeografía comparada
FING	maximum width of terminal disc of the third finger
FL	Femur lenght
GBIF	Global Biodiversity Information Facility
GTR	General Time-Reversible model of evolution
GTR-G	General Time-Reversible model plus Gamma
HL	Head length
HPD	Highest Posterior Density (interval)
HW	Head width
IAvH	Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
ICN	Instituto de Ciencias Naturales de la Facultad de Ciencias de la Universidad Nacional de Colombia
IGUN	Instituto de Genética de la Universidad Nacional de Colombia
IOD	Inter-orbital distance
IUCN	International Union for Conservation of Nature
IT	Integrative Taxonomy
masl	Meters above the sea level
MAV	Mario Alfonso Vargas – acrónimo de colección
MCZ	Museum of Comparative Zoology
ML	Maximum Likelihood
mm	Milimeters
MMV	Middle Magdalena Valley of Colombia
MNHM	Museo Nacional de Historia Natural de Madrid, España
mnsm	Metros de altitud sobre el nivel medio del mar
mPTP	multi-rate Poisson Tree Processes phylogenetic inference
MVR	Mario Vargas Ramírez
MTD	Museum für Tierkunde - Senckenberg Dresden, Germany
MVZ	Museum of Vertebrate Zoology, the University of California - Berkeley
Mya	Million years ago (before present)
NCR	Nicolás Castillo Rodríguez – acrónimo de colección
NNP	National Natural Park
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
PCR	Reacción en cadena de la polimerasa

Abreviatura	**Término**
PERMANOVA	Permutational multivariate analysis of variance
PP	Posterior probability
PPI	Proyectos piloto de investigación para *fracking*
Rho	Rhodopsin nuclear gene
RNA	Ribonucleic acid
RUNAP	Registro Único Nacional de Áreas Protegidas
SDM	Species Distribution Model
SINAP	Sistema Nacional de Áreas Protegidas de Colombia
s.l.	*sensu lato*
SMNS	Naturkundemuseum Stuttgart, Germany
SSIGMOL	Servicio de Secuenciación y Análisis Molecular, Instituto de Genética, Universidad Nacional de Colombia
SVL	Snout-vent length
TD	Maximum tympanum diameter
TI	Taxonomía integrativa
TL	Tibia length
Tyr	Tyrosinase nuclear gene
VU	Vulnerable, categoría de la IUCN
UCS	Unconfirmed Candidate Species
ZSM	Zoologische Staatssammlung München, Germany

Introducción

La distribución de las especies y sus límites responden a tres condiciones principales: 1) que las áreas que deben habitar puedan ser ocupadas, 2) que las especies sean capaces de ocuparlas y 3) que las especies puedan permanecer viviendo dentro de un área determinada. La Región Neotropical (RN) concentra casi la mitad de las especies conocidas en la Tierra (Jenkins, Pimm, & Joppa, 2013) y la mitad (226) de los ecosistemas más recientemente contabilizados (Sayre et al., 2020). Sin embargo, aún se desconocen muchas especies y su relevancia a efectos de conservación -porque sus distribuciones son restringidas o sus hábitats están amenazados- (Pimm et al., 2014). En consecuencia, siempre se subestiman sus relaciones ecológicas con los ecosistemas que habitan y los valores económicos, culturales o políticos que pueden representar para las poblaciones humanas (Tundisi & Matsumura-Tundisi, 2008).

Para superar esa realidad, se han seleccionado taxones indicadores que se utilizan como *proxies* para evaluar la salud de los ecosistemas, la diversidad de las especies y su distribución (D. Rapport, 1998). Los anfibios se consideran excelentes indicadores dada su gran sensibilidad a los cambios ambientales (Bielby, Cooper, Cunningham, Garner, & Purvis, 2008; Jenkins et al., 2013) y su estrecha y antigua relación con las poblaciones humanas (Xiao, Liu, & Lai, 2011). Los anfibios también se consideran el grupo de vertebrados más amenazado (Hof, Araújo, Jetz, & Rahbek, 2011).

A pesar de las ventajas mencionadas, las relaciones evolutivas y la taxonomía de los bioindicadores suelen representar un cuello de botella para los fines de conservación (Swenson et al., 2012) o para la planificación de la investigación básica a medio y largo plazo (Nori et al., 2015). Los anfibios son una fuente ampliamente reconocida de diversidad críptica (Bickford et al., 2007; Funk, Caminer, & Ron, 2011; Motta, Menin, Almeida, Hrbek, & Pires Farias, 2018), especialmente en el Neotrópico, y los esfuerzos por desentrañar esa diversidad y sus consecuencias son aún precarios (Fouquet et al., 2021, 2012; Rojas, Fouquet, De Carvalho, et al., 2018; Vacher et al., 2017).

El énfasis geográfico de esta investigación son las tierras bajas del noroeste de Sudamérica (cis-andinas y trans-andinas), pero se detallan especialmente Colombia y su biodiversidad poco conocida en general --y su taxonomía de anfibios desactualizada en particular. Colombia no representa un vacío de conocimiento en las recientes evaluaciones taxonómicas y sistemáticas regionales y globales de anfibios, sino un enorme cráter de información que debe ser llenado colectivamente (tal vez en la excepción de Dendrobatoidea y el trabajo que ha sido realizado por el laboratorio de Tarant Grant en la Universidad de Sao Paulo, Brasil, pero véanse por ejemplo M. A. Caminer *et al.*, 2017; M. Caminer & Ron, 2014; Fouquet *et al.*, 2021; Rojas, Fouquet, Ron, *et al.*, 2018; J.-P. Vacher *et al.*, 2020). Brasil y Colombia son los dos países más ricos en número de especies de anfibios conocidas (Acosta-Galvis, 2021; Segalla et al., 2016) y los territorios andino-amazónicos del norte de Sudamérica, en los que se centra esta investigación, son una de las principales fuentes esperadas de novedades taxonómicas en anfibios y otros grupos biológicos (Jenkins et al., 2013).

Cerca del 25% de los anfibios conocidos en Colombia están incluidos en una de las tres categorías de amenaza de la Unión Internacional para la Conservación de la Naturaleza (UICN): VU para "vulnerable", EN para "en peligro" y CR para "en peligro crítico". Otra cuarta parte de la diversidad de anfibios ha sido considerada recientemente como Datos Insuficientes (DD, Urbina-Cardona, com. pers.), lo que significa que la mitad de la fauna actual de ranas de Colombia podría estar en peligro. Alrededor del 30% de la diversidad de anuros del país pertenece a clados dentro de Terrarana, que tienen un desarrollo directo (sin estado larvario), por lo que son fuertemente dependientes de la humedad ambiental y de la cobertura forestal para su supervivencia en los primeros estadios. Otro 30% de la diversidad (40% si se restringe a las zonas con altitudes inferiores a 1000 msnm, o tierras bajas), pertenece a la familia Hylidae (Lynch, 2006). Esta familia se considera muy diversa; su taxonomía y sistemática han sido ampliamente discutidas y constantemente actualizadas (W. E. Duellman, Marion, & Hedges, 2016; Faivovich, Haddad, García, Frost, & Wheeler, 2005; Faivovich et al., 2018; Feng et al., 2017; R A Pyron & Wiens, 2013; Wiens, Kuczynski, Hua, & Moen, 2010) y sus relaciones filogenéticas se han hecho explícitas pero con posiciones contrastantes (ver referencias anteriores).

Los patrones de distribución de los hílidos de tierras bajas también se han estudiado a partir de registros de presencia/ausencia, al igual que su correlación con variables ecológicas como la estacionalidad o la dependencia de la humedad (Caminer et al., 2017; Escalona, Prieto-Torres, & Rojas-Runjaic, 2017; Ferrão et al., 2016; Fouquet, Santana Cassini, Fernando Baptista Haddad, Pech, & Trefaut Rodrigues, 2014; Gehara et al., 2014; Giovanelli, de Siqueira, Haddad, & Alexandrino, 2010; Haddad & Prado, 2005; Nowakowski et al., 2018; Vacher et al., 2020). Esto hace que los hílidos sean muy buenos candidatos para evaluar cómo los procesos históricos y ecológicos han moldeado la distribución y diversificación de las especies en el Neotrópico, y para ayudar en la identificación de áreas naturales bajo una perspectiva de biogeografía histórica (Harold & Mooi, 1994).

A pesar de la diversidad ampliamente mencionada, un análisis de la información bibliográfica disponible sobre la incidencia de la taxonomía en las decisiones de conservación durante la primera década del siglo XXI (Arbeláez-Cortés, 2013) mostró que ni la taxonomía de los bioindicadores ni sus distribuciones habían sido suficientemente cubiertas. Además, la información mínima que sería necesaria para sustentar las decisiones de conservación, o para definir estrategias de gestión, o para identificar y gestionar las principales amenazas sobre las especies y los ecosistemas no está disponible públicamente. Eso significa que las evaluaciones de la biodiversidad siguen elaborándose a partir del "juicio de los expertos" en lugar de basarse en datos.

A lo largo de esta disertación, se utilizaron seis especies nominales de ranas arborícolas como modelos para investigar tres preguntas:

1) ¿Cuántos linajes de ranas viven realmente en el noroeste de Sudamérica bajo las seis especies nominales seleccionadas? ¿Cuál es la expresión fenotípica de la variabilidad genética en las poblaciones de ranas estudiadas?
2) ¿Qué factores determinan la diversidad de los hílidos modelo en las tierras bajas del norte de Sudamérica? ¿Cómo se relacionan las distribuciones actuales y la evolución de esta fauna de ranas?
3) ¿Cómo el estado actual de conocimiento (o ignorancia) sobre la diversidad de ranas de la región puede impactar en las decisiones de conservación y gestión de la biodiversidad?

Taxonómicamente, los modelos seleccionados comprenden dos especies semiacuáticas (*Scarthyla goinorum* y *Sphaenorhynchus lacteus*) y cuatro especies principalmente terrestres (*Scarthyla vigilans*, *Scinax rostratus*, *S. ruber* y *S. wandae*), las cuales tienen distribuciones amplias y contrastantes en el norte de Suramérica, pero pueden compartir áreas de distribución en las planicies transandinas y cisandinas de Colombia. *Scarthyla goinorum* se distribuye a lo largo del río Amazonas desde Bolivia hasta Colombia. *Sphaenorhynchus lacteus* habita en los cursos de los ríos y estanques a lo largo de la cuenca del Amazonas-Orinoco, desde Bolivia hacia el norte hasta Trinidad y Tobago y las Guayanas costeras. *Scarthyla vigilans* y *Scinax rostratus* se limitan a las tierras bajas del Caribe (es decir, desde Urabá en Colombia o el este de Panamá hasta Guayana), incluyendo los valles interandinos y el norte de la Orinoquia de Colombia. *Scinax ruber* es el *Scinax* más ampliamente distribuido, y ocupa desde Costa Rica hasta el norte de Argentina en una variedad de ecosistemas por debajo de los 2000 msnm. Por último, *S. wandae* está restringida a los llanos colombo-venezolanos y al estado adyacente de Amazonas en Venezuela, pero únicamente en áreas de sabana.

Todos los *Scinax* tiene reproducción explosiva y renacuajos de vida libre que se desarrollan en aguas lénticas, mientras que se sabe poco sobre la reproducción de *Scarthyla* y *Sphaenorhynchus* más allá de sus renacuajos en pantanos (ya sea dentro del bosque o de las sabanas para *Scarthyla goinorum* y *S. vigilans*, respectivamente) o debajo de praderas flotantes en el curso del río (Angela M. Suárez-Mayorga & Lynch, 2001a, 2001b). Las relaciones entre *Scarthyla, Scinax* y *Sphaenorhynchus* son contradictorias hasta la fecha: Faivovich *et al.*, (2005) reconocieron estos tres géneros como miembros de la tribu Dendropsophini dentro de Hylinae; Pyron & Wiens (2011) recuperaron dos clados diferentes y relacionados dentro de Hylinae, siendo (*Sphaenorhyncus* + *Scinax*) el grupo hermano de ((*Pseudis*+*Scarthyla*+*Lysapsus*)+*Dendropsophus*)), equivalente a Pseudinae + Dendropsophinae en la taxonomía actual. Duellman *et al.* (2016) erigieron la familia Scinaxinae para (*Scinax* + *Sphaenorhynchus*) y confirmaron la posición de *Scarthyla* dentro de Pseudinae. También propusieron *Julianus* para el clado *Scinax uruguayus*, y *Ololygon* para el clado *Scinax catharinae* sin sustento evolutivo (Conte, Araujo-Vieira, Crivellari, & Berneck, 2016). En la actualidad, los análisis moleculares parecen coincidir con (Lourenço et al., 2016) en que dichos cambios nomenclaturales no eran necesarios ni apropiados.

Metodológicamente, se utilizaron dos enfoques para empezar a resolver las preguntas de investigación: la Taxonomía Integrativa (TI) y la Filogeografía Comparada (FC). El término Taxonomía Integrativa se acuñó formalmente en 2005, y simplemente recuerda la necesidad de integrar líneas de evidencia múltiples y complementarias para decidir más rápido y, con suerte, mejor (o más cerca de una realidad objetiva), sobre cómo se ven las diferentes unidades taxonómicas operativas (OTU) y cómo las diferencias identificadas pueden tener algún significado evolutivo (adaptado de Schlick-Steiner *et al.*, 2010). Se ha argumentado (Vinarski, 2020) que la TI no es un campo de estudio novedoso sino una reconstrucción de la sistemática del siglo pasado (Mayr, 1982). La aplicación de análisis combinados e interdisciplinarios podría ayudar sin duda a la planificación de la conservación para la toma de decisiones, la bioprospección y la educación (Bickford et al., 2007).

La **filogeografía**, por su parte, es el estudio de la disposición espacial de los linajes evolutivos, tanto dentro de las poblaciones de una misma especie y de especies estrechamente relacionadas, como entre ellas (Avise, 2000). Combina la genética de las poblaciones (a escala microevolutiva) y la sistemática filogenética (enfoque macroevolutivo) bajo un claro alcance espacial. La filogeografía permite responder a preguntas relacionadas con la biogeografía, la conservación de la biodiversidad y la sistemática de los organismos (Hickerson et al., 2010). Así, la base de la evaluación filogeográfica es la identificación del número de poblaciones que constituyen una especie --entendida como un linaje evolutivo diferenciado-- y las condiciones en las que se mantienen tales linajes evolutivos, así como sus distribuciones.

La taxonomía integrativa y la filogeografía son la base del primer capítulo (artículo científico que se presentará a la revista Zookeys), en el que los autores desentrañan la confusa taxonomía vigente por más de 50 años y la diversidad críptica de una única especie nominal: lo que se ha identificado como "*Scinax ruber*" en el área de estudio. El resultado más importante de este capítulo es la aclaración de todo el marco taxonómico (incluida la diferenciación morfológica y ecológica) de la especie de *Scinax* más ampliamente distribuida, y la única hasta la fecha considerada útil para las poblaciones humanas por la presencia de péptidos antimicrobianos en su piel. Desgraciadamente, las poblaciones registradas con esta característica son endémicas de una parte de Colombia y su supervivencia está probablemente ya amenazada.

En los dos primeros capítulos se realizó un modelamiento básico de distribución de especies para algunos de los nuevos linajes definidos integralmente, con el objetivo de comparar los registros conocidos disponibles con los rangos de distribución calculados y las inferencias ecológicas que allí se discuten. Ese ejercicio, y la superposición de los modelos obtenidos a las capas de "impacto" (el mapa oficial de áreas protegidas de Colombia según el Registro Nacional de Áreas Protegidas - RUNAP, y los proyectos de explotación de petróleo y gas en curso y con licencia, incluyendo el fracturamiento hidráulico o *fracking*, según la Agencia Nacional de Hidrocarburos - ANH en el Magdalena Medio) permitieron identificar preferencias de hábitat aparentes, procesos de diversificación hipotéticos y amenazas potenciales sobre la conservación de especies y ecosistemas.

El tercer capítulo comprende los principales análisis comparativos filogenéticos, filogeográficos y biogeográficos, basados en filogenias datas y análisis de haplotipos nucleares de los linajes macrosimpátricos llaneros-caribeños (*Scarthyla vigilans*, *Scinax rostratus* y *Scinax wandae*), así como de los dos linajes semiacuáticos amazónicos y supuestamente poco estructurados poblacionalmente (*Scarthyla goinorum* y *Sphaenorhynchus lacteus*).

La combinación de los métodos descritos y las inferencias obtenidas con ellos permitió descubrir respuestas sólidas a las preguntas inicialmente planteadas, dado que los análisis integrados arrojaron resultados similares para las diferentes especies y grupos taxonómicos operativos (terrestres, acuáticos, ligados al bosque, ligados a tierras bajas abiertas, transandinos, cisandinos y sus combinaciones), acogiendo el principio biogeográfico de que la evolución de las especies refleja la evolución de las áreas. Con la excepción de *Scarthyla vigilans* y *Sphaenorhynchus lacteus*, que se revelaron como linajes evolutivos de amplia distribución, todos los taxones nominales aquí considerados estaban compuestos por más de un linaje evolutivo críptico diferente (de dos a trece linajes por especie nominal). *Scarthyla goinorum* se compone de al menos tres linajes evolutivos diferentes. El clado "*Scinax rostratus*" comprende de 15 a 17 linajes, de los cuales 4 a 5 están contenidos en la morfología y el hábitat estrictos de *Scinax rostratus*, más dos linajes de *Scinax kennedyi*. "*Scinax garbei*", que se ha confundido con *S. rostratus* por sus similitudes morfológicas pero es una especie fundamentalmente ligada al bosque, se compone de cuatro linajes distantes. "*Scinax ruber*" en este muestreo se compone de 14 linajes en la cuenca noroccidental del Amazonas, más otro linaje en la selva central peruana, mientras que el restringido y casi endémico (a Colombia y Venezuela) *Scinax wandae* es en realidad una combinación de elementos amazónicos y llaneros, incluyendo dos especies no descritas de la región de Chiribiquete que fueron referidas previamente como *S. cruentomma*, y cuatro linajes de "*S. wandae*" asociados a barreras conformadas por los ríos y, aparentemente, la composición del suelo. Todos los linajes recién identificados se enumeran, con una visión general de sus distribuciones, en la Tabla 1.

Las fechas y escenarios de diversificación fueron similares entre los taxones no relacionados, apoyando así el precepto básico de la biogeografía histórica según el cual la historia de las especies refleja la historia de las zonas. Cuatro eventos geológicos fundamentales parecieron determinar la diversidad real y los rangos de distribución de los hílidos modelo en las áreas andina amazónica y guayanesa occidental: 1) la elevación inicial de la Cordillera Oriental y la separación de los drenajes del Magdalena Medio y de los Llanos durante el Oligoceno (hace 25-30 Ma), que inicialmente separó las especies más acuáticas y realmente extendidas de las terrestres, incluyendo los Pseudinae del Caribe (*Pseudis*, probablemente *Scarthyla vigilans*), y determinó el desarrollo posterior de los valles interandinos; 2) la primera intrusión marina a lo largo de los Llanos durante el Mioceno temprano (18-17 Ma), que podría haber separado los elementos centroamericanos-chocoanos (Hyliane, *Scinax elaeochroa*) de los cisandinos orientales (Dendropsophinae, por ejemplo). 3) Una segunda transgresión posterior relacionada con el sistema de Pebas contribuyó en la explosión de la biodiversidad durante el Mioceno medio (12,4-16,1 Ma) al proporcionar nuevos hábitats disponibles y diferentes barreras a la migración, principalmente en ambientes abiertos. 4) Los clados de bosque, como *S. garbei* (amazónico) y Scinax3 (transandino), pudieron haberse separado de los clados de áreas abiertas/humedales por los efectos integrados del asentamiento hacia el este de la Cordillera Oriental de Colombia y la formación del arco del Vaupés (9-10 Ma); y 5) el levantamiento final de la Cordillera Oriental y la consolidación de los ríos Meta y Orinoco durante el Plioceno (a partir de ca. 5 Ma), que configuraron evidentes diferencias de paisaje dentro del extenso piedemonte amazónico. Ello debió favorecer la diversificación local, que es evidente y temporalmente coincidente en los clados amazónicos de *Scarthyla goinorum* y *Scinax garbei*, y en los clados de *S. kennedyi*, *Scinax sp. gr. ruber* (linajes Scinax1 y Scinax5) y *S. wandae* de los Llanos colombo-venezolanos. En los tres últimos grupos, el río Meta está estructurando claramente las poblaciones de los Llanos del Norte de Colombia hasta el límite extremo del Escudo Guayanés en el Vichada.

Tabla 1. Recuento de los nuevos linajes identificados a través de este trabajo

Identificación del linaje	**Ubicación taxonómica**	**Distribución geográfica**	**Referencias en el documento**
Scarthyla goinorum A	Pseudinae	Perú, Cusco Amazónico	Capítulo 3
Scinax1	*Scinax* gr. *ruber*	Arauca-Casanare Llanos of Colombia	Capítulos 1-2
Scinax2	*Scinax* gr. *ruber*	Alto Manacacías, Colombia	Capítulo 1
Scinax3	*Scinax* gr. *ruber*	Middle Magdalena valley of Colombia	Capítulos 1-2
Scinax4	*Scinax* gr. *ruber*	Bolivia	Capítulo 1
Scinax5	*Scinax* gr. *ruber*	Cumaribo, Colombia	Capítulo 1
Scinax6	*Scinax* gr. *ruber*	Prinoco piedmont, Colombia	Capítulo 1
Scinax6C	*Scinax* gr. *ruber*	Chiribiquete, Colombia	Capítulo
Scinax7	*Scinax* gr. *ruber*	Perú	Capítulo 1
ScinaxX	*Scinax* gr. *ruber*	Middle Magdalena valley of Colombia	Capítulos 1-2
Scinax kennedy A	*Scinax* gr. *rostratus*	Arauca Llanos of Colombia	Capítulo 3
Scinax rostratus A	*Scinax* gr. *rostratus*	Chocó-Magdalena province, Colombia	Capítulo 3
Scinax rostratus B	*Scinax* gr. *rostratus*	French Guiana	Capítulo 3

Tabla 1.
Continuación

Identificación del linaje	**Ubicación taxonómica**	**Distribución geográfica**	**Referencias en el documento**
Scinax garbei A	*Scinax* gr. *rostratus*	Bolivia	Capítulo 3
Scinax garbei B	*Scinax* gr. *rostratus*	Leticia, Colombia	Capítulo 3
Scinax garbei C	*Scinax* gr. *rostratus*	Leticia, Colombia	Capítulo 3
Scinax garbei D	*Scinax* gr. *rostratus*	Perú-Colombia border	Capítulo 3
Scinax wandae A	*Scinax* gr. *wandae*	Arauca, Colombia	Capítulo 3
Scinax wandae B	*Scinax* gr. *wandae*	Orinoco piedmont, Colombia	Capítulo 3
Scinax wandae C	*Scinax* gr. *wandae*	Vichada, Colombia	Capítulo 3
Scinax wandae D	*Scinax* gr. *wandae*	Casanare, Colombia	Capítulo 3
SW1	*Scinax* gr. *wandae*	Chiribiquete, Colombia	Capítulo 3
SW2	*Scinax* gr. *wandae*	Amazonas, Colombia	Capítulo 3
SW3	*Scinax* gr. *wandae*	Chiribiquete, Colombia	Capítulo 3

Finalmente, con el fin de evaluar el impacto del desconocimiento en las decisiones de conservación de la biodiversidad de Colombia, se elaboraron modelos de distribución de especies para los nuevos linajes identificados del clado "*Scinax ruber*" en el país. Posteriormente, esos modelos se superpusieron al mapa oficial de áreas protegidas de Colombia (Unidad Administrativa Especial de Parques Nacionales Naturales, https://www.parquesnacionales.gov.co/portal/es/sistema-nacional-de-areas-protegidas-sinap/mapa-sinap/) y a las capas de explotación de petróleo y gas (incluyendo los proyectos piloto de fracking propuestos) de la Agencia Nacional de Hidrocarburos de Colombia (www.anh.gov.co). Ese ejercicio simplemente confirmó que dichas explotaciones de hidrocarburos se han desarrollado exactamente sobre los rangos de distribución de por lo menos tres especies endémicas dependientes del agua y ambientalmente sensibles, y que la tendencia de degradación está planeada para continuar hacia los restos de los bosques del Magdalena Medio y Bajo, lo que representa una gran amenaza sobre las especies, las comunidades y los ecosistemas de la región.

Objetivos y predicciones

Objetivo general

Dilucidar los patrones y procesos evolutivos a nivel específico y poblacional que han influido en la distribución y diversificación actual de tres clados de ranas (Hylidae) de amplia distribución en las tierras bajas del norte de Sudamérica (*Scarthyla*, el clado *Scinax ruber* y *Sphaenorhynchus lacteus*).

Objetivos específicos e hipótesis correspondientes

- **Objetivo 1**. Describir la variación histórica y actual de las poblaciones y los hábitats de las especies bajo estudio.

Hipótesis 1.1: Las áreas de distribución actuales de las especies incluidas en el clado terrestre son coherentes con las historias de diversificación en macrosimpatría de las poblaciones.

Predicción 1.1: Las áreas de distribución de las especies consideradas son pequeñas y están especializadas en torno a variaciones ecofisiológicas y ambientales.

Hipótesis 1.2: La diversificación en el clado acuático se ha producido debido a la migración a lo largo de los cursos de agua y a la aparición de barreras geomorfológicas.

Predicción 1.2: Existe una estructura genética dentro de cada especie entre los extremos de las distribuciones conectadas por los cursos de agua.

Hipótesis 1.3: Los tiempos de divergencia de los clados terrestres considerados serán más tempranos que los de los clados acuáticos, coincidiendo con la historia paleobiogeográfica conocida de las barreras geográficas que pueden haber delimitado sus áreas de distribución.

Predicción 1.3: Los intervalos de confianza de los tiempos de divergencia de los clados considerados incluirán las fechas estimadas de formación de las barreras.

- **Objetivo 2.** Evaluar el impacto de la presencia de bosques y sus variables ambientales relacionadas (precipitación, humedad y temperatura) en la formación de los límites de las distribuciones de los anuros de tierras bajas cisandinas y transandinas de Colombia.

Hipótesis 2.1: Las distribuciones actuales de las especies consideradas estarán limitadas por barreras ambientales que pueden estar separando diferentes linajes genéticos dentro de cada área.

Predicción 2.1: Las áreas de distribución de las especies consideradas en la propuesta estarán fragmentadas en las distribuciones de los linajes evolutivos que sean identificados.

- **Objetivo 3.** Resolver los interrogantes taxonómicos que se han planteado sobre la identidad y distribución de *Scinax* del grupo *rostratus*, *S. ruber* y *S. wandae* para Colombia.

Hipótesis 3.1: *Scinax rostratus*, *S. ruber* y *S. wandae* están compuestos cada uno por más de un linaje evolutivo diferenciado (especie) en Colombia, de la misma manera que se ha visto para Brasil y Guyana.

Predicción 3.1.1: La diversidad de anuros de la familia Hylidae para Colombia y otros países de la región andino-amazónica aumentará significativamente con especies crípticas pertenecientes a *Scinax*.

Predicción 3.1.2: Algunas especies que han sido sinonimizadas a lo largo de la historia reciente serán válidas en el contexto de la diversidad neotropical.

Hipótesis 3.2: *Scinax rostratus* no habita en las selvas tropicales de los países andinos; está restringida a las sabanas secas y estacionales del extremo norte de Sudamérica.

Predicción 3.2: Se encontrará más de un linaje evolutivo distinto dentro de los organismos identificados como *Scinax rostratus* de Brasil, Colombia y Venezuela, y algunos de ellos probablemente serán simpátricos en el Magdalena Medio de Colombia o en el piedemonte de los Llanos Orientales, donde convergen los ecosistemas de sabana y de bosque.

1. Capítulo 1: Cryptic diversity of “*Scinax ruber*” (Anura: Hylidae) in Andean South America: using integrative taxonomy to frog-leap out of a deep information hole to evidence-based taxonomy and conservation.

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1.1 Abstract

Species richness and endemicity have been traditionally used for describing and defining conservation areas, implementing changes in policy recommendations, or deciding on management alternatives. However, throughout the lowlands of the Andean-Amazonian countries the effectiveness of such decisions is limited by the largely demonstrated underestimation of species level biodiversity and the absence of a robust taxonomic baseline.

To support the need of an evolutionary-based taxonomy, we use the nominal species of tree frog "*Scinax ruber*" as a model of ignorance. Our different lines of evidence (integrative taxonomy, phylogeography, species distribution modelling, and certain ecological traits) support that "*Scinax ruber*" is not a widely distributed species. It is, rather, a large species complex comprising at least eight philopatric and morphologically distinguishable evolutionary lineages in Colombia, Bolivia, and Perú, plus the topotypic *Scinax ruber* s.s., hereby restricted to a portion of Guiana, Suriname (the type locality) and Tobago. About one half of the newly identified lineages might be already at risk of extinction because of their interdependence with their habitats and the perfect match between their actual and potential distributions and the trends of degradation of their ecosystems.

Finally, we elaborate on the importance of an integrative taxonomic approach to support conservation and management decisions, remarking on the importance of digging in the information hole that this region´s biodiversity still supports.

1.2 Introduction

The definition of “conservation targets” is fundamental for establishing conservation areas (Margules et al. 2002). While the delimitation of areas to be conserved is the main goal, taxonomy is the main criterion of decision: species richness, endemicity, and the threat status of species inside the proposed areas (Figueredo Cardona & Acosta Cantillo, 2008; Reyes Gutiérrez, 2006; TNC, 2003) are a priori criteria for deciding whether a place is worth protecting. However, when resources for conservation are limited, the definition of biodiversity indicators (i.e. populations of species whose eco-physiological conditions, population sizes, population trends, and genetic variability allow predictions for their ecosystems) is also crucial (D. J. Rapport, 1992). The best trade-offs between ecosystem conservation and human well-being should be inferred from the best trade-offs between reliable information and the amount of investment to obtain them.

Amphibians are excellent biodiversity indicators and preferred conservation targets. Their sensitivity to environmental changes (Bielby et al., 2008; Collins & Storfer, 2003; Crawford, Lips, & Bermingham, 2010) and their relationship to local or indigenous communities, either as food source (Bonilla González, 2015) or as an important part of their traditional knowledge (Sánchez Núñez, 2006), make them desirable as conservation objects based on community conservation strategies. However, the continuously changing taxonomy of amphibians often represents a serious caveat for the delimitation of protected areas (Swenson et al., 2012), especially in megadiverse regions. In Andean South America, for instance, extensively detailed and systematic taxonomic inventories are lacking. Many putatively widely distributed lowland species are actually complexes of cryptic species, which makes it difficult to properly define conservation areas or to design accurate and effective management strategies (Angulo & Icochea, 2010; Bickford et al., 2007).

The genus *Scinax* Wagler, 1830 is one of the most common, conspicuous, and cryptic genera of tree-frogs of the amphibian fauna of the lowlands (below 1000 masl) throughout tropical America (Ferrão et al., 2016; Fouquet, Vences, et al., 2007; Menezes et al., 2016). *Scinax* taxonomy has been a matter of research since 2002 (Faivovich, 2002), but the genus is also a subject of constant revisions. Since the taxonomic Möbius ring of Duellman *et al*., (2016) and the subsequent precisions on the *Scinax uruguayus* and *S. catharinae* clades (Faivovich et al., 2018; Lourenço et al., 2016, respectively), the genus currently comprises around 120 described species (Frost, 2021). However, it may include more than 300 species (Faivovich J, pers. comm., 2020).

Although *Scinax* tree frogs are common and not attention worthy, they constitute an important food source and pest control agent (Astwood-Romero et al., 2016; Muñoz-Guerrero, Serrano, & Ramírez-Pinilla, 2007). Several species of the genus produce anti-microbial peptides (AMPs) which make them a potential source of antibiotics useful to human populations (Groot et al., 2012). A large proportion of lowland *Scinax* species are explosive breeders in ponds or swamps, but they are almost impossible to detect outside the reproductive season. At the beginning of the rainy season they are literally everywhere (Corzo et al., 2010), which makes them normally overlooked in terms of conservation management because they are regarded as weedy species. Each species seems to be intimately related to a single habitat type, at least concerning forested/non-forested (open) areas (Ferrão, de Fraga, Moravec, Kaefer, & Lima, 2018; Román-Palacios et al., 2016).

The *Scinax ruber* group was defined by Faivovich (2002) to include all the species of Scinax that neither could be included in the *S. catharinae* group, nor belonged with any previously recognized species group. Faivovich (*ibidem*) acknowledged that the "*Scinax ruber* group" was not monophyletic but contained at least one monophyletic group (the *S. rostratus* clade) and several non-monophyletic groups, including the *S. staufferi* and *S. perpusillus* groups. Faivovich et al. (2005, p. 80, Figure 11) reformulated the *S. ruber* clade and identified *Scinax uruguayus* as its sister taxon, also recovered that way by Ron *et al.*, (2018). Duellman *et al.* (2016) used *S. uruguayus* plus "*Hyla pinima*" (as *Scinax*) to erect the genus *Julianus*, but that proposal has no phylogenetic support! (see above). All the *Scinax* of the *S. catharinae* group were transferred to *Ololygon*. But both changes were reversed later and changed again by Julián Faivovich and colleagues (Araujo-Vieira et al., 2019; Lourenço et al., 2016).

Based on those studies, the following can be concluded: 1) "*Scinax ruber*" is a species complex and it is not monophyletic; 2) the intrageneric relationships of *Scinax* are neither properly described nor clear; and 3) every publication after 1993 emphasizes the latter, but few studies contribute to solving the confusion (Fouquet *et al.*, 2007 is an exception).

After Faivovich (2005) no later publication includes extensive sampling of the widely distributed "*S. ruber*" from the Andean-Amazonian countries (Bolivia, Colombia, Ecuador, Perú and Venezuela). Following the influential monograph by Duellman & Wiens (1993), small hylids of approximately 30 mm snout-vent length from South American lowlands are simply identified as *S. ruber* when they have a rounded or acutely rounded snout, reduced/absent webbing between toes I and II, yellow or orange concealed surfaces of thighs, and groins marked with dark spots, freckles or caterpillar-like patterns, and no dark "X" dorsal markings.

Vacher et al. (2020) recently published a large phylogeny of Amazonian and Guianan frogs using 16S RNA sequences, which confirms that the diversity of many Amazonian genera is vastly underestimated (but see Chan et al., 2020 for a critical stance with regard to this approach). Unfortunately, their taxonomic analysis is rather superficial, and their mapping/modelling scale is too coarse compared to the size of the habitats and the acknowledged crypticity (see Discussion below). Also, their "Guiana province" definition is well beyond the general understanding of Guianan ecosystems: Vacher et al. (2020) include the northwestern Amazon (Leticia), and the northern Llanos of Colombia in their Guiana province. Both regions represent a mixture of Andean piedmont, northern Amazonia, and the Caribbean coastal plains and have been extensively separated from the Amazonian and Guianan domains/provinces/subregions (see Morrone, 2014 for a summary and comparisons of classifications).

Another explanation for the weak representation of "*S. ruber*" in the recent research efforts of Andean-Amazonian countries could be the Andean Decision 391 (Comunidad Andina de Naciones - CAN, 1996). Thanks to that law, the above cited countries suffered until at least 2014 (some of them still suffer) from several delays and normative difficulties in sorting out their legislations in order to protect the biodiversity knowledge and the access to indigenous genetic resources while allowing local research. Even if the legislation is intended to allow the use of molecular approaches for taxonomic research, several bio-political battles had to be fought during the first two decades of the 21st century to admit the use of molecular evidence for biodiversity assessments.

In the present study we used integrative taxonomy to disentangle the cryptic diversity of "*Scinax ruber*" in the northern South America, particularly inside Colombia. In doing so, we apply phylogenetic, phylogeographic, and species delimitation analyses based on mitochondrial and nuclear DNA sequences; we analysed distributional patterns, habitat associations, and examined the morphology and morphometry of museum specimens. We discussed taxonomic characters used so far within the group to describe the cryptic diversity found inside our geographic coverage and sampling. We also proposed phylogeographic inferences indicating patterns of diversification, and we highlighted some insights regarding the conservation of that species complex in the current political and economic framework of the region.

1.3 Materials and Methods

1.3.1 Study area

The lowlands of northern South America for this research include the following: northwestern Amazon (extreme northern Bolivia, northwest Brazil, Colombia, Ecuador and Peru), the Biogeographic Chocó of Colombia, the Caribbean coast of Colombia and Venezuela, the Colombian-Venezuelan Orinoquia, the area of the Guiana shield (middle-eastern tip of Colombia, northwestern Brazil, western Guyana, French Guiana, Suriname and southwestern Venezuela according to Giraldo-Cañas, 2014), Trinidad and Tobago, and the inter-Andean valleys of the Cauca and Magdalena rivers in Colombia, approximately north of 13º south (-13.00) in latitude and west of 60º W in longitude. This study area is nearly equal to Müller’s (1973) dispersal centres Nos. 8-22 and the north-western part of his No. 25 (as modified by Morrone, 2014 in his Figure 8). Colombia is the only country in the region containing portions of nearly all these areas. Thus, the present work focused on the continental territory of Colombia including trans-Andean and cis-Andean savannas and forests below 1000 masl. Those areas correspond geographically and biologically to the biogeographical provinces III, V, VI, VII and VIII of Hernández-Camacho *et al.* (1992).

1.3.2 Data collection

The wide distributional range of what is currently understood as “*Scinax ruber*” is illustrated in **Figure 1-1**. It incorporates the IUCN distributional map (solid blue) according to the 2020 species fact-sheet of *Scinax ruber* (Solís et al., 2010) and the point layer of filtered and cleaned occurrence records of the Global Biodiversity Information Facility (GBIF, 2020) data portal (purple dots). In order to inspect coincidences between the IUCN map and the publicly available records of the species, we first downloaded and reviewed the raw collection data available through GBIF. Next, we built a “*Scinax ruber*” occurrence database containing nearly 5000 records from 17 countries in America (all georeferenced). We downloaded, filtered, and cleaned the database to one record per locality and eliminated the untrusted records according to the “Present” filter in the GBIF portal query and our actual knowledge of the group and we overlapped the resulting layers (**Figure 1-1**). Afterwards, we contacted biological collections in Colombia, Brazil, Ecuador, Germany, Peru, and Spain which could host and share specimens or tissues with us. We obtained 80 tissue samples mainly from museum specimens (only 5 without a collection voucher) in seven collections of Colombia, Germany, and Spain. We included in our research the available tissues and/or specimens identified as *S. chiquitanus, S. funereus, S. ictericus, S. iquitorum, Scinax oreites*, *Scinax ruber*, *S.* aff. *ruber*, *S.* cf. *ruber*, “*S. ruber*-like” or *S. x-signatus* (**Table S 1-1**).

Figure 1-1: Comparison of the distribution of “*Scinax ruber*” sensu lato according to the International Union for Conservation of Nature, IUCN (grey polygon), and the occurrence records provided through the Global Biodiversity Information Facility (GBIF). Points represent collection localities of occurrences databased under “*Scinax ruber*”. Map sources: IUCN, Conservation International and NatureServe (2008).

1.3.3 Molecular data

All tissue samples were processed for obtaining sequences of three mitochondrial (12S, 16S, COI) and two nuclear (Tyr, Rho) genes, unless specified otherwise (**Table S1-1**). The majority of the Colombian tissues and specimens were obtained from the “Banco de ADN y Tejidos de la Biodiversidad Colombiana” [Biodiversity Tissues Bank] (BTBC) of the Instituto de Genética (IGUN), the Colección de Anfibios [Amphibian Collection] of the Instituto de Ciencias Naturales (ICN-Anfibios) of the Universidad Nacional de Colombia, the Colección Zoológica de Prácticas de Docencia of Universidad del Valle (CPZ-UV), and the Tissue Collection at Biomics Lab, Universidad de los Andes, Colombia. Most of the Peruvian and Bolivian tissues were shared by the Museo Nacional de Historia Natural (MNHN) of Madrid, Spain, and the Zoologische Staatssammlung München (ZSM), Germany. Tissues from Brazil, French Guiana, Peru, Tobago and Surinam (topotypic) were provided by the Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, Germany (MTD), the Naturkundemuseum Stuttgart [State Museum of Natural History Stuttgart] (SMNS) and the ZSM, Germany. Twenty-three additional GenBank sequences with similar identities as well as four outgroup samples (see **Table S1-1** for details) were used to strengthen the geographical sampling.

DNA extractions of the tissues from the European collections were performed in the Molecular Genetics Laboratory of the MTD using the innuPREP DNA Mini Kit of Analytics Jenna ™. DNA extractions for the Colombian samples were performed with equivalent kits (Smobio™ and ThermoScientific™ in the Molecular Ecology Lab of the Instituto de Genética, Universidad Nacional de Colombia. All extractions were PCR-amplified using the primers L25195 fwd and H2916 rev for the 12S fragment (Vences et al., 2000), 16S A and 16S B for 16S fragment (Vences, Thomas, van der Meijden, Chiari, & Vieites, 2005), dgLCO1490 and dgHCO2198 for the COI gene fragment (Vences et al., 2005), Rho1A and Rho1C primers (Bossuyt & Milinkovitch, 2000) for the nuclear Rho gene fragment, and TyrC and TyrG for the nuclear Tyr gene fragment (Bossuyt & Milinkovitch, 2000). PCRs were performed using an initial denaturation step at 95°C for 5 min, followed by 37 cycles of denaturation at 95ºC for 30 sec, annealing at 58ºC for 1 min and extension at 72ºC for 1 min and a final extension step at 72ºC for 8 min. All PCR products were cleaned with the ExoSap-IT PCR product cleaning agent (Thermo Fisher Scientific ™) and sequenced in the respective sequencing facilities of the Senckenberg Molecular Genetics lab and the Servicio de Secuenciación y Análisis Molecular [Sequencing and Molecular Analysis Service] - SSIGMOL of the Instituto de Genética, Universidad Nacional de Colombia.

1.3.4 Morphological and morphometric data

The morphological and morphometric data were obtained by direct examination of the voucher specimens of the sequenced material, when available, plus 146 additional specimens of the above-mentioned collections. In addition, digital photographs of the syntypes of *Scytopis alleni* were examined from the Museum of Comparative Zoology (MCZ) and the Academy of Natural Sciences of Drexel University (ANSP). After filtering for collection localities (up to five specimens/locality were considered) and maturity (only adults were considered for the morphometric analysis), the morphometric database contained data of 140 specimens (58 females, 73 males and 9 undetermined) of which 131 were selected for the calculations (**Table S1-2**) based on the state of preservation.

The following standard measurements (following Watters *et al.*, 2016) were taken to the nearest 0.05 mm (**Table S1-2**): eye diameter (ED), eye-snout distance (ES), head length (HL), head width (HW), inter-orbital distance (IOD), snout-vent length (SVL), maximum tympanum diameter (TD), and tibia length (TL). For every specimen the following characters were recorded: presence and shape of pattern on the hidden (anterior and posterior) surfaces of thighs and groin; shape of snout (rounded, acutely rounded, protruding or pointed, truncated); and sex/age category (female = adult female, male = adult male, juv = juvenile, indet = specimen inspected, but sex not recorded or identified, not indicated = specimen not inspected and sex not reported in the database; **Table S1-2**). Sex-age categories were determined by gonad inspection when allowed; in case of unopened specimens, the adult males were identified by the presence of opened vocal slits, and the females by the absence of opened vocal slits and/or large sizes and eggs/oviducts visible through the body wall. Each specimen was also assigned to a georeferenced sampling site when coordinates were available, or a pair of coordinates was approximated based on the locality data after a Google Maps™ search. For the search, we visually estimated the central point and the radius of the circle including the polygon referred to the closest depicted toponym.

1.3.5 Phylogenetic and phylogeographic inferences

All sequences were edited with BioEdit (Hall, 2005) and aligned iteratively with the ClustalW algorithm as implemented in BioEdit, Muscle as implemented in MegaX (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) and adjusted by visual inspection. The protein-coding DNA fragments were checked for the correct reading framework through the ExPASy web portal (Artimo et al., 2012). The best combination of partition scheme and substitution models for the complete evidence and mitochondrial and nuclear alignments was obtained using PartitionFinder (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2017) under the corrected Akaike Information Criterion (AICc; Table S3). Phylogenetic relationships were inferred for a data set of 99 sequences of "*Scinax ruber*" and allies plus three outgroups (*Pseudis paradoxa*, *Scinax boulengeri* and *S. uruguayus*; **Table S1-1**). We then ran an exploratory Maximum Likelihood (ML) analysis with RAxML 8 (Stamatakis, 2014) followed by a Bayesian extensive analysis as implemented by MrBayes 3.2 (Ronquist et al., 2012). We tested multiple outgroups iteratively as proposed by Grant (2019) until finding the actual combination.

MrBayes was run using the following settings: for the complete evidence analysis, 30 million generations, 4 chains, burn-in 25%, sampling every 2000 generations, and printing every 1000 generations. For the mitochondrial analysis we ran 15 million generations, while for the nuclear evidence we ran 25 million generations (same settings as for the complete evidence). Convergence was checked in the average standard deviation of split frequencies (<0.01) with the minimum and average estimated sample size (ESS) provided by the program, and graphically with the plots of the generation (x-axis) versus the log probability of observing the data (y-axis). The trees resulting from the analyses were edited using FigTree (Rambaut, 2018), Power Point ™and Inkscape (GPL license, version 0.92.5) at www.inkscape.org.

1.3.6 Haplotype network analyses

Nuclear alignments were phased with the phase algorithm as implemented in DNAsp (Rozas et al., 2017), and the phased haplotypes were used to build statistical parsimony networks as implemented in TCS (Clement, Posada, & Crandall, 2000) and PopArt (Leigh & Briant, 2015). The final edition of the network and labelling were performed using Inkscape (GPL license, version 0.92.5) at www.inkscape.org. The criterion used by Rakotoarison *et al.* (2017) of coherence between 16S *p*-distances higher than 4% and at least one unique nuclear haplotype to consider any evolutionary lineage as a full species was acknowledged but modified, given that several taxonomic revisions during the last decade have shown that in Amazonian frogs the *p*-distance threshold for mitochondrial fragments may be lower (below 2%, M. Caminer & Ron, 2014; S. Ron *et al.*, 2012; J.-P. Vacher *et al.*, 2020) and that both nuclear fragments might be subject to adaptive mutations (Larmuseau, Huyse, Vancampenhout, Van Houdt, & Volckaert, 2010; Yokoyama, 2000). That means that a combined interpretation of the nuclear fragments selected would be mandatory: the tyrosinase (Tyr) contributes to determining the disposition of melanin that forms the external colour pattern and the rhodopsin (Rho) is involved in the way that frogs perceive colour –or a given pattern. Hence, we propose that a threshold above 1.5% and in concordance with the presence of unique haplotypes in both nuclear fragments for the same samples indicated divergent lineages with distinct evolutionary histories.

1.3.7 Species delimitation

Based on the complete evidence phylogram resulting from our Bayesian analysis (mitochondrial plus nuclear fragments, 2376 bp) and the recorded distributions (**Table S1-2**), we defined Operational Taxonomic Units (OTUs) and assigned every individual measured to one OTU according to its collection site. Afterwards, we performed an exploratory analysis of the morphometric data by calculating boxplots and scatterplots in RStudio (RStudio Team, 2019). A PCA of selected morphometric variables (SVL, TL, HL, HW, TD, ES, IOD) was carried out to check the morphometric assignment of individuals to any of the genetically confirmed populations and to visualize the significance of evident morphological differences. The graphic analyses and the PCAs were calculated and plotted using the R packages "ggbiplot" (Vu, 2011), "lattice" (Sarkar, 2008), and "vegan" (Oksanen et al., 2019).

The barcode gap for 16S, a standard barcode marker for frogs (Vences et al., 2005), was explored using the ABGD algorithm (Puillandre, Lambert, Brouillet, & Achaz, 2012) and the ML approach of mPTP (Kapli et al., 2016). To compare the assignments made by the two algorithms, within-group and between-group distance matrices were calculated for the 16S fragment of all the sequences where it was available using Mega X (Kumar et al., 2018). Consequently, every individualized linage was assigned to one of these three categories, following Vieites *et al*. (2009):

1. **Confirmed Candidate Species (CCS)**: We assigned pairwise genetic divergence for all other described species > 1.5% in 16S RNA, unique nuclear

haplotypes and concordant ecological, morphometric, morphological and/or distributional differences. Each CCS was identified by the name *Scinax* followed by an Arabic number or a capital letter in the phylogeny, indicating a newly identified independent evolutionary lineage of *Scinax*. Given that the combination was not a scientific name, it was not written in italics.

2. **Deep conspecific lineages (DCL)**: pairwise genetic divergence to all other described species > 1.5% in 16S RNA, but no evident morphological differences or differences known to vary interspecifically (e.g. bones colour).
3. **Unconfirmed candidate species (UCS):** pairwise genetic divergence to all other described species > 1.5% in 16S RNA gene, but bioacoustic, ecological or morphological/ morphometric information not available. This was also used for lineages with genetic divergences below the threshold, but with pronounced morphological and morphometric differences. Each UCS is identified in the phylogeny by the letter U followed by specific letters referring to any characteristic of the lineage.

1.3.8 Distribution modelling

Species distribution models (SDM) were calculated for each of the identified CCS with more than five records. GBIF records were tentatively assigned to those taxonomic units when the records fell geographically within genetically verified records. A final record database of 1145 georeferenced records from Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Tobago, and Venezuela was used to feed the SDM. The models were built with MaxENT (Phillips, Dudík, & Schapire, 2020) and the geographic management infrastructure of ArcGIS (ESRI, 2012) following the approach described in Vargas-Ramírez *et al.* (2016).

1.3.9 Integrative approach

Finally, the definition and diagnosis of candidate species was performed by integrating the results from the Bayesian phylogram of complete molecular evidence, phylogeographic evidence, and the inferences of the barcode gap algorithms with (i) genetic distances, (ii) morphometry, (iii) the morphological assessment, (iv) inferences derived from the SDMs and, in particular, the thermal physiology data obtained during and (v) the life-history characteristics available from the literature, field notes or our own experience.

1.4 Results

1.4.1 Phylogenetic and phylogeographic analyses

The Bayesian phylogeny and the corresponding distribution map of the referred lineages are presented in **Figure 1-2** and **Figure 1-3**, respectively. The final Bayesian phylogeny contains 10 well-supported major clades –posterior probabilities (PP) = 0.9 or above, composed as follows (top to bottom in **Figure 1-2**):

Open lowlands clade: This clade is comprised of two reciprocally monophyletic subclades, lineages **Scinax1** and **ScinaxX**, plus the GenBank sequence KU359530.1 (COI, voucher specimen UFMG347) identified as *Scinax x-signatus* from Minas Gerais, Brazil. That sample is sister of both open-lowland CCSs (PP = 1, bootstrap = 99 %, **Table S1-3**) and will be referred to as **UA** from this point. ScinaxX inhabits the Western slope of the Cordillera Oriental of Colombia in the Middle Magdalena Valley (MMV) while Scinax1 lives on the Eastern slope of the mountain, but only in the Northern Orinoquia section. Both reciprocally monophyletic subclades were robustly supported (PP = 1, bootstrap = 100%), even though they were separated by an uncorrected p- distance in the 16S fragment of only 1.6% (**Table S1-3**). Their morphology was characteristic of non-forested areas (see below), but morphological differences were slightly noticeable from one slope of the Andes to the other, apart from the size and position of the vocal sac in males. Both nuclear haplotype networks (**Figures 1-4** and **1-5**) showed unique haplotypes for the samples JPH 1016 (ScinaxX) and AFAG 440 (Scinax1), but they still showed a mixture of haplotypes from both versants of the Cordillera Oriental of Colombia.

Forest lowlands clade: This large clade contained eight subclades of cis-Andean and trans-Andean elements, apparently linked by forest ecosystems (gallery forest and mature forest in a tropical savanna matrix). From top to bottom, the Forest lowlands clade is comprised of:

- **Alto Manacacías subclade:** it is represented by the sample AFAG 429 from the Eastern piedmont of the Cordillera Oriental in Arauca, Colombia. It was the basal sister group of all the remaining members of the clade (PP = 1, bootstrap 95%), and will be referred from now on as **Scinax2**. Its uncorrected *p*-distance to the members of the Open Lowlands clade ranged from 9.2% to 9.6%, while distances to the members of the Eastern cis-Andean lowlands clades (Amazonian slope of the Andes and Guiana region) ranged from 1% to 11.9%. The macro-sympatric Orinoquia clades (lineages Scinax5, Scinax6 and Scinax6C) were separated by *p*-distances as small as 1.6%. Both nuclear haplotype networks showed an incomplete segregation of haplotypes for this OTU (**Figures 1-4** and **1-5**) but the voucher specimen of AFAG 429 was not available for a more extensive description.

- **Trans-Andean Forest subclade**: contained a single well-supported OTU, the monophyletic (PP = 1, bootstrap =100%) and morphologically distinguishable **Scinax3** lineage. Its morphology was clearly of the forest, thus, we anticipated that it was distributed also in the northern Chocoan lowlands. The uncorrected *p*-distance within this clade was 0.3%, and the distances between Scinax3 and the OTUs inside its sister Guianan-Amazonian subclade (PP = 0.95) ranged from 8% (to Scinax4) to 11.7% (members of the UR). There were several connected unique haplotypes in the samples of this clade for Tyr (**Figure 1-5**) and only one unique haplotype in the Rhodopsin (Rho) network (**Figure 1-4**). The sample MAV 326 contained unique haplotypes in both networks.

- The well-supported **Guianan subclade** (PP = 1, bootstrap = 87%), below, was the sister group of the Amazon jungle subclade (PP = 0.73). The former contained a single sample of the topotypic *Scinax ruber s.s.*, from Suriname, and its closest relative from Tobago (MTD-TD 7518 and ZSM 1607/2006, respectively; PP = 0.88, bootstrap = 60%). Despite the minimal uncorrected *p*-distance in the 16S fragment (below 0.5%) within the entire Guiana clade, there were unique haplotypes in both nuclear networks for the samples MTD-TD 8753 and 8766, both from Iwokrama Forest, Guiana, as well as unique haplotypes for the Suriname sample in Rho. The diversity of haplotypes inside **UG** seemed to be proportional to the number of samples, and in the Rho network all the haplotypes inside the OTU varied largely in the number of mutational steps and connections to other haplogroups.

Considering that ABGD and mPTP barcoding algorithms recovered a single unit for all the Guianan samples, and that there were no noticeable morphological differences between most of the samples, we were not able to restrict *Scinax ruber s.s*. to Suriname and Tobago at this point. However, we considered it very likely that the nominal species was, in fact, possibly endemic to the former, and that the still not disentangled crypticity required a more extensive genetic and morphological sampling.

Figure 1-2: BI phylogram of complete molecular evidence (three mitochondrial plus two nuclear fragments), and species delimitation according to ABGD and mPTP. A: Part I. B. Part II. C. Part III. Node values are posterior probabilities. Outgroups (Scinax boulengeri MVZ 207215 from Costa Rica, Scinax pinimus CFBH 5788 BR, Pseudis paradoxa MACN 38642 and MNRJ 33859) not shown.

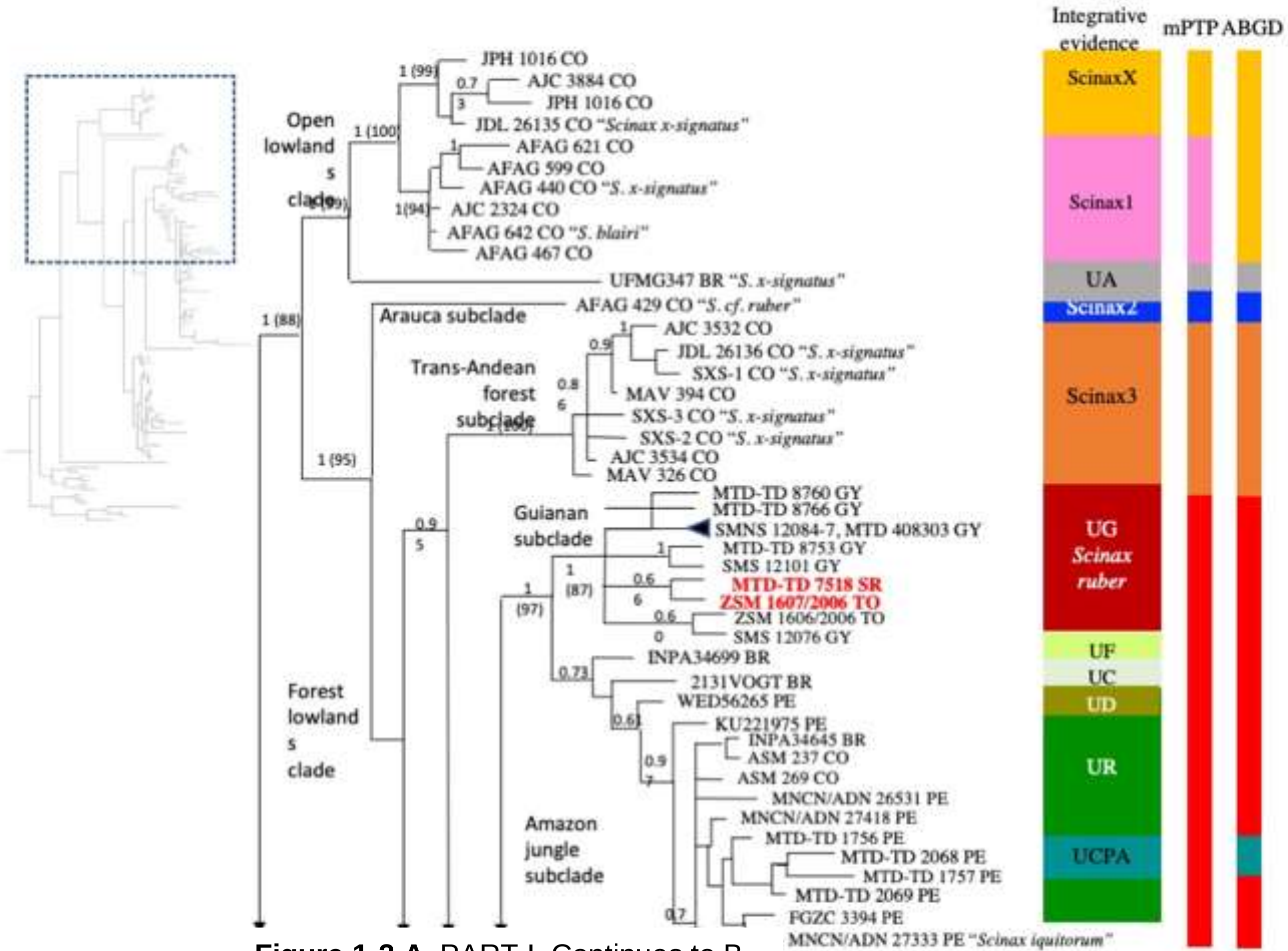
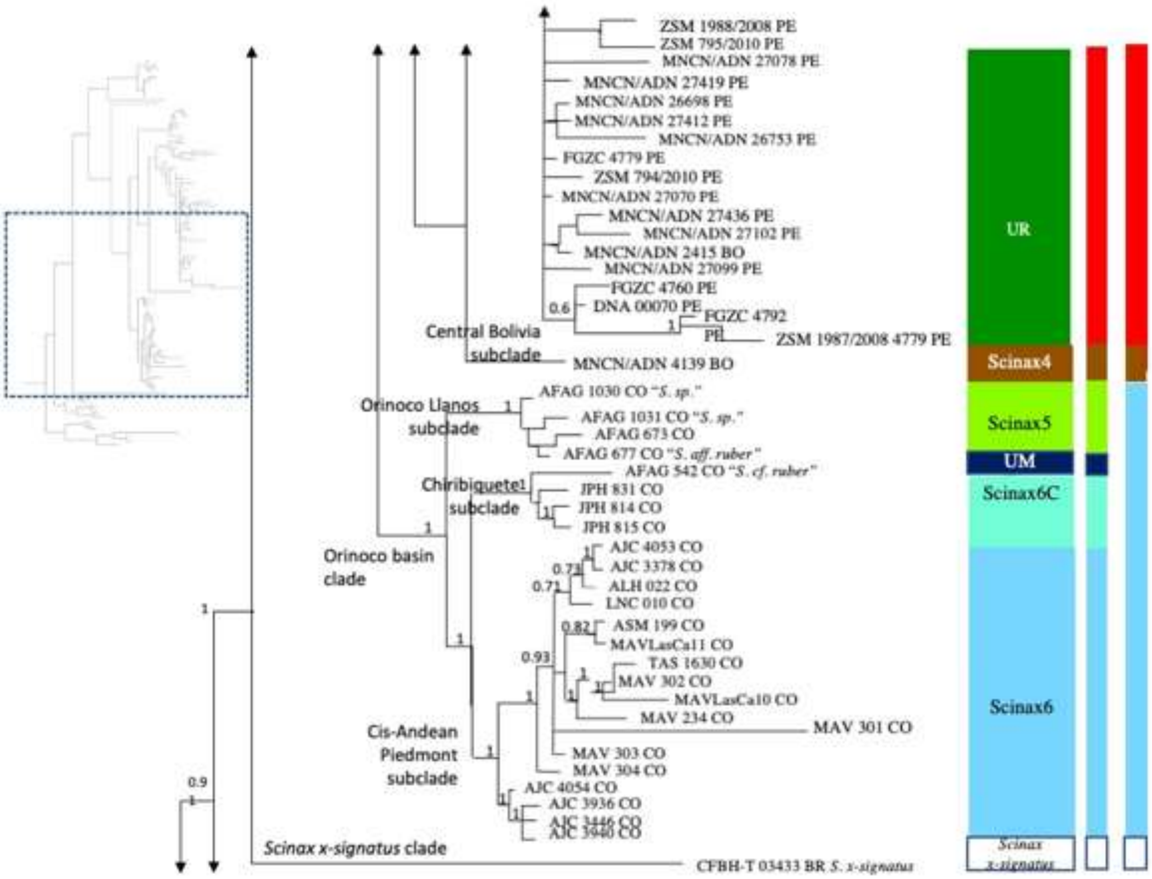


Figure 1-2 A. PART I. Continues to B

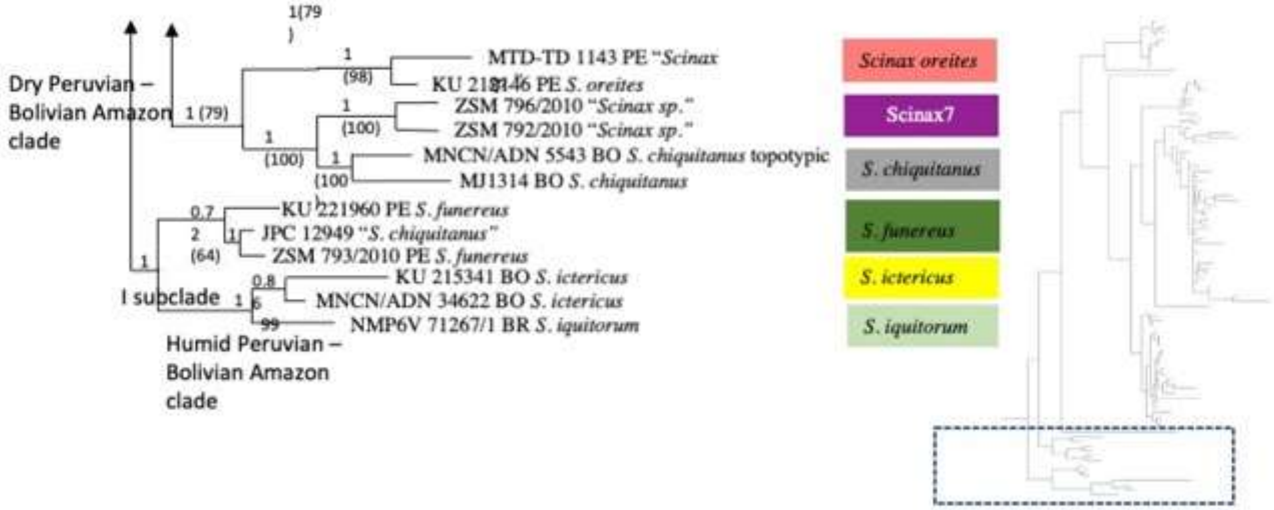
- The subclade below that Guianan subclade is the **Amazon jungle subclade**. It is composed of three nested lineages plus a large polytomy of samples from the cis-Andean lowland forests of Bolivia, Brazil, Colombia and Perú (Eastern slope). The sister taxon of the strict polytomy (referred herein as **UR**) was the GenBank sequence AY326034.1 (WED 56265, 12S and 16S) from the Northern Perú, which is referred to as **UD** here –because it was collected by W. Duellman. In turn, the sister taxon of the group formed by UR plus UD was named **UC**, and it contains a single sample with three GenBank sequences: JN690793.1 12S, JN691399.1 16S and JN692122.1 Tyr. It was collected near the Madeira river (Brazil), one of the expeditions to South America during the ith a previously described taxon (the uncorrected *p*-distance between this lineage and the UR clade is 3.4%, **Table S1-3**). However, none of us reviewed the specimen, so it remains as an UCS until new evidence becomes available.

Figure 1-2 B. PART II. Continues to C



The sister taxon of all the remaining entities (UC+UD+UR) in the clade was **UF** (GenBank sequence KU317399 16S, specimen INPA-H 34699), named from Ferrao’s definition (Ferrão et al., 2016), who regarded it as a candidate species. The uncorrected *p*-distance between this lineage and the immediately more internal taxon (UC) was 1.8%, while to the largest Amazonian clade of the phylogram (UR) it was 3.0% (**Table S1-3**). With that evidence at hand, it was impossible to decide on whether the sequence corresponded to a morphologically distinct population or lineage, or to one of the morphs of the populations examined and measured inside UR.

Figure 1-2 C. PART III. Bottom of the figure.



- Inside the **UR** there were four samples from the Central Peruvian Amazon (**UCPA**) nested in the polytomy, which were recovered together by the barcode gap algorithms as a different entity. There was one more sample from the northwestern Amazonia (GenBank sequence KU317404.1 from Cusco Amazónico), which was nested inside the strict polytomy with some Leticia-Iquitos samples. The mean uncorrected *p*-distance within UR and within UCPA was large (1.4% each), while the distance between both clades was 1.8%. When UCPA-**1** and UCPA**-2** are studied as different units (according to the phylogram, sample MTD-TD 1756 could be the sister clade of MTD-TD 1757 and MTD-TD 2068 plus MTD-TD 2069), the uncorrected *p*-

distance between them was 4.4%, leading to the conclusion that those groups were distinct evolutionary lineages. That was also indicated by the distribution of Rho haplotypes in the network: MTD samples were distributed in two different OTUs (**Figure 1-4**, blue-green circles), MTD-TD 1757 was completely separated from the UR haplotypes (14 mutational steps to the node) and from MTD-TD 2069. In the Tyr network the haplotypes of the MTD samples were shared with individuals of the UR *sensu stricto,* except from MTD-TD 1757 which was separated by a single mutational step.

- The last subclade within this "Forest lowlands" clade was represented by the **Central Bolivia subclade and sample** (MNCN/ADN 2415 from Bolivia, Chalalán). The uncorrected *p*-distance of this sample/clade to the remaining members of the clade ranged from 5.5% (the UR clade) to 9.0 % (to UCPA-2, **Table S1-3**, **Figures 1-2** and **1-3**, respectively). It contained unique haplotypes in the Rho network (Tyr sequence not available) and will be referred as **Scinax4** from now on.

Figure 1-3: Geographic distribution of the clades depicted in Figure 1-2 plus three other *Scinax* lineages and species to which the former clades have been related. See conventions on the map. Colour code follows the one in the phylogeny of Figure 1-2.

The Amazon jungle subclade was reciprocally monophyletic to the **Orinoco basin** clade. It contained three monophyletic subclades distributed in the more open forest/savanna mixed areas of the Andean piedmont, including the Colombian-Venezuelan llanos, and the southernmost ecotone between Llanos and the Amazon forest inside the Chiribiquete region of Colombia. From north-east to south-west, it comprised the following subclades:

- The monophyletic **Orinoco Llanos subclade** (PP = 1, bootstrap < 60%), comprised four samples (AFAG 673, AFAG 677 and AFAG 1030-1) from the north-eastern border of Colombia (Vichada, Cumaribo) at the Western Guiana shield (**Figure 1-3**). It will be referred to here as **Scinax5**. This clade was reciprocally monophyletic to the Orinoquia piedmont subclades, Scinax6 and Scinax6C, to which it was separated by uncorrected *p*-distances of 1.2% and 2.2%, respectively. The uncorrected *p*-distance within the clade was 0.3%. This OTU was also well-identifiable in both nuclear haplotype networks (**Figures 1-4** and **1-5**, lime green circles), with unique haplotypes for sample AFAG-673.

- **Chiribiquete subclade**: it contained samples from Serranía de Chiribiquete National Natural Park (NNP) - Mixed World Heritage Site, and from San Martín (Meta, Colombia) at the base of the Serranía de la Macarena –another nationally protected area. The mitochondrial barcode gap calculated with mPTP, as same as the nuclear haplotype networks (**Figures 1-4** and **1-5**, lime green circles) supported the splitting of this subclade into two independent evolutionary lineages: **UM**, represented by the sample AFAG 542 from San Martín, Meta, and **Scinax6C**, restricted to the Serranía de Chiribiquete NNP in the extreme northwestern Amazon basin around the Equator, at the limit between the Llanos and Amazon forests. Both nuclear haplotype networks showed unique haplotypes for JPH 831, while AFAG 542 (UM) contained a well-separated haplotype for Rho that was not nested neither in the Scinax6 nor in the Scinax6C groups.

- **Cis-Andean Piedmont subclade**: The members of this subclade occurred along the Orinoquian piedmont of Colombia and seemed to have no special preference for a forest/non-forest habitat on the geographic scale. Nonetheless, their morphology is closer to the "forest" type. Despite the various localities with tissue samples and collection vouchers, the topology and the haplotype networks revealed it as a compact group of populations (uncorrected *p*-distance in the 16S fragment within the clade = 0.3%) in which samples MAV 304 and ASM 199 showed unique haplotypes for both nuclear fragments. Nonetheless, two individuals from San Martín, Meta (IGUN-MAV 234 and IGUN-MAV LasCa 11) perhaps represented a different and distant lineage: There was only one nuclear sequence for each of them (Rho and Tyr, respectively) so it was not possible to check for coincidences between the networks. However, neither haplotype was connected to other haplotypes of the given group.

The sister species in our sampling of all the clades described above (which we call the actual *Scinax ruber*-like clade) was *Scinax x-signatus* topotypic, from Bahia, Brazil (GenBank sequences No. KU495577.1 16S and KU494784.1 COI; **Figure 1-3** blank square). Its uncorrected *p*-distance to any of the *S. ruber*-like OTUs was huge (ranging from 12.8% to 20.6%, **Table S1-3**), being Scinax1 and UCPA-2 the inferior and superior limits of the range.

Outside the "*Scinax ruber*-like" section of the phylogeny, there were two clades representing contrasting biogeographic units: the Dry and the Humid Peruvian-Bolivian Amazon clades. The former clade comprised three monophyletic internal subclades, which morphologies should appear *Scinax ruber*-like (i.e. patterned posterior thighs) based on their diagnoses, but their distributions perhaps represented drier habitats (W. E. Duellman & Wiens, 1993). Neither was in fact a member of the *Scinax ruber*-like group, provided that the uncorrected *p*-distances to external clades ranged from 11.2 % (*S. chiquitanus* to UG) to 19.5% (*S. chiquitanus* to UD, **Table 1-3**). The **Dry Peruvian-Bolivian Amazon clade** comprised three subclades:

- ***Scinax oreites* subclade**: It was composed of two samples: MTD-TD 1143 from Perú, Huánuco, and its sister taxon within this sampling, *Scinax oreites* (GenBank sequence GQ896260.1, of solely 12S). Distances within the clade could not be calculated, and the mean uncorrected *p*-distances between MTD-TD 1143 and its closest clades (Scinax7, *Scinax chiquitanus* and *Scinax funereus*) were 9.5%, 9% and 10.7%, respectively.

- **Scinax7 subclade**: This comprised two sympatric samples (ZSM 792/2010 and 796/2010) from Estación Biológica Panguana, Huánuco, Perú. The voucher specimens of these samples were not reviewed in the ZSM Museum but three other samples from the same locality were (ZSM 1988/2008, ZSM 794/2010 and ZSM 289/2016), the former was collected in the same collection event. This clade showed a large within-group uncorrected p-distance (2.2%); still below the 3% threshold proposed for anurans but large enough to be considered different lineages in this study. The uncorrected *p*-distance between Scinax7 and its sister clade *Scinax chiquitanus* was 6.1%, while distances to the members of the *Scinax ruber*-like clade ranged from 11.1% to 16.2% (Scinax2 and *Scinax x-signatus*, as the lower and higher limits). In the Tyr haplotype network, the sample ZSM 796/2010 had a unique and independent haplotype, but no Rho sequences were available. With that evidence it was not possible to identify whether the other tissue sample of the clade (ZSM 792/2010) was effectively conspecific.

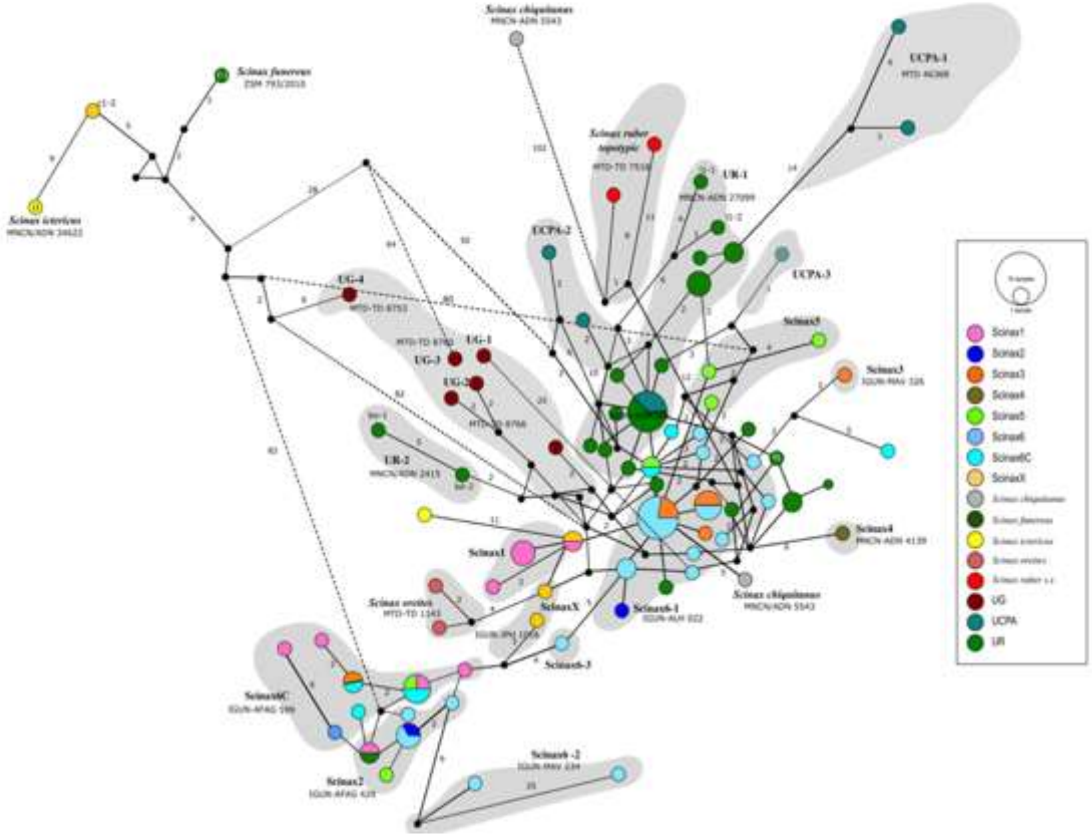
- ***Scinax chiquitanus* subclade**: This small clade was composed of nearly topotypical samples of *S. chiquitanus* (MNCN:H 43718 plus the GenBank sequence JF789945.1 16S). There was no orange/brown/black pattern on the posterior surfaces of thighs of this species (**Figure 1-17**), even though its dorsal coloration was very similar to members of the UR lineage from the western Amazonas department of Colombia. The mean uncorrected p-distance within this clade was 1.3% (quite large for the group), while the distance between this species and the Amazonian *Scinax ruber*-like group (UR) was 12.2%. The Rho haplotype network showed a *S. chiquitanus* unique and completely separated (120 mutational steps) haplotype.

Lastly, the phylogenetic analyses revealed the **Peruvian-Bolivian Amazon South clade** as the sister group of all the other clades mentioned above, and the southernmost one. The reviewed specimens in this clade lacked patterns on the posterior surfaces of thighs, however the individual ZSM 793/2010 had spotted flanks and/or groins (light and black). The clade comprised two reciprocally monophyletic, though fairly supported, subclades: *S. funereus* subclade (PP = 0.72) and I subclade (PP = 1) under the Bayesian inference. The ML topology was slightly different at this point, because S. ictericus was the sister species of the clade comprised of *Scinax funereus* + *S. iquitorum*, with low support. Posterior probabilities of the internal nodes were high and similar between topologies (**Figure 1-2**).

The two subclades inside the **Peruvian-Bolivian Amazon South clade** were:

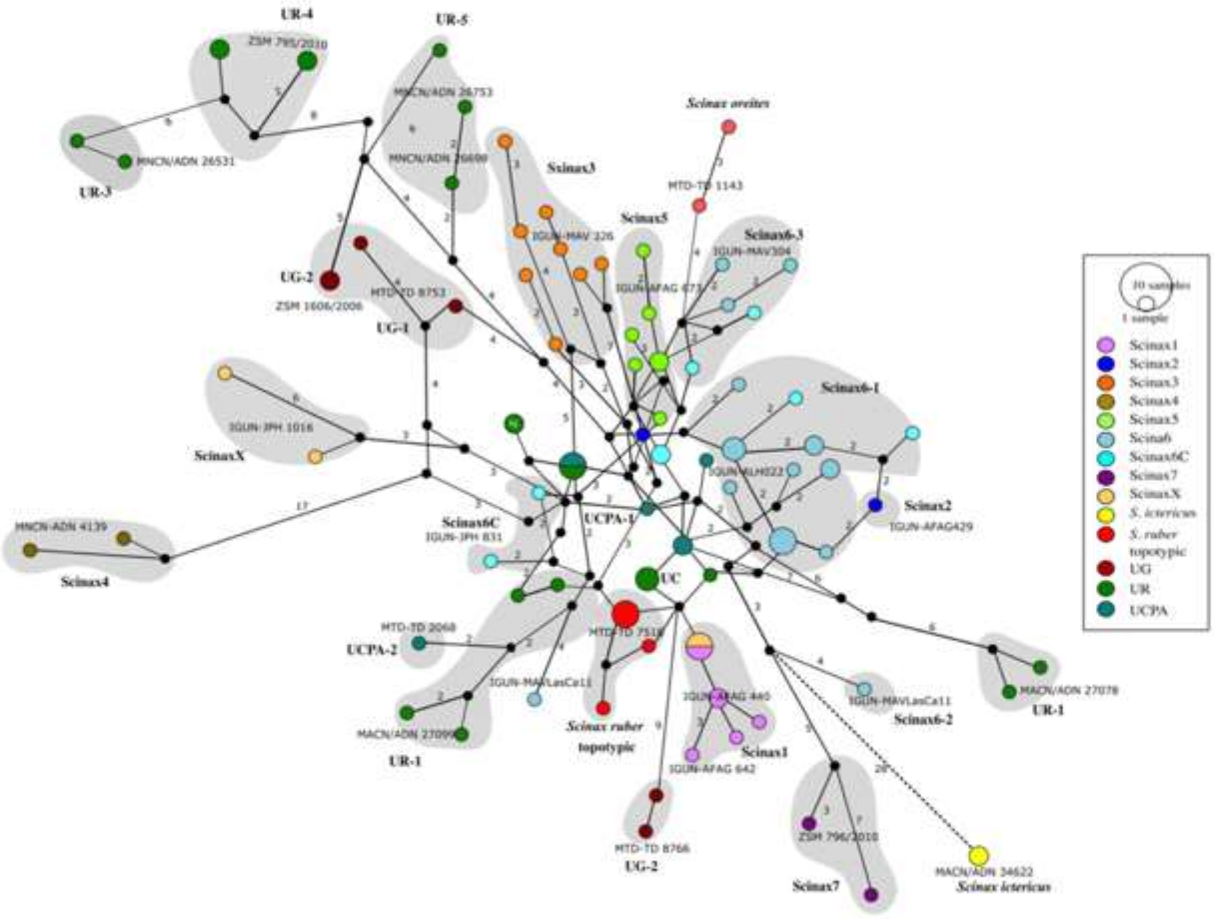
- The ***Scinax funereus* subclade**: It was represented by the sample ZSM 793/2010 from Perú, and the GenBank sequences GQ896256.1 12S and GQ896253.1 12S (the recorded identity of the later in GenBank was "Scinax chiquitanus"). The mean uncorrected *p*-distances between this lineage and the members of the Dry Peruvian-Bolivian Amazon clade ranged from 9.9% to 10.7%. The sample ZSM 793/2010 had a unique haplotype for the Rho fragment (**Figure** 1-5), which was close to *Scinax icterićus* in the network. No nuclear haplotypes were available from the remaining GenBank samples.

Figure 1-4: Statistical parsimony haplotype network for the rhodopsin (Rho) fragment of the molecular alignment and the possible species distribution along them. Colour code follows the lineages of Figure 1-2 (see text). Solid black dots are inferred haplotypes, the numbers below/besides the connectors are the numbers of mutational steps and the shaded areas represent the correspondence between haplotypes and the taxonomic inference of OTUs proposed herein. Stippled connectors represent Nos. of mutational steps > 50.



- The **I subclade**: This clade contained the GenBank sequence KU317397.1, which was one of the paratypes of *Scinax iquitorum* (Moravec, Arista Tuanama, Pérez, & Lehr, 2009) as the sister group of the *Scinax ictericus* samples (GenBank sequence GQ896258.1 12S and the sequence MNCN/AND 34622, both from Bolivia). The mean uncorrected *p*-distance between the two subclades was 7.8%, but the distance within *S. ictericus* could not be calculated. The only sample for which nuclear sequences were obtained (MNCN/AND 34622) showed two separated and distant haplotypes for Rho, which were connected in every case to ScinaxX haplotypes (by 9 and 11 mutational steps). In turn, the same sample contained a single haplotype for Tyr, separated from Scinax7 by 28 mutational steps.

Figure 1-5: Statistical parsimony haplotype network for the Tyrosinase (Tyr) fragment of the molecular alignment and the possible species distribution along them. Colour code follows the lineages of **Figure** 2 (see text). Solid black dots are inferred haplotypes, the numbers below/besides the connectors are the numbers of mutational steps and the shaded areas represent the correspondence between haplotypes and the taxonomic inference of OTUs proposed herein. Stippled connectors represent Nos. of mutational steps > 50.



1.4.2 Delimitation of candidate species and the real *Scinax ruber*

After the integrative analysis, it was evident that under the name "*Scinax ruber*" in our study area there were many more taxa than the ones that we were able to identify initially. The following taxonomic and distributional outlook referred to the taxa in which specimens were reviewed by us:

- **ScinaxX**: Our sampling comprised four distant localities in open areas of the Magdalena and Cauca valleys of Colombia (Magdalena province of Morrone, 2014): the semi-arid plains of Huila, the low northern Andean mountain ranges such as the Serranía de San Jerónimo and Ayapel and the Southwestern Perijá, as well as the mixed areas of wetlands and Caribbean coastal savannas (**Figure 1-3**). The SDM for ScinaxX was presented in **Figure 1-6** (dark yellow areas), showing an approximate Caribbean distribution, including the coastal savannas and the dry sections of the inter-Andean valleys of Colombia. This CCS does not enter either the xerophytic areas in La Guajira or the humid western Chocoan area, but according to the SDM it could enter the savannas between Serranías of Perijá and Mérida and vicinities of the Maracaibo Gulf in Venezuela.

  The specimens of ScinaxX were small to moderate sized, they had a dorsal pattern composed of X or V-shaped brownish markings on a tan dorsum (which may not be present), light brown transverse bars on dorsal thighs or shanks, acutely-rounded and short snout in dorsal aspect, and rather circular (slightly laterally expanded) disks on fingers and toes. Members of this clade had patterned concealed surfaces of thighs and shanks, with an orange/dark brown caterpillar-like pattern (**Figure 1-7**) that is common among individuals of both sexes. Individuals within this clade were rather small with respect to other populations described herein, with adult males around 30 mm SVL and females up to 38 mm (**Figure 1-8**, **Table S1-2**).

  Scinax1: This lineage inhabited open areas on the "Altillanura" [hilly savannas] and the foothills of the Cordillera Oriental, north of the Meta river (Casanare and Meta departments). It was apparently restricted to the Savanna Province of Morrone (2014) (**Figure 1-9**, pink areas). The morphology of Scinax1 was similar in size and markings to that of ScinaxX, but individuals tended to be smaller (at least the ones genetically confirmed), more decorated dorsally and laterally and with more acuminated snouts (**Figure 1-7 C-D**).

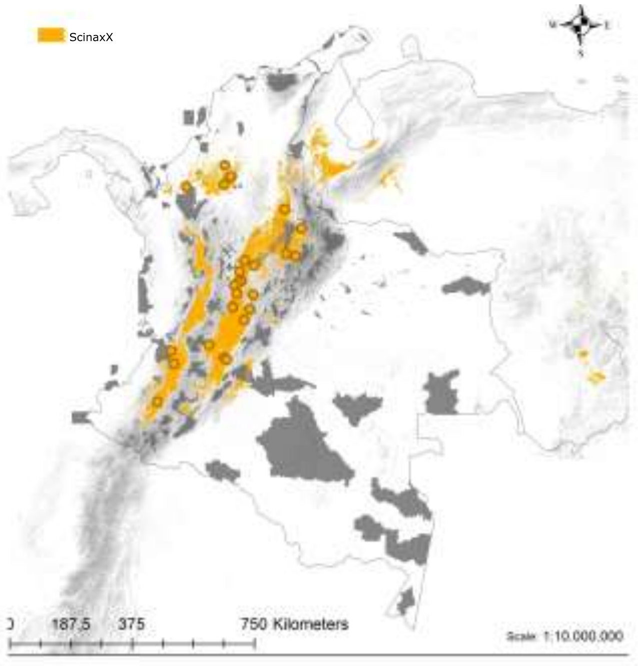


Figure 1-6: Species distribution model (SDM) of the lineage ScinaxX in Colombia and vicinity. Dark yellow areas represent suitable habitat (based on 10 WorldClim variables), while grey polygons represent the protected areas of the National System of Protected Areas of Colombia (SINAP) according to the National Registry of Protected Areas (RUNAP). Credits of the map: Carlos Gantiva.

- **Scinax4**: The only representative of this clade was a sample collected by J. M. Padial (JMP 364) in Bolivia. It was smaller than the Amazonian specimens from other localities (subadult female SVL = 30.3), and has almost no pattern on hidden thighs, but small black spots on the flanks and a truncated snout (Figure 1-11D). It was morphologically distinguishable from any other Scinax ruber-like sample identified here.

- **Scinax5**: Specimens of this CCS were medium-sized, brightly coloured frogs, recalling the forest morphologies of Scinax3 (**Figure 1-15**). The known representatives of this taxon were very green dorsally, but without dark webbing on the feet and hands and slightly pointed snouts. A SDM for this CCS is presented in **Figure 1-16**, showing that the taxon was restricted to the Colombia-Venezuela boundary around the Orinoco river in extreme eastern Vichada. That distribution is intimately associated with the Meta and Tomo rivers, and the gallery forests along the main drainages of the Colombian “Orinoquia”, in the limits between the Imeri, Llanos and Pantepui provinces of Morrone (2014). However, these data do not support any of those provinces. There is also a suitable habitat patch in the western slope of the Andes, in the limits between Chocó and Valle del Cauca precisely where there is a climatic transition from the hyper-humid Chocoan forests of the north, and the slightly dryer and open lowlands in the Cauca river valley. Scinax5 will not extend there, but potentially a sibling lineage could be found in that largely under-sampled habitat.

Figure 1-7: Specimens of Scinax of the ScinaxX and Scinax1 lineages. A. ScinaxX, La Dorada, Caldas, Colombia (ASM195). Picture credits: Yeison Tolosa through NaturaLista (CC https://www.inaturalist.org/photos/33374342). Note dorsal x-marked pattern, fully coloured posterior surfaces of thighs, and body shape. B. ScinaxX, ICN 11803. C-D. Scinax1, Cravo Norte, Arauca, Colombia. C. General view. D. Detail of the flank and anterior hidden thighs. Note greenish dorsum with a brown X-marking, patterned flank and hidden thighs and snout shape. Picture credits: Andres Felipe Aponte Gutiérrez.

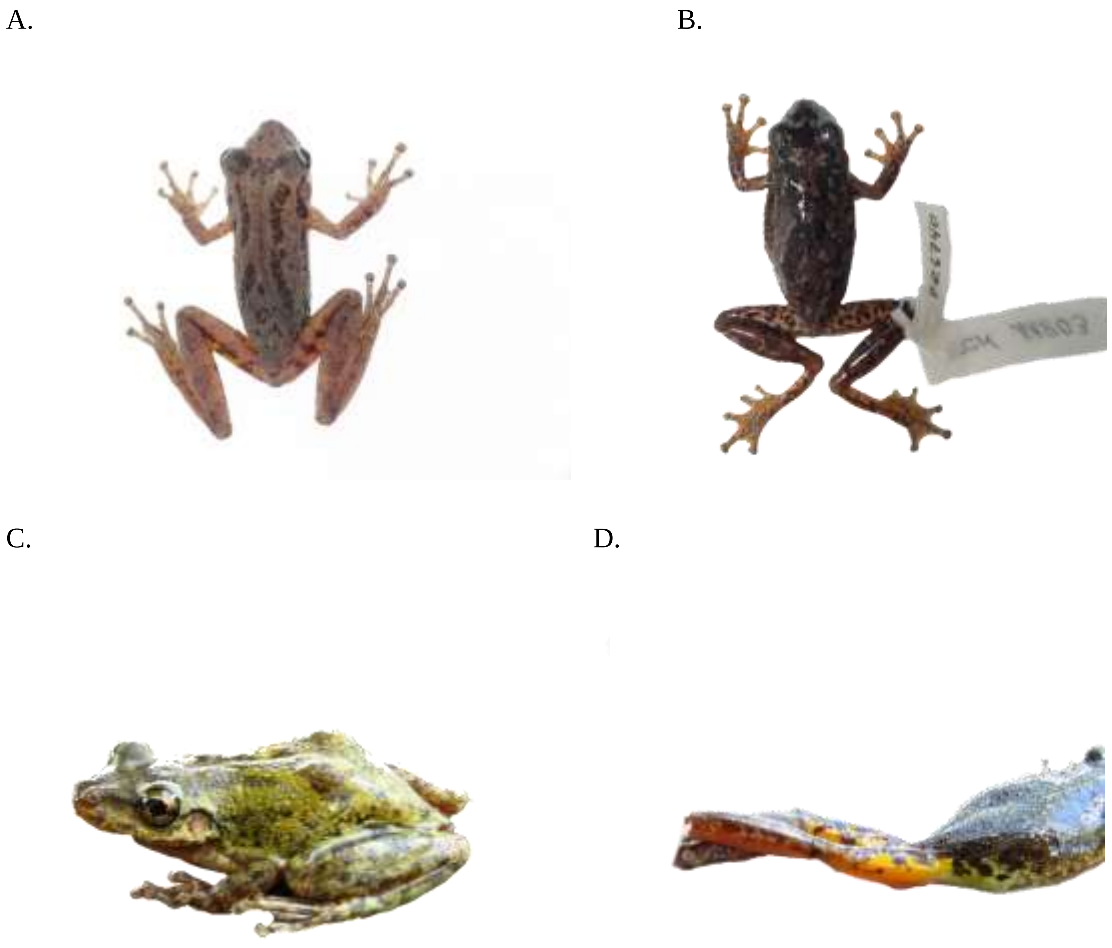
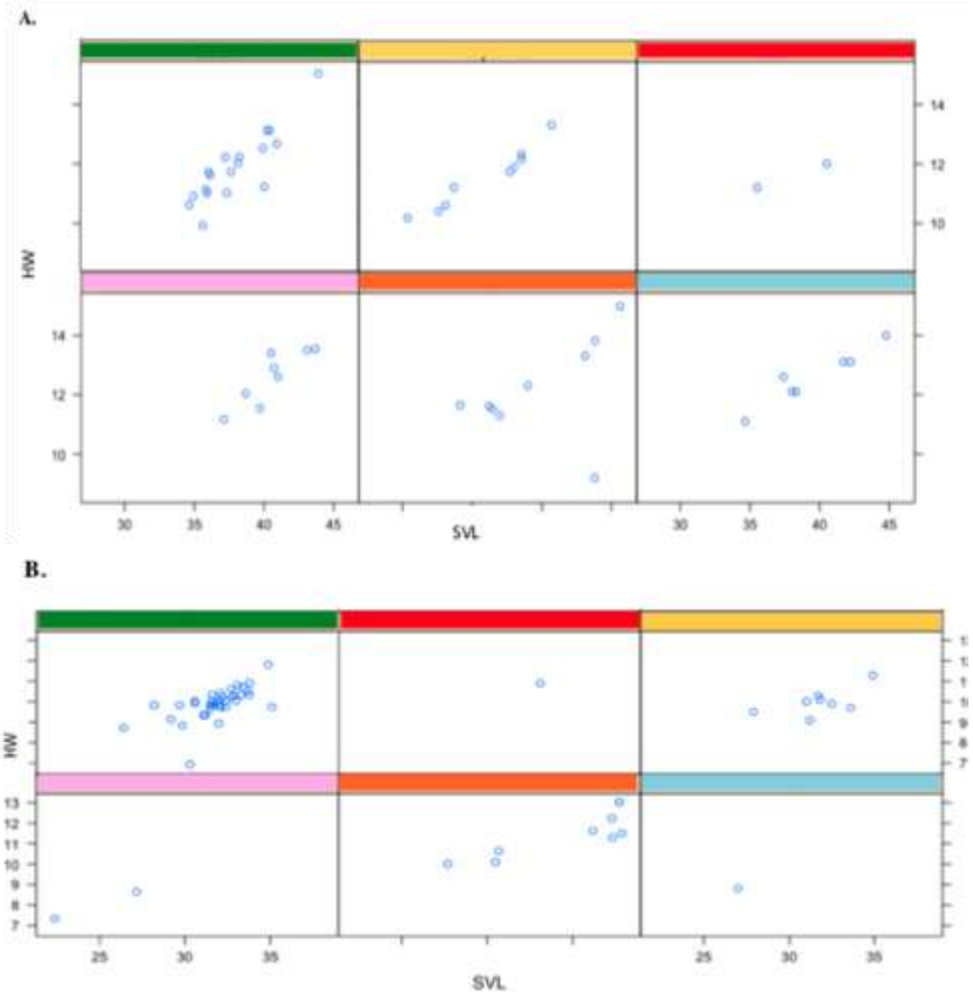


Figure 1-8: Visual comparison of sizes (expressed as the rate between HW and SVL) among the different candidate species of *Scinax* identified in the analysis (see text). The colours of the candidate species follow the color chart in the phylogeny of Figure 2. Colour code: green = UR; dark yellow = ScinaxX; red = Scinax ruber topotypic.; pink = Scinax1; orange = Scinax3; light blue = Scinax6. A. Females only. UR (n=18), ScinaxX (n=9), Scinax ruber UG (n=3), Scinax1 (n=8), Scinax3 (n=9), Scinax6 (n=7). B. Males only. UR (n=18), ScinaxX (n=9), Scinax ruber UG (n=3), Scinax1 (n=8), Scinax3 (n=9), Scinax6 (n=7).



- **Scinax6**: The actual distribution of this lineage appeared supplementary to that of Scinax6C, occupying the Orinoquian piedmont at slightly higher elevations north to the Serranía de la Macarena NNP (see SDM in **Figure 1-16**). Individuals of this clade were slender, with acutely rounded snouts and light dorsal patterns (Figure 17 A-B, F), while their thighs were deep orange with brown thick markings, but normally without rounded spots (Figure 17 C). This poorly decorated dorsal pattern (in exception of sparse golden spots on a tan dorsum on the dark phase of the coloration, Figure 15F) could help in distinguishing Scinax6 from the partially sympatric S. blairi, which was described as having reddish-brown flecks on the dorsum and the anterior surfaces of thighs, but with clear posterior surfaces on the thighs.

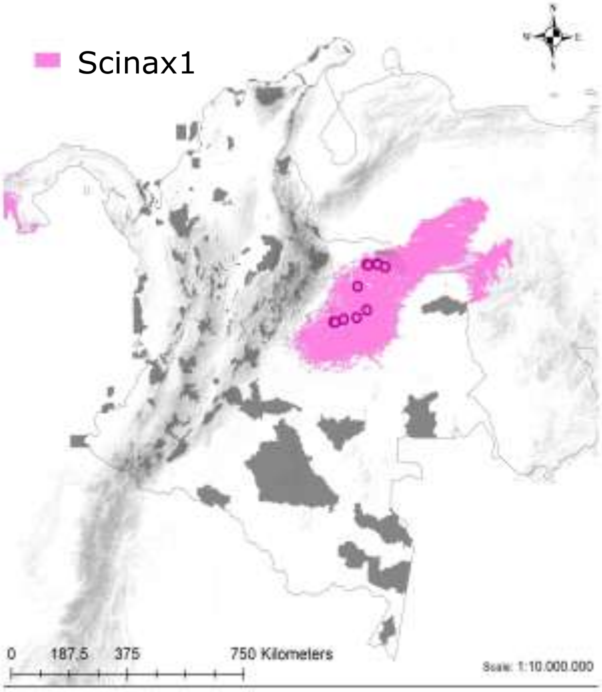


Figure 1-9: Species distribution model (SDM) for the Scinax1 lineage. The potential distribution in pink indicates suitable habitat, based on 10 WorldClim variables. In turn, grey polygons represent the protected areas of the National System of Protected Areas of Colombia (SINAP) according to the National Registry of Protected Areas (RUNAP). Credits of the map: Carlos Gantiva.

Figure 10.

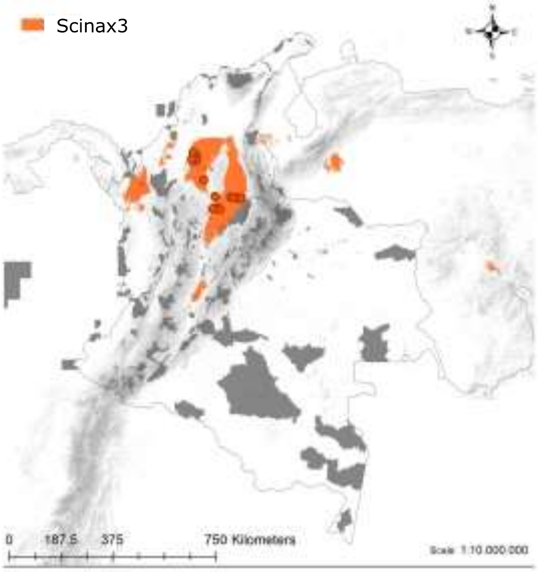
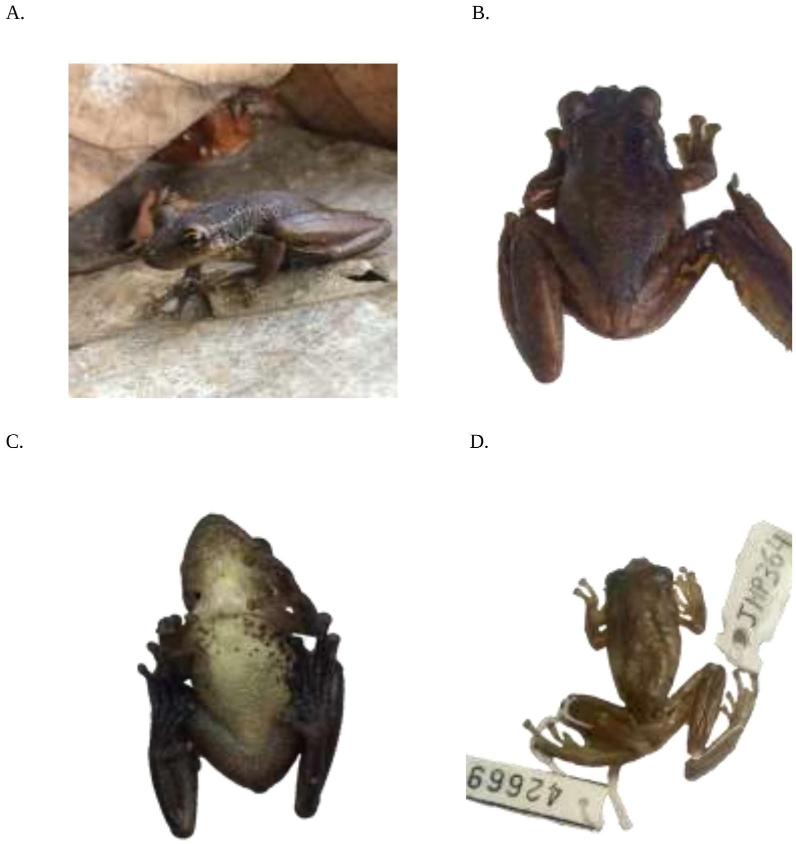


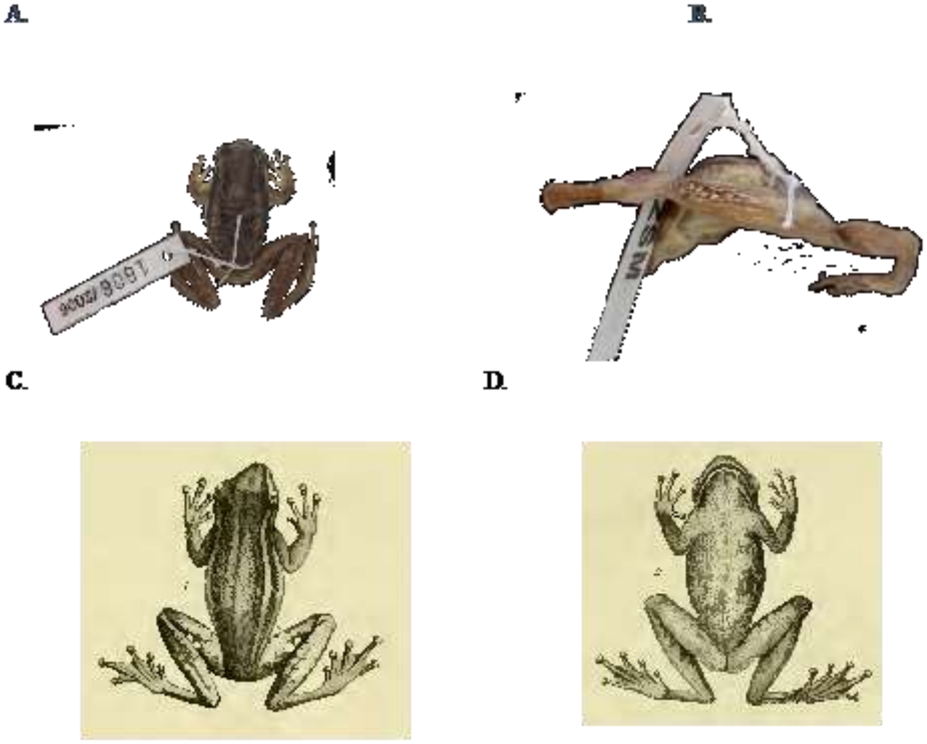
Figure 1-10: Species distribution model (SDM) for the Scinax3 lineage. The potential distribution (orange areas) represents suitable habitat based on 10 WorldClim variables, while grey polygons represent the protected areas of the National System of Protected Areas of Colombia (SINAP) according to the National Registry of Protected Areas (RUNAP). Credits of the map: Carlos Gantiva.

Figure 1-11: Specimens of *Scinax* of the Scinax3 and Scinax4 lineages. A-C. Scinax3 from Reserva El Silencio, Yondó, Colombia. A. Lateral view. B. Detail of posterior hidden thighs. C. Ventral aspect. Note the clear circled pattern on thighs, shanks and tarsus, mottled throat, and the nearly rounded snout. D. Scinax4 (MNCN 42669) from Bolivia, Balliván. Note the truncated snout in dorsal view and the general dark coloration. Pictures: ASM.



- **Scinax6C**: This lineage could be considered the main support of the protected areas established in the extreme northwestern Amazonia of Colombia. Its potential distribution (SDM presented in **Figure 1-16**) comprises the Serranía de Chiribiquete, Serranía de la Macarena and Tinigua NNPs plus the western portion of the Guiana Shield basement in Colombia, including the Nukak National Reserve in Guaviare. Provided that the Serranía de la Macarena is an Andean arm derived from the Cordillera Oriental of Colombian connecting the Orinoco-Amazon boundary (Jaramillo-Justinico & Rangel-Ch, 2014), the fact that this taxon is phylogenetically related to the ones in the Andean piedmont (Scinax6) was somehow expected. Specimens of this clade were dorsally tan with no or very light colour patterns and a caterpillar-like pattern of orange spots on the dark brown posterior surfaces of the thighs (**Figure 1-15 D-E**), a very "open areas" pattern. The snout shape varied from acutely rounded to rounded in the dorsal aspect, The taxon was said (according to the collector) to be common if present, but it is reported for only two (out of 10) localities inside Chiribiquete NNP, which is about 4 million hectares (Á. M. Suárez-Mayorga & Lynch, 2017).

Figure 1-12: *Scinax ruber s.s***.** and UG clade morphology. A-B ZSM 1606/2006 from Tobago. Note the cream dorsolateral stripes bordered by brownish ones, whitish circular spots over brown hidden thighs, and the acutely rounded snout. C-D. Hyla rubra Daudin, 1802. Copied from Plate IX, page 28 in Daudin (1802) of the digital document provided by Biodiversity Heritage Library (BHL, www.bhl.org). Note the concordant dorsal and hidden limbs pattern, acutely rounded snout, and general body shape. Pictures: ASM.



- **UG and the topotypic *Scinax ruber***: The 13 samples within this clade (**Figure 1-2**) occurred at three moderately distant localities in Guiana, Suriname and Tobago (**Figure 1-3**), and they are morphologically homogenous. Their dorsal pattern was very similar and perfectly congruent with the second description of *Hyla rubra* by Daudin (1802), based on the specimen deposited in Seba’s cabinet, which was said to be the base of the original description by Laurenti (1768) (**Figure 12 C-D**). The colour pattern of those populations consisted of dorsolateral white lines bordered by brownish lines on a light tan dorsum, sub-acuminated snout, and hidden thighs painted with small whitish (orange in life) and connected spots, like a large caterpillar on a brown surface. There was, however, a small difference in the dorsal skin texture between the reviewed samples from Tobago and the Guiana UG samples, the later were somewhat granular. The specimens of this clade recalled those of ScinaxX from Colombia, except for the dorsolateral white lines. They were medium-sized: the only male measured was ca. 33 mm SVL, while the two females were between 35 and 40 mm (**Figure 1-8**).

- **UR**: Inside the polytomy there were at least three morphologically different groups of specimens (Figures 13-14): 1) the small ones (females SVL < 38 mm, Figures 11A, 12), with hidden thighs largely painted with circular orange spots surrounded by dark brown lines. Their dorsum was dark with lighter dorsolateral stripes sometimes present, and their snout was nearly truncated (Figure 13 A-B); 2) the medium-size group (females SVL 37-42 mm, Figure 13 C-D, like the topotypic S. ruber and the majority of UR), with acutely-rounded snouts, light dorsum and "forest" pattern on hidden thighs (orange circled spots connected by thick dark brown borders); 3) the large-sized specimens (females SVL > 42 mm, Figure 13 E) with hidden thighs either heavily painted with orange large circular spots surrounded by dark brown circles or dark brown with scarce yellow markings. Their snout was rounded to truncated, never acute.

- **UCPA**: This cryptic clade was defined for specimens from Chanchamayo, Perú, in the MTD collection. Within the specimens reviewed by ASM, MTD 46368 (MTD-TD 1757) was described to have a *S. x-signatus* pattern on dorsum, with scattered inguinal spots and light anterior thighs" (which is uncommon in forest specimens). On the contrary, specimens MTD 46367 and 46486, from Junín and Pasco, Perú, respectively, were described as having a fully ornamented pattern on the anterior and posterior surfaces of the thighs. Beyond that, the uncorrected *p*-distances between MTD-TD 1757 and the members of its sister clade were huge (4.4%), indicating that it can be a completely different lineage.

Figure 1-13: Specimens of *Scinax* of the UR clade. A. MNCN 45165 from Perú, Loreto, Pucarquillo. B. ICN 47539 from Colombia, Amazonas, Leticia; C. MNCN 15780 from Ecuador, Napo. D. ICN 51828 from Colombia, Amazonas, Leticia. E. ICN 36599 from Colombia, río Caquetá near La Pedrera. F. IGUN-ASM 572 from Colombia, El Retorno, Guaviare. Note the "jungle" pattern with heavily pigmented and contrasting flash marks on posterior hidden thighs. Pictures: ASM.

- ***Scinax iquitorum*** : The specimen MNCN:H 44948 from Iquitos, Perú, is totally compliant with the distinctive morphology described for this taxon by Moravec et al. (2009, Figure 14). Its almost entirely black posterior thighs with lighter markings behind the knees and around the cloacal area, and the black spotted groins and posterior flanks, make this species unmistakable. However, apart from the colour pattern, its size, body shape, snout shape and the colour of the bones (not indicated to have a green dye, as many of the taxa in the clades outside the *Scinax ruber*-like group) would allow its inclusion as a member of the UCSR- small size group. The sequences obtained from the tissues shared with ASM by the MNCN regarded this specimen as a member of the UCSR Clade, both in the phylogeny and in the haplotype networks (there were not unique haplotypes for this sample in any network), but neither sequence was phylogenetically related to the GenBank sequence KU317397.1 16S, corresponding to one of the paratypes in the original description (and obtained years after the publication of the new species). Clearly one of the sequences is misidentified, but with the evidence available it is impossible to identify which one.

Figure 1-14: *Scinax iquitorum* MNCN:H 44948 from Perú, Loreto, Iquitos. A. Dorsal view. Note the large black longitudinal dash on the posterior surface of thighs. B. Lateral view, showing black-spotted groin, flank, axilla, and proximal arm area. C. Ventral view, with immaculate venter and anterior thighs like the pattern of a member of the *Scinax rostratus* group. Pictures: ASM.

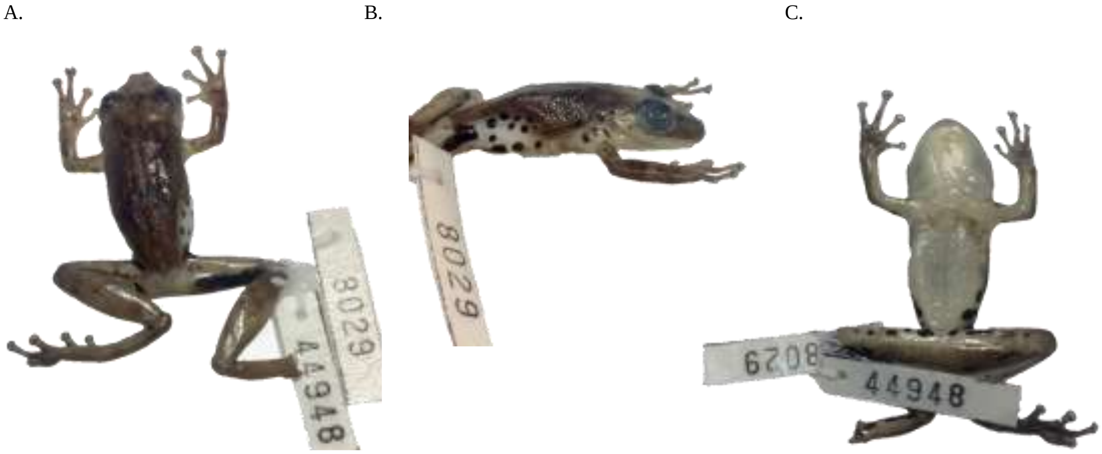


Figure 1-15: External aspect and distribution of the Scinax5 lineage. A. Live aspect, lateral view. B. Species distribution model (SDM). Lime green areas represent suitable habitat (based on 10 WorldClim variables), while grey polygons represent the protected areas of the National System of Protected Areas of Colombia (SINAP) according to the National Registry of Protected Areas (RUNAP). Credits of the map: Carlos Gantiva.

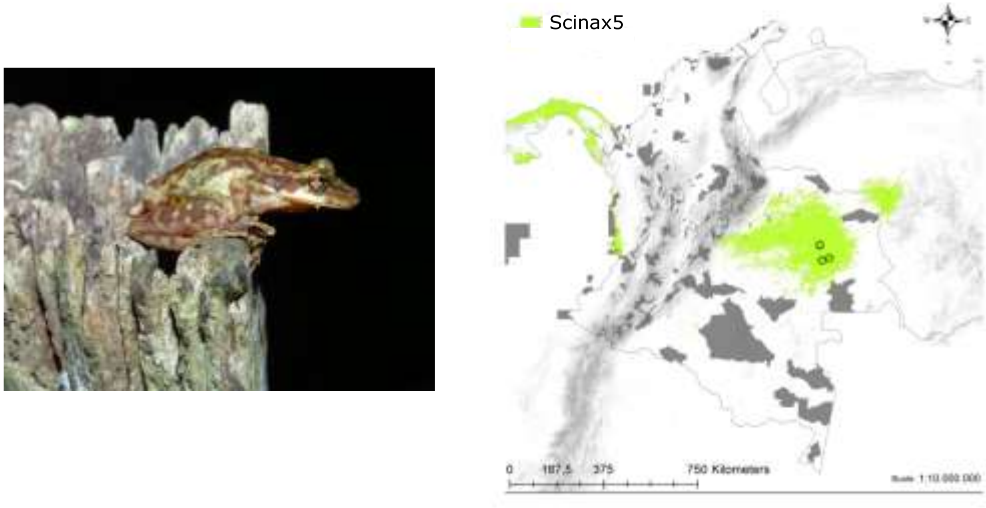


Figure 1-16: Species distribution models (SDM) of lineages Scinax6C and Scinax6. Potential distribution (turquoise areas) represents suitable habitat based on 10 WorldClim variables, while grey polygons represent the protected areas of the National System of Protected Areas of Colombia (SINAP) according to the National Registry of Protected Areas (RUNAP). A. Scinax6C. B. Scinax6. Credits of the map: Carlos Gantiva.

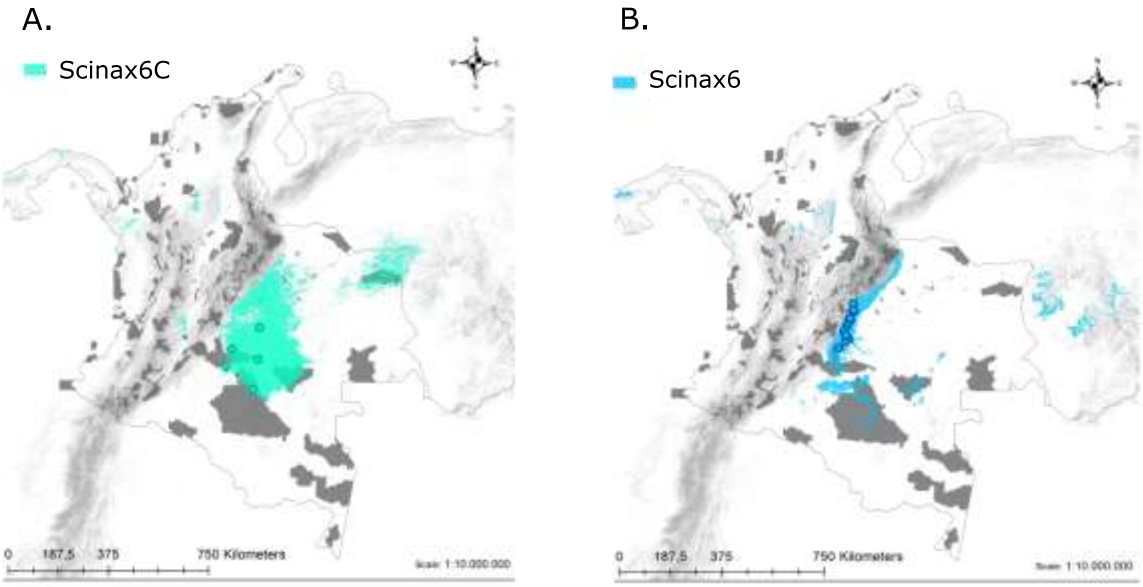
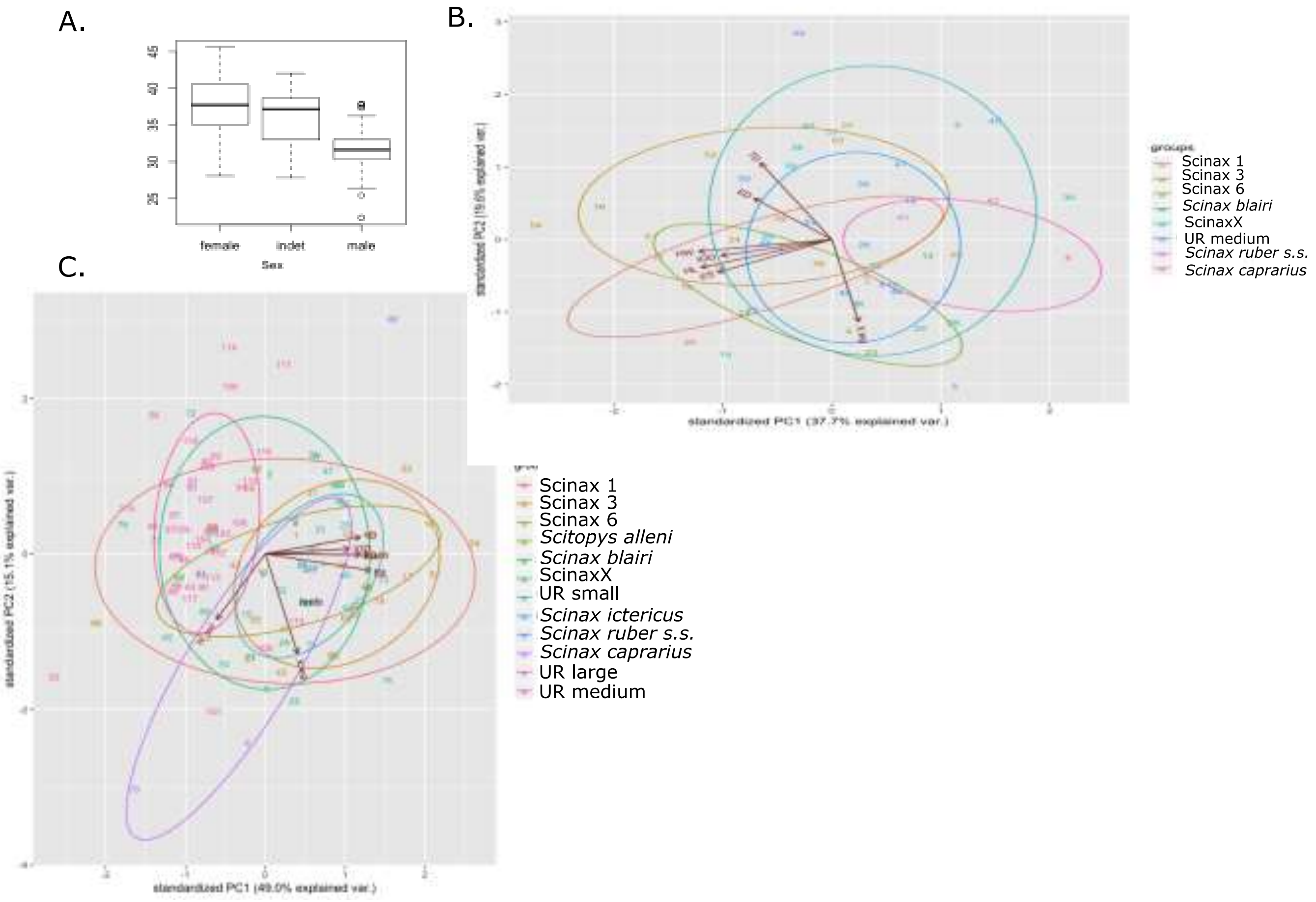


Figure 1-17: Specimens of *Scinax* of the Scinax6 and Scinax6C lineages. A-C. ZSM 314/1937 from Caño Grande [Meta], Colombia (Scinax6c), collected by Hellmich in 1938. A. Dorsal view. B. Ventral view. C. Posterior surfaces of thighs. D-E. JPH1016 from Chiribiquete National Natural Park, Guaviare, Colombia (Scinax6C lineage). D. Lateral view. E. Dorsal pattern and hidden thighs. Picture: Juan Pablo Hurtado. F. LNC-010 from Medina, Cundinamarca. Picture: Leonardo Niño.

Figure 1-18: A. Boxplot comparing the sizes (SVL) of all the adult specimens of *Scinax* considered herein, discriminated by sex. B-C. PCA for all the morphometric variables measured (8), discriminated by sex. B. Females. C. Males. Some already described (and confused) species were including in the measurements for comparison purposes: *Scinax blairi*, *S. caprarius*, *S. ictericus*, and the museum specimen A1519, holotype of *Scytopis alleni* of MVZ collection.



1.5 Discussion

1.5.1 Cryptic diversity

The phylogenetic and phylogeographic analyses combined with the extensive revision of morphology, morphometry and the distributions of "*Scinax ruber" s.l.* and allied (or previously confused with) species revealed the presence of eight confirmed candidate species (CCS) and eight unconfirmed candidate species (UCS) *sensu* Vieites et al. (2009) inside the "*Scinax ruber*-like" group (**Figure 1-2**), plus one CCS in the Dry Peruvian-Bolivian Amazon clade. Of the CCSs, seven are restricted to Colombia, one is restricted to a single locality in Beni, Bolivia (historically a forested area), and yet another one from a dry forest in Perú.

All the evolutionary lineages identified here were robustly supported in the phylogeny based on complete molecular evidence, even though the 16S separation of clades supported by the integrative approach may be as low as 1.0% (Scinax6-Scinax6C). The phylogenetic analyses (**Figure 1-2**) showed that the populations from the Orinoquia piedmonts (Scinax5, Scinax6 and Scinax6C) were clearly monophyletic, although only slightly divergent (uncorrected *p*-distances = 1.2% between CC5 and CCS6, 1.5% between CCS5 and CCS6-C, and 1.0% between CCS6 and CCS6-C). The species delimitation algorithms differed in the discrimination of potential species, from only one entity (ABGD) in the entire Amazonian piedmont (**Figure 1-2**, light blue) to four entities (mPTP, **Figure 1-2**), indicating that geographically isolated populations such as the Chiribiquete are reproductively isolated (different evolutionary lineages).

Contrary to the traditional taxonomic use (or abuse) of colours as diagnostic characters in lowland hylids, many *Scinax ruber*-like populations appear to be polymorphic and several are also cryptic (Fouquet, Gilles, et al., 2007; Funk et al., 2011). The colour patterns on hidden surfaces of thighs and shanks, combined with the shapes of snouts and finger disks, and head dimensions such as HW and TW, seem to be conserved and characteristic within populations. There exists a diagnostic colour pattern on the posterior surfaces of thighs which might be genetically determined with adaptive consequences. All the open area populations (i.e., ScinaxX, Scinax1, Scinax6C) have a dominant caterpillar-like pattern with light base colour and thin brown/dark yellow markings. All the forest-covered area populations have either large spot ("balls") pattern, with a dominant orange/brown interface, or a dark brown phase, slightly marked with orange flash-marks of different sizes and shapes.

This flash-mark coloration could be related to the need for being located efficiently and quickly by mates in the middle of the forest. According to fieldnotes of the collectors of museum specimens and to John Lynch (pers. comm.), the forest lineages are canopy dwellers that will come down eventually to low vegetation and the forest floor or swamps for reproduction after heavy rains. They are nocturnal and explosive breeders and lay their eggs on vegetation near ponds inside the forest or directly on the water surface (Somma, 2019). Thus, it is possible that hidden limb patterns help them to locate potential mates under low light conditions of the lower levels of the forest. In the open areas, those intense flash-marks may not be necessary or could be even dangerous, because predators can also see them. It is also likely (and supported by the diversity and structure of the nuclear haplotypes) that members of different evolutionary lineages hybridize. The presence of intermediate colour morphotypes and mixed nuclear haplotypes or incomplete lineage sorting precisely where there are sympatric landscape units, also supports this idea. Besides, time runs against the successful reproduction of explosive pond breeders because mating is life costly: they must mate quickly before the reproductive resource is occupied by other species, and before being discovered by predators that will have a banquet in every pond after the rain. The senior author and John D. Lynch (JDL) have reported males from molecularly confirmed different evolutionary lineages (assigned to the same OTU in the field but separated by colour pattern at the field station) calling side by side in a 1.5 m diameter swamp. Calls were not distinguishable to the human ear.

Regarding morphometry, our survey showed that the lineages identified here are sexually dimorphic (Figure 1-18A), but there is no clear pattern of morphometric separation between lineages (Figure 1-18 B-C). In females, 38.7% of the morphometric variation is explained by the first component of the PCA, and 19.7%, by the second. The sizes of adult females are scattered through the PCA landscape, and they seem to be very similar (Figure 1-18B), being the transverse lengths of head components (ED, HW, TD) responsible for most of the variation in the first principal component (PC). Conversely, the proportion between TL and SVL is the main source of variation in the second PC. Male sizes are better discriminated, perhaps due to the larger number of specimens sampled per group in a smaller number of groups; and the transversal measurements (ED, HW, TD) are more important for explaining the variation of the second component.

The UR Amazonian clade is named as it is because R is the first letter of “revuelto”, which means “a mess” in Spanish. Based on the low mitochondrial divergences and the very high haplotypic, morphological and habitat variability, it is impossible to support a CCS in the entire Amazon basin of Bolivia, Brazil, Colombia, Ecuador, and Peru. At least two different and concordant lines of evidence are necessary for every morphologically distinctive population, and a more extensive sampling that includes more than a pair of specimens per locality. This might be illustrated by the presence of specimens with green bones/white bones inside the same morphologically and genetically confirmed population in Araracuara, Colombia. Taboada et al. (2017) explains such differences by the concentrations of biliverdin in bones and other tissues that can vary along ecological, behavioural, or health condition gradients, etc. In this case, the combination of different lines of evidence, and an integrative view are fundamental, because there are so many particularities of the habitat and of the lineages and their ecology and evolution that may obscure the real relationships between the populations.

Outside the *Scinax ruber*-like clade, our analyses allowed us to identify one CCS and a probable UCS (specimen MTD-TD 1143 inside the Bolivian-Peruvian clades no. 9 and 10; Figure 2) that are morphologically quite different from the *Scinax ruber*-like clade. Nevertheless, the monophyly of S. chiquitanus, S. funereus and S. ictericus was confirmed and well-supported.

With our still limited knowledge, we could confirm that haplotypes, morphology, and microhabitats were consistently different between minimally divergent clades (not separated by any of the algorithms examining the barcode-gap). This suggests that, at least in tree-frog populations, the single use of the barcode marker 16S for disentangling the diversity of such a megadiverse and complex family is not enough, especially without a detailed knowledge of the ecology, the reproductive biology, and the population structure of the taxa under study. That is evident in Vacher *et al*. (2020), where the level of differentiation between hylids (aka *Boana calcarata*) is said to be lower than that of the forest-floor groups like *Leptodactylus* or *Rhinella*, based on the "standard" 3% threshold. However, the authors are not citing relevant taxonomic and phylogenetic revisions of widely distributed genera or species groups inside the Amazonian Hylidae, demonstrating specific separation below that value (i.e., Caminer *et al*., 2017; Caminer & Ron, 2014; Rivadeneira *et al*., 2018; Rivera-Correa & Orrico, 2013).

1.5.2 Implications of integrative taxonomy

It is worth recalling that the original description and locality for *Hyla rubra* Laurenti, 1768 were based on the illustration and the description provided by A. Seba (Tab. LXVIII, No. 5 in Seba, 1735) that depicts a reddish frog extracted from the stomach of a snake (a green pit-viper) in initial decomposition (according to the text). For a trained eye it is impossible to distinguish from the picture any diagnostic morphological trait of what today should be considered a *Scinax* (i.e., the reduction of webbing between toes I and II, the expanded disks on feet and hands or the depressed snout). However, the reddish frog could have been any medium-sized hylid with reddish-tan coloration. After the re-description of that same material by Daudin (1802), who provided information about the location of the former holotype ("au Museum d'Histoire Naturelle de Paris et il provient du cabinet de Seba") and an illustration (pl. IX, p. 28), the morphology of the real *Hyla rubra* was finally characterized, and its distribution was described more precisely and concisely. Later on, Duellman & Wiens (1993) restricted the type locality to Paramaribo, Suriname.

In spite of acknowledging the levels of crypticity inside *Scinax* in general (Ferrão et al., 2016; Menezes et al., 2016; S. R. Ron et al., 2018), and "*Scinax ruber*" in particular (Fouquet, Vences, et al., 2007; Vacher et al., 2020), nobody had devoted effort to solving the "R-mess" of this polymorphic and cryptic nominal taxon from a large distributional and biogeographic perspective, perhaps because nothing seemed to be exciting or interesting from the very common "*Scinax ruber*", or because the sampling along the Andean Amazonian countries was (and still is to some extent) limited and administratively expensive. An important exception was the unpublished BSc monograph of Jeannette Nieto at the Universidad Nacional de Colombia in 1996. Her extensive analysis yielded the diagnosis of two new species: "spA", today described as *Scinax caprarius* Acosta-Galvis 2018, and "sp2", described in part as *Scinax manriquei* (Barrio-Amorós, Orellana, & Chacón-Ortiz, 2004). She separated the *Scinax ruber*-like species of Colombia into *S. ruber* and *S. x-signatus*, proposing a cline in size from the northwest to the southeast of the country. Clearly, she was also convinced by Duellman & Wiens (1993) in confusing the diagnostic combination of characteristics of the species with characters of evolutionary utility, thus maintaining the crypticity of the species complex. Fortunately, the techniques, the procedures, and the overall knowledge of the evolution of those characters have also evolved, allowing us to review what can be considered evolutive (potential synapomorphies) and adaptive traits.

1.6 Conclusions

Integrative evidence shows that there are more than 10 evolutionary differentiated lineages in the Andean countries of northern South America under the name “*Scinax ruber*”, each of them genetically supported by mitochondrial plus nuclear evidence, and most of them morphologically distinguishable. Neither the dorsal pattern, the green bones, the iris colour (Rivera-Correa & Faivovich, 2013) nor the sonograms of recordings made under different conditions at different localities (Vargas-Salinas, Angarita-Sierra, Ospina-L., Rocha-Úsuga, & Rueda-Solano, 2019) alone should be trusted as concluding evidence of diversification. They should be taken with caution when performing integrative taxonomic analyses with an evolutionary basis. However, the real integration of different lines of evidence such as bioacoustics (when methods and variables are explicit), habitat assessments, life histories, molecules, morphology, morphometrics, or population ecology can unveil a convincing state of knowledge. Based on what has been presented here, it is evident that very few knew or know what *Scinax ruber* is as a taxon (a Guianan endemic with high invasive potential, currently distributed from Suriname to Guiana, Trinidad, Tobago, and Puerto Rico) and the implications of its huge cryptic diversity. It is not a “species without pattern”, as proposed by Lynch & Suárez-Mayorga (2004), even if, being an opportunistic sit-and-wait predator and potential invader, it can enjoy the company of humans and the light bulbs (and preys) that their houses provide (Somma, 2019). In fact, the cryptic species inside the updated known distribution of the nominal taxon are easily encountered in the vicinity of humans, but that does not mean that they are open-areas inhabitants. Instead, “*Scinax ruber*” is a complex of many species with multiple unknown life histories.

Strictly from the taxonomic perspective inside the geographic coverage of this research, some urgent changes should be made:

- We must abandon the name “*Scinax ruber*” for identifying every tan frog with painted hidden surfaces of thighs, especially if it occurs outside the Guiana plateau plus Tobago. *Scinax ruber* does not occur in the Andean countries, and probably not in Brazil either.

- We must stop calling “*Scinax x-signatus*” every *Scinax* that is dorsally x-patterned from the same Andean countries, especially because the neotype of *Scinax x-*

signatus is completely different morphologically and deeply divergent genetically from what we have in the NW South America. In that regard, the senior author has recently reviewed the extant types of the two recognized synonyms of *Hyla x-signata* (Spix, 1824), namely *Hyla affinis* and *Hyla caerulea* in the ZSM collection, while the Molecular Genetic Laboratory at Museum für Tierkunde, Senckenberg Dresden, obtained two mitochondrial sequences (12S and 16S) of the former. From the manuscript in preparation, we can anticipate here that those sequences place that type far from its morphologically expected taxonomic and evolutionary position.

- Based on the pictures of the types in ANSP and MVZ museums reviewed by ASM, it is evident that *Scytopis alleni* is a member of the *Scinax ruber*-like clade, but with the evidence at hand it is not possible to know whether it belongs to one of the CCSs or UCSs identified here. According to the pictures, and based on our review, the MVZ type from Pará and the ANSP type from Loreto (Pebas) do not seem to be conspecific.

- After the careful revision by ASM of the Spix' (1824) types deposited in the SZM and the restrictions in taxonomy and distributions presented here, the only true synonym of *Scinax ruber* hosted by that museum is *Hyla lateristriga* Spix, 1824. The specimen is in very poor condition, but its morphology is completely consistent with the Guianan species. Moreover, considering the level of detail of many of Spix's descriptions, including even the tree or shrub in which the specimen was collected, we consider the indication of Brazil as the original locality for this species doubtful.

1.7 Implications for conservation

When we selected this group as a model, one of the objectives of the senior author was to identify whether the absence of data from north-western South America was a pitfall for accurate conservation decisions. Now that the cryptic taxonomy of the group is starting to be clarified and ordered, we can confirm that many conservation decisions have been made without the minimal base line. Our data suggest that the members of the *Scinax ruber*-like clade are absolutely philopatric, beyond their polymorphism and cryptic diversity and would be very useful as indicators of biodiversity richness and ecosystem quality.

There are clearly forest-dwelling species with forest morphologies inside the *Scinax ruber*-like complex that do not leave the forest (**Figures 1-10** and **1-16**), and there are open-areas inhabitants (Orinoquia and Middle Magdalena valley savannas, dry coastal areas, Guiana plateau) that do not enter the forest unless they are forced to do so. The only exception based on the SDMs discussed above is Scinax6, which seems to be comfortable in both (but it might be a sampling effect). That condition has severe consequences for the conservation of some of the cryptic species, provided that the most intervened areas in the Andean countries, subject to extensive monocultures, cattle, oil and gas mining, are precisely the ones that were thought not to contain any "worth preserving" biological entity.

Scinax species are important predators of Arthropoda and important prey items for reptiles, birds, and mammals, hence helping in the maintenance of the ecological balance of the ecosystems they inhabit. Their philopatry and their vulnerability to environmental changes in temperature, humidity, and water availability make them useful for understanding and monitoring the distribution of the biodiversity in their megadiverse habitats, and its threats. Besides, their capacity to produce anti-microbial peptides (AMPs) makes the frogs studied herein potentially useful for improving human health. However, much of the diversity of the group –and its consequent genetic diversity—is located either inside the forested areas that are every day more scarce and less preserved or in open areas of the Orinoquian and inter-Andean valleys of Colombia and the coastal savannas of Caribbean South America, where human settlements and crops develop rapidly. Those ecosystems are threatened and so are the species living in them. Besides, most of the distributional ranges of open-habitats taxa (see SDMs of **Figures 1-6**, **1-9** and **1-15**) fall outside protected areas. In Colombia, a minimum of three endemic species in the known range of oil exploitations are present; the same is true for Bolivia with the CCS4. We can anticipate, then, that the hydrocarbons production, as well as infrastructure development will negatively impact this diversity that we are just beginning to recognize.

This is just the beginning of a long-lasting quest. A lot is still pending for discovery about diversification patterns and processes of this species complex. But considering the potential usefulness of at least the savannas CCS for human well-being and understanding what is going to be lost due to infrastructure or mining, our findings should become warnings.

Table S1-1: Tissue samples and GenBank sequences used for the molecular analyses, ordered by taxonomic identity. The “Recorded identity” corresponds to the identity recorded in the collection’s catalogue or in the documented database from which the sample was obtained. The column Tissue Code includes the GenBank codes for the sequences used, as well as the code for the herein provided sequences. Catalogue No. and Collector No. refer to the Darwin Core concepts “Catalogue Number” (for the unique identifier of the specimen catalogued in a biological collection) and “Collection Number” (for the code given to the specimen by its collector in the field).

GenBank No./ tissue code	**Catalogue No.**	**Collection No.**	**Recorded identity**	**Sex/age**
AY843740.1 12S, 16S AY844727.1 Rho AY844167.1 Tyr	MACN 38642		*Pseudis paradoxa*	not indicated
MK293738.1	MNRJ:33859		*Pseudis paradoxa*	not indicated
XXXX_S67	IGUN-BATB 3934	AFAG 642	*Scinax blairi*	male
AY843755.1 12S, 16S AY844741.1 Rho AY844177.1 Tyr	MVZ 2017215		*Scinax boulengeri*	not indicated
XXXXX_A1	MNCN 43718	IR 4672	*Scinax chiquitanus*	male
JF789945.1 16S	MNK:A:9481	MJ1314	*Scinax chiquitanus*	not indicated
GQ896253.1 12S		JPC 12949	*Scinax chiquitanus*	not indicated
GQ896256.1 12S	KU 221960		*Scinax funereus*	not indicated
fuPEHuaZ2	ZSM 793/2010		*Scinax funereus cf.*	not indicated
GQ896258.1 12S	KU 215341		*Scinax ictericus*	not indicated
XXXXX_A14	MNCN: H 44781 MNCN/ADN 34622	JMP 5159	*Scinax ictericus*	male
XXXX_A16	MNCN:H 44948 MNCN/ADN 27333		*Scinax iquitorum*	male
KU317397.1 16S	NMP6V 71267/1		*Scinax iquitorum*	not indicated
GQ896260.1 12S	KU 212146		*Scinax oreites*	not indicated
KP149177.1 COI KP149379.1 16S	ANDES-A 1040	AJC 4053	*Scinax ruber*	not indicated
KP149236.1 COI KP149442.1 16S	ANDES-A 1046	AJC 4054	*Scinax ruber*	not indicated
KP149491.1 16S	ANDES-A 1165	AJC 2324	*Scinax ruber*	not indicated
KP149149.1 COI KP149347 16S	ANDES-A 1210	AJC 3532	*Scinax ruber*	not indicated
KP149255.1 COI KP149466.1 16S	ANDES-A 1290	AJC 3446	*Scinax ruber*	not indicated

Table S1-1: Continues.

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Sex/age
KP149100.1 COI KP149295.1 16S	ANDES-A 1449	AJC 3534	*Scinax ruber*	indet
KP149243.1 COI KP149452.1 16S	ANDES-A 1504	AJC 3378	*Scinax ruber*	not indicated
KP149133.1 COI KP149330.1 16S	ANDES-A 1810	AJC 3884	*Scinax ruber*	male
KP149099.1 COI KP149294.1 16S	ANDES-A 1811	AJC 3936	*Scinax ruber*	not indicated
KP149124.1 COI KP149320.1 16S	ANDES-A 1812	AJC 3940	*Scinax ruber*	not indicated
XXXXXX_S22	IGUN-BATB 3286	ASM 237	*Scinax ruber*	male
XXXXXX_S23	IGUN-BATB 3290	ASM 269	*Scinax ruber*	indet
XXXXXX_B22	ICN 48667	JDL 26135	*Scinax ruber*	female
XXXXXX_B23	ICN 48668	JDL 26136	*Scinax ruber*	indet
XXXXXX_S4	IGUN-BATB 3787	AFAG 429	*Scinax ruber*	not indicated
XXXXXX_S8	IGUN-BATB 3805	AFAG 467	*Scinax ruber*	male
XXXXXX_S1	IGUN-BATB 3856	AFAG542	*Scinax ruber*	not indicated
XXXXXX_S7	IGUN-BATB 3903	AFAG 599	*Scinax ruber*	juvenile
XXXXXX_S6	IGUN-BATB 3921	AFAG 621	*Scinax ruber*	male
XXXXXX_S66	IGUN-BATB 3947	AFAG 673	*Scinax ruber*	not indicated
XXXXXX_S29_	IGUN-BATB 3383	ALH 022	*Scinax ruber*	not indicated
XXXXXX_S32	IGUN-BATB 3280	ASM 199	*Scinax ruber*	not indicated
XXXXXX_S27	IGUN-BATB 2152	JDL 32662	*Scinax ruber*	male
XXXXXX_S15	IGUN-BATB 3317	JPH 1016	*Scinax ruber*	male
XXXXXX_S16C	IGUN-BATB 2249	JPH 814	*Scinax ruber*	not indicated
XXXXXX_S17C	IGUN-BATB 2250	JPH 815	*Scinax ruber*	not indicated
XXXXXX_S18C	IGUN-BATB 2266	JPH 831	*Scinax ruber*	not indicated
XXXXXX_S30	IGUN-BATB 3382	LNC 010	*Scinax ruber*	not indicated
XXXXXX_S14	IGUN-BATB 3363	MAV 234	*Scinax ruber*	not indicated
XXXXXX_S38b	IGUN-BATB 3366	MAV 301	*Scinax ruber*	not indicated
XXXXXX_S39	IGUN-BATB 3370	MAV 302	*Scinax ruber*	not indicated
XXXXXX_S40	IGUN-BATB 3361	MAV 303	*Scinax ruber*	not indicated
XXXXXX_S41	IGUN-BATB 3360	MAV 304	*Scinax ruber*	not indicated
XXXXXX_S38a		MAV 394	*Scinax ruber*	female
XXXXXX_S10		MAVSR10	*Scinax ruber*	not indicated
XXXXXX_S11		MAVSR11	*Scinax ruber*	not indicated
XXXXXX_S12	IGUN-BATB 4863	TAS 1630	*Scinax ruber*	not indicated
KU317404.1 16S	INPA-H34645		*Scinax ruber*	not indicated

Table S1-1: Continues.

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Sex/age
KU317399.1	INPA-H34699		*Scinax ruber*	not indicated
AY326034.1 12S, 16S	KU 207622	WED 56265	*Scinax ruber*	not indicated
AY819447.1	KU 221975		*Scinax ruber*	not indicated
XXXXXX_S13	IGUN-BATB 3365	MAV 326	*Scinax ruber*	male
XXXXXX_A3	MNCN 45161 MNCN/ADN 26531	JMP 1411	*Scinax ruber*	male
XXXXXX_A17	MNCN:H 42669 MNCN/ADN 4139	JMP 364	*Scinax ruber*	juv
XXXXXX_A4	MNCN:H 42981 MNCN/ADN 2415		*Scinax ruber*	male
XXXXXX_A8	MNCN:H 45151 MNCN/ADN 27412	IR 8110	*Scinax ruber*	male
XXXXXX_A7	MNCN:H 45153 MNCN/ADN 27418	IR 8116	*Scinax ruber*	female
XXXXXX_A6	MNCN:H 45154 MNCN/ADN 27419	IR 8117	*Scinax ruber*	male
XXXXXX_A5	MNCN:H 45156 MNCN/ADN 27436	IR 8134	*Scinax ruber*	female
XXXXXX_A10	MNCN:H 45163 MNCN/ADN 26698	JMP 1579	*Scinax ruber*	male
XXXXXX_A9	MNCN:H 45166 MNCN/ADN 26753	JMP 1633	*Scinax ruber*	male
XXXXXX_A13	MNCN:H 45171 MNCN/ADN 27070	JMP 2476	*Scinax ruber*	female
XXXXXX_A12	MNCN:H 45176 MNCN/ADN 27078	JMP 2484	*Scinax ruber*	female
XXXXXX_A15	MNCN:H 45186 MNCN/ADN 27099	JMP 2505	*Scinax ruber*	male
XXXXXX_A11	MNCN:H 45188 MNCN/ADN 27102	JMP 2508	*Scinax ruber*	male
XXXXXX_S96	MTD-TD 1756	MTD 46367/ RVM 401	*Scinax ruber*	male
rXXXXXX_S97	MTD-TD 1757	MTD 46368	*Scinax ruber*	male
XXXXXX_S98	MTD-TD 2068 MTD 46487	MLI 063 (MLI 064 in notes)	*Scinax ruber*	male
XXXXXX_S99	MTD-TD 2069 MTD 46489	MLI 065 (MLI 066 in notes)	*Scinax ruber*	female
XXXXXX_S102	MTD-TD 7518 MTD 48119		*Scinax ruber*	not indicated
XXXXXX_S105	MTD-TD 8753 MTD 48302		*Scinax ruber*	not indicated
XXX48303MTD	MTD 48303		*Scinax ruber*	
XXXXXX_S106	MTD-TD 8760 MTD 48309		*Scinax ruber*	not indicated
XXXXXX_S107	MTD-TD 8766 MTD 48315		*Scinax ruber*	not indicated
XXXX12076SM	SMNS 12076		*Scinax ruber*	

Table S1-1: Continues.

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Sex/age
XXXX12084SM	SMNS 12084		*Scinax ruber*	
XXXX12085SM	SMNS 12085		*Scinax ruber*	
XXXX12086SM	SMNS 12086		*Scinax ruber*	
XXXX12087SM	SMNS 12087		*Scinax ruber*	
XXXX12101SM	SMNS 12101		*Scinax ruber*	
XXXXXX_ Z12	ZSM 1606/2006		*Scinax ruber*	male
XXXXXX_Z7	ZSM 1607/2006		*Scinax ruber*	not indicated
XXXXXX_Z15	ZSM 1987/2008		*Scinax ruber*	not indicated
XXXXXX_Z10	ZSM 1988/2008		*Scinax ruber*	male
XXXXXX_Z8	ZSM 794/2010		*Scinax ruber*	indet
XXXXXX_Z9	ZSM 795/2010		*Scinax ruber*	not indicated
JN690793.1 12S JN691399.1 16S JN692122.1 Tyr		2131VOGT	*Scinax ruber*	not indicated
XXXXXX_Z4		FGZC 3394	*Scinax ruber*	female
XXXXXX_Z1		FGZC 4779	*Scinax ruber*	not indicated
XXXXXX_Z13		FGZC 4760	*Scinax ruber*	not indicated
XXXXXX_Z3		FGZC 4792	*Scinax ruber*	not indicated
XXXXXX_S56	IGUN-BATB 3949	AFAG 677	*Scinax ruber-like*	not indicated
XXXXXX_Z6	DNA 00070		*Scinax sp.*	not indicated
XXXXXX_S25	IGUN-BATB 4210	AFAG 1030	*Scinax sp.*	not indicated
XXXXXX_S26	IGUN-BATB 4211	AFAG 1031	*Scinax sp.*	not indicated
XXXXXX_S95	MHNSM 19820	MTD-TD 1143	*Scinax sp.*	not indicated
XXXXXX_Z5	ZSM 792/2010		*Scinax sp.*	indet
XXXXXX_Z14	ZSM 796/2010		*Scinax sp.*	indet
AY843681.1 12S, 16S AY844674.1 Rho AY844123.1 Tyr		CFBH 5788	*Scinax uruguayus*	not indicated
XXXXXX_S28	IGUN-BATB 3973	AFAG 440	*Scinax x-signatus*	indet
XXXXXX_S34		SXS1	*Scinax x-signatus*	not indicated
XXXXXX_S35	.	SXS2	*Scinax x-signatus*	not indicated
XXXXXX_S36	.	SXS3	*Scinax x-signatus*	not indicated
KU359530.1 COI	UFMG347		*Scinax x-signatus*	not indicated
KU495577.1 16S KU494784.1 COI	CFBH-T 03433		*Scinax x-signatus*	not indicated

Table S1-2: Voucher specimens measured for the morphometric analysis of the populations sampled in the Northern South America. Unless juvenile specimens were measured, their measurements were not considered for comparison purposes. Standard abbreviation of measurements taken are as follows: eye diameter (ED), eye-snout distance (ES), head length (HL), head width (HW), inter-orbital distance (IOD), snout-vent length (SVL), maximum tympanum diameter (TD) and tibia length (TL). When the corresponding measurement was not taken, the content of the cell is NA.

Catalogue No.	Collection No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ANDES-A 1919	AJC 3952	female	CCS6	3.2282	-73.8518	38.00	19.30	NA	NA	2	NA	12.1	NA
ANDES-A 1920	AJC 3937	female	CCS6	3.2282	-73.8518	38.30	19.30	NA	NA	2	NA	12.1	NA
ANDES-A 2010	AJC 3938	female	CCS6	3.2282	-73.8518	37.40	19.40	NA	NA	2	NA	12.6	NA
ANDES-A 2011	AJC 3948	female	CCS6	3.2282	-73.8518	41.70	22.20	NA	NA	2	NA	13.1	NA
ANDES-A 2107	AJC 4740	female	UCSR	-4.1033	-69.9749	37.30	21.10	NA	NA	1.8	NA	12.2	NA
ANDES-A 896	JMP 2141	female	UCSR	-4.1241	-69.9413	41.00	21.00	NA	NA	2	NA	12.65	NA
Cope,1974		female	UCSR	-4.4896	-73.5899	44.00	NA	15.00	NA	NA	NA	15	NA
CPZ-UV3461	CAH029	female	CCSX	2.0790	-77.0100	36.80	19.20	12.50	3.80	1.90	6.10	10.70	3.60
CPZ-UV4892	JKT022	female	CCSX	5.7257	-74.7245	28.10	16.20	10.70	4.80	2.20	5.10	8.80	3.00
CPZ-UV660	WB1408	female	CCSX	3.3694	-76.5545	33.10	16.40	11.30	4.00	2.50	4.80	10.60	4.00
ICN 11207	PR 6867	female	UCSR	-4.1936	-69.9390	35.90	19.30	12.30	4.40	2.00	5.50	11.10	4.60
ICN 11287		female	UCSR	-3.7655	-70.3884	37.70	20.70	13.40	4.10	2.20	5.60	11.70	4.40
ICN 11884		female	blairi	-0.6276	-72.2505	34.50	17.60	12.60	4.60	1.70	5.40	11.60	3.60
ICN 22230		female	UCSR	2.5062	-72.6704	40.30	21.60	12.70	5.30	2.20	6.50	13.10	3.90
ICN 308		female	CCSX	6.6205	-73.9181	40.70	22.50	14.20	5.50	1.80	7.10	13.30	4.10

Table S1-2: Continues.

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ICN 36599	MC 7701	female	UCSR	-1.3149	-69.5886	34.70	17.70	14.30	4.50	2.20	5.40	10.60	3.10
ICN 37111		female	CCS6	-0.6351	-72.2849	44.75	22.50	15.40	5.30	2.50	7.10	14.00	4.70
ICN 37122		female	CCS1	6.3095	-70.2139	43.70	22.90	14.90	4.50	2.60	7.45	13.55	4.30
ICN 37122		female	CCS1	6.3106	-70.2157	43.10	23.10	14.65	4.50	2.50	6.70	13.50	4.00
ICN 37123		female	CCS1	6.3106	-70.2157	41.00	21.20	14.70	4.50	2.20	6.60	12.60	5.50
ICN 37124		female	CCS1	6.3078	-70.2088	40.50	21.00	15.40	3.80	2.10	7.10	13.40	4.90
ICN 37124		female	CCS1	6.3106	-70.2157	40.70	20.60	14.70	3.90	2.30	6.20	12.90	3.90
ICN 37378	LR-VI 044	female	CCS6	4.0407	-73.7074	42.20	22.50	14.60	4.20	2.20	6.60	13.10	4.40
ICN 37383		female	CCS6	4.0407	-73.7074	34.65	18.20	13.00	4.30	1.50	6.00	11.10	4.90
ICN 37384		female	CCS3	4.0407	-73.7074	39.00	20.00	13.60	5.00	2.20	6.70	12.30	4.70
ICN 39867		female	UCSR	1.1305	-76.5791	40.50	20.30	13.80	4.90	2.00	6.40	13.10	4.00
ICN 39868		female	UCSR	1.1305	-76.5791	36.20	18.50	12.90	4.80	1.90	6.00	11.60	3.85
ICN 39901		female	UCSR	1.0303	-76.5505	38.30	20.00	13.60	4.90	1.85	6.90	12.20	4.80
ICN 48660	JDL 25675	female	CCS3	8.4089	-75.8906	33.70	18.50	11.50	4.30	1.80	5.70	11.20	3.90
ICN 48661	JDL 25766	female	CCS3	8.7217	-75.9906	32.60	18.10	12.20	4.80	1.80	6.10	10.40	4.00
ICN 48667	JDL 26135	female	CCSX	8.4400	-74.9794	30.37	15.53	10.66	3.77	2.08	5.09	10.18	2.80
ICN 48769	JDL26773	female	CCS3	8.4000	75.2833	36.99	18.23	13.03	4.42	2.65	5.94	11.30	3.56
ICN 51508	JDL 23603	female	UCSR	-4.0839	-70.0108	37.40	19.30	13.80	4.80	1.80	5.65	11.00	3.95
ICN 51807	JDL 23602	female	UCSR	-4.0839	-70.0108	40.00	20.30	13.90	4.70	2.10	5.30	12.50	4.40
ICN 52760	JMR 1594	female	UCSR	-4.2054	-69.9471	36.10	18.40	12.90	5.00	2.20	5.50	11.70	3.70
ICN 55179	HMR 366	female	CCS3	8.0085	-76.1202	43.80	22.80	15.00	4.90	2.50	7.00	9.20	4.20
ICN37444	MC8244	female	CCS3	5.8297	-74.2801	45.59	23.44	15.28	5.20	2.70	7.71	14.98	4.92
ICN39201	CMV 673	female	CCS3	8.0227	-76.2095	36.20	19.60	13.55	4.52	2.40	6.53	11.62	3.78

Table S1-2: Continues.

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ICN43268	46 Chap	female	CCSX	3.6453	-75.5942	36.45	18.75	12.23	4.95	2.68	6.31	11.51	3.43
ICN44392	JDL22135	female	CCSX	10.6167	-75.2333	37.71	18.08	12.99	5.04	2.22	5.64	11.73	3.92
ICN44394	JDL22137	female	CCSX	10.6167	-75.2333	38.01	18.77	13.27	4.91	2.46	6.21	11.89	3.82
ICN44399	JDL22142	female	CCSX	10.6167	-75.2333	38.55	18.75	13.21	4.93	2.26	6.57	12.16	3.73
ICN44403	JDL22146	female	CCSX	10.6167	-75.2333	38.53	18.87	13.21	4.77	2.59	6.43	12.32	3.12
ICN47103	JVR142	female	CCS3	7.7960	-77.1298	43.83	23.34	14.19	5.33	2.53	6.97	13.83	4.54
ICN47105	JVR144	female	CCS3	7.7960	-77.1298	43.10	21.70	13.20	5.40	2.50	6.00	13.30	4.70
ICN52451	MOM1078	female	caprarius	6.1667	-74.8500	34.17	18.03	11.60	4.23	2.37	5.74	11.52	3.88
IGUN-AFAG599	AFAG 599	juv	CCS1	6.3668	-70.7135	30.47	15.33	11.61	4.28	1.98	5.41	10.05	3.39
IGUN-MAV394	MAV 394	female	CCS3	6.4000	-70.4314	34.14	19.03	12.29	4.53	2.27	5.27	11.66	2.86
MNCN:H 15780		female	UCSR	-0.4516	-76.9956	38.20	20.00	14.20	4.60	2.40	6.65	12.00	4.10
MNCN:H 45153	IR 8116	female	UCSR	-4.1934	-73.4774	28.50	14.60	12.20	4.00	1.70	4.50	8.70	3.55
MNCN:H 45156	IR 8134	female	UCSR	-4.1934	-73.4774	35.70	17.10	9.50	4.00	2.30	5.90	9.90	3.70
MNCN:H 45171	JMP 2476	female	UCSR	-5.0575	-73.8540	35.00	19.00	10.40	4.30	2.10	6.60	10.90	4.60
MNCN:H 45176	JMP 2484	female	UCSR	-5.0575	-73.8540	36.00	18.30	10.80	4.70	2.30	5.40	11.00	4.50
MTD 40903		female	ruber	4.9428	-52.2994	40.50	19.00	10.60	5.30	2.70	5.70	12.00	4.70
MTD 49060		female	ruber	4.1344	-59.0332	35.50	19.50	13.60	4.30	1.60	6.20	11.20	4.40
MTD-TD 2069	MTD 46488/ MLI 065	female	UCSR	-10.0705	-75.5558	40.10	20.90	13.40	4.20	2.00	6.60	11.20	4.10
ANDES-A 1209	AJC 3465	male	CCS6	3.2295	-73.8603	27.00	13.00	NA	NA	1.50	NA	8.80	NA

Table S1-2: Continues.

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ANDES-A 1810	AJC 3884	male	CCSX	7.3496	-73.4981	31.00	15.45	NA	NA	1.60	NA	10.00	NA
ANDES-A 1834	AJC 3562	male	CCSX	5.2627	-74.8894	31.20	16.40	NA	NA	2.05	NA	9.10	NA
ANDES-A 1835	AJC 3554	male	CCSX	5.2627	-74.8894	33.60	17.90	NA	NA	1.90	NA	9.70	NA
ANDES-A 2105	AJC 4717	male	UCSR	-4.1033	-69.9749	32.20	17.10	NA	NA	1.70	NA	10.20	NA
ANDES-A 2106	AJC 4738	male	UCSR	-4.1033	-69.9749	30.60	16.00	NA	NA	1.55	NA	10.00	NA
ANDES-A 2108	AJC 4555	male	UCSR	-4.1033	-69.9749	31.50	16.20	NA	NA	1.60	NA	9.80	NA
ANDES-A 2108	AJC 4835	male	UCSR	-4.1033	-69.9749	32.10	16.10	NA	NA	1.25	NA	9.70	NA
ANDES-A 651	JMP 1851	male	UCSR	-4.1195	-69.9510	31.80	15.90	NA	NA	1.65	NA	9.90	NA
ANDES-A 652	JMP 1852	male	UCSR	-4.1195	-69.9510	32.10	15.70	NA	NA	1.60	NA	9.80	NA
ANDES-A 658	JMP 1858	male	UCSR	-4.1195	-69.9510	32.80	17.20	NA	NA	2.10	NA	10.20	NA
ANDES-A 663	JMP 1869	male	UCSR	-4.1195	-69.9510	32.10	17.10	NA	NA	1.90	NA	10.40	NA
ANDES-A 897	JMP 2142	male	UCSR	-4.1241	-69.9413	33.00	16.10	NA	NA	1.60	NA	10.00	NA
CPZ-UV3200	WB1983	male	caprarius	5.5337	-75.0407	25.40	14.80	9.90	3.50	1.20	4.40	8.80	2.80
CPZ-UV3822	EB30	male	CCSX	5.3674	-74.7935	32.50	15.10	11.10	3.20	2.10	5.05	9.90	3.10
CPZ-UV3823	EB031	male	CCSX	5.3674	-74.7935	31.80	16.45	11.90	3.70	2.20	4.40	10.10	3.00
ICN 11286		male	UCSR	-3.7651	-70.3869	33.80	16.30	11.20	4.30	1.70	4.80	10.30	3.30
ICN 11947	ARA 048	male	blairi	3.3346	-73.4216	31.40	16.40	10.90	3.10	1.70	5.00	9.90	3.10
ICN 20541	MC 141	male	UCSR	3.4604	-73.6241	32.40	16.50	10.60	4.00	1.60	5.40	9.70	3.00

Table S1-2: Continues.

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ICN 35722	MC 7359	male	UCSR	-3.3752	-68.2009	33.10	16.20	11.20	4.10	1.70	5.40	10.80	3.70
ICN 36604	MC 7709	male	UCSR	-1.3149	-69.5886	31.60	16.00	11.50	4.10	1.50	4.40	10.00	3.10
ICN 37105	JDL 19812	male	UCSR	-0.6237	-72.3856	29.20	15.00	10.90	3.80	1.60	4.80	9.10	3.30
ICN 37112		male	UCSR	-0.6218	-72.3828	28.20	14.20	9.70	3.60	1.40	4.60	9.80	3.30
ICN 37425	CMV 521	male	UCSR	-0.6325	-72.2500	29.70	15.10	10.80	3.70	1.70	4.80	9.80	3.10
ICN 39869		male	UCSR	1.1305	-76.5791	32.90	16.40	11.40	4.15	1.40	5.30	10.30	3.50
ICN 39871		male	UCSR	1.1305	-76.5791	33.30	16.70	11.70	4.30	1.70	5.20	10.30	3.20
ICN 41321	JDL 21646	male	CCSX	8.0178	-76.2147	30.50	16.20	12.00	4.50	1.80	5.70	10.10	3.60
ICN 42297	JC 011	male	CCSX	9.7614	-75.4748	34.91	16.67	12.22	5.10	2.51	5.78	11.29	3.37
ICN 42362		male	UCSR	1.0304	-76.6238	31.00	14.80	11.20	3.90	1.60	5.20	10.50	2.90
ICN 42363		male	UCSR	1.0436	-76.6111	32.00	16.40	11.70	4.10	1.60	4.60	10.90	3.10
ICN 47539	MC 11479	male	UCSR	-4.1195	-69.9505	32.40	15.60	11.10	4.00	2.00	4.90	10.00	2.90
ICN 48685	JDL 26773	male	CCSX	8.7217	-75.9906	31.70	15.90	11.00	4.30	1.60	5.20	10.30	2.90
ICN 49169	JDL 24660	male	CCSX	8.6000	-75.1500	27.90	13.80	10.00	3.30	1.50	4.50	9.50	2.10
ICN 51812	JDL 23607	male	UCSR	-4.0839	-70.0108	32.00	18.20	11.40	4.10	1.60	5.00	10.00	3.00
ICN 51828	JDL 24186	male	UCSR	-4.0819	-69.9963	31.60	15.10	11.30	4.00	2.00	4.90	9.80	3.20
ICN 52769	JMR 1660	male	UCSR	-3.6943	-70.2284	30.60	15.50	10.65	4.40	1.70	4.80	9.90	2.80
ICN 54574	JDL 27947	male	CCS3	7.3500	-73.5000	37.90	18.10	14.70	4.00	2.40	4.80	11.50	3.60
ICN 55181	HMR 368	male	CCSX	8.0085	-76.1202	27.70	14.10	9.40	3.80	1.70	4.60	10.00	3.90
ICN00932		male	CCS3	8.0155	-76.2032	37.34	19.84	13.42	4.95	2.24	6.70	11.29	4.04
ICN39198	CMV 670	male	CCS3	8.0227	-76.2095	37.31	19.04	12.89	4.79	2.74	6.61	12.23	3.94
ICN39199	CHV 671	male	CCS3	8.0227	-76.2095	36.20	19.60	13.55	4.52	2.40	6.53	11.62	3.78
ICN39202	CMV 698	male	CCS3	8.0227	-76.2095	37.75	19.92	13.10	4.80	2.63	6.65	13.02	3.34

Table S1-2: Continues.

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
ICN39204	CMV 676	male	CCS3	8.0227	-76.2095	37.75	19.92	13.10	4.80	2.63	6.65	13.02	3.34
ICN41321	JDL 21646	male	CCS3	8.0227	-76.2095	30.69	16.29	11.32	4.45	2.04	5.32	10.64	3.25
IGUN-AFAG467	AFAG 467	male	CCS1	6.3897	-70.4317	22.37	11.14	NA	3.26	1.40	3.97	7.35	2.59
IGUN-AFAG642	AFAG 642	male	CCS1	6.3994	-70.4359	27.16	12.90	8.83	3.38	2.00	4.23	8.64	2.80
MCZ:Herp:A-1519		male	UCSR	-1.4500	-48.4833	33.50	16.50	11.50	4.00	2.20	4.30	10.80	4.50
MNCN:H 15781		male	UCSR	-0.4516	-76.9956	33.80	18.40	12.40	4.30	2.00	5.10	10.90	3.80
MNCN:H 42981		male	UCSR	-14.1598	-68.5196	31.20	15.70	10.20	4.10	1.80	4.70	9.30	NA
MNCN:H 43718	IR 4672	male	chiquitanus	-13.1967	-70.5558	30.75	15.40	11.10	3.85	1.70	4.30	9.00	3.40
MNCN:H 44781	JMP 5159	male	ictericus	-12.6731	-68.7092	29.70	14.10	12.80	3.70	1.80	4.40	8.70	3.80
MNCN:H 44948		male	UCSR	-5.0575	-73.8540	34.90	17.80	11.40	NA	NA	NA	11.80	NA
MNCN:H 45151	IR 8110	male	UCSR	-4.1346	-73.4616	31.10	15.15	12.00	3.90	1.85	5.00	9.30	3.00
MNCN:H 45154	IR 8117	male	UCSR	-4.1934	-73.4774	29.85	14.70	11.40	4.00	1.60	5.30	8.80	3.70
MNCN:H 45161	JMP 1411	male	UCSR	-3.3309	-71.9168	32.70	15.10	9.80	4.35	1.90	5.55	10.60	4.50
MNCN:H 45163	JMP 1579	male	UCSR	-3.3309	-71.9168	33.40	16.20	11.55	4.40	1.80	5.30	10.70	3.75
MNCN:H 45166	JMP 1633	male	UCSR	-3.3309	-71.9168	32.10	16.35	12.30	3.60	1.85	5.00	9.70	4.10
MNCN:H 45186	JMP 2505	male	UCSR	-5.0575	-73.8540	33.70	15.00	11.00	4.40	1.80	4.60	10.50	3.90

Table S1-2: Continues

Catalogue No.	Col No.	Sex	Proposed group	Latitude	Longitude	SVL	TL	HL	ED	TD	ES	HW	IOD
MNCN:H 45188	JMP 2508	male	UCSR	-5.0575	-73.8540	26.40	12.35	10.90	3.30	1.60	4.40	8.70	3.40
MTD-TD 1756	MTD 46367/ RVM 401	male	UCSR	-11.0846	-75.3263	35.10	17.50	10.30	4.00	1.70	5.30	9.70	4.80
MTD-TD 1757	MTD 46368	male	UCSR	-11.0846	-75.3263	31.50	15.80	13.00	3.80	1.60	4.90	9.60	4.00
MTD-TD 2068	MTD 46486/ MLI 063	male	UCSR	-10.0705	-75.5558	32.00	15.30	10.60	3.70	1.80	4.90	8.90	3.50
ZSM 1606/2006		male	ruber	11.2886	-60.6706	33.10	17.45	NA	4.70	1.90	5.25	10.90	4.60
ZSM 289/2016		male	UCSR	-9.6167	-74.9333	31.60	17.00	11.20	4.10	1.90	5.80	10.00	5.20

Table S1-3. Best partition schema and respective evolution model selected by PartitionFinder for the complete evidence alignment (Lanfear et al., 2017).

Subset	**Best Model**	**MrBayes**	**# sites**	**subset id**	**Partition names**
1	GTR+G	GTR+G	565	7b298c837680186aae356daf4fcdbd48	Gene1
2	GTR+G	GTR+G	369	3e772a77276d326ffc348c09f7510f90	Gene2
3	TIM+I+G	GTR+G+I	220	8fb2803aa6897f181b0662e3012d7aac	Gene3_pos1
4	TRN	GTR	219	201378950fa8d82a0d3d41e36bfd4ed6	Gene3_pos2
5	TRNEF+G	GTR	219	213f168fcee1c0236daa77121eee9e2e	Gene3_pos3
6	GTR+I+G	GTR+I+G	104	c9efdf927b6e76a7798cc9375bf9f751	Gene4_pos1
7	GTR+G	GTR+G	103	38d205a19a26ba0aab6c815b8121cdd8	Gene4_pos2
8	TVMEF+I+G	GTR+I+G	103	2c26cf0a2c55067f244e883293ca5f29	Gene4_pos3
9	TVM+G	GTR+G	158	6452fec7c4fbd5773a56243c76167cc5	Gene5_pos1
10	TRNEF+G	GTR+G	158	f435d1214ad124e750f80614b8f099cd	Gene5_pos2
11	GTR+G	GTR+G	158	811acffea2d61d04d3dbf26b57df4c20	Gene5_pos3

Table S1-4. Mean p-distances within and among the "Scinax ruber" groups recovered in the phylogram and delimited after the revision of the nuclear haplotype networks. A. Distance within groups. B. Distances between groups. Taxon codes are identified in the text and in the phylogram of Figure 2.

A.

Taxon	mean p-distance within group (%)
CCSX	0.0
CCS1	0.1
CCS2	N.A.
CCS3	0.3
CCS4	N.A.
CCS5	0.0
CCS6	0.3
CCS6-C	1.6
CCS7	2.2
Scinax chiquitanus	1.2
Scinax funereus	N.A.
Scinax ictericus	N.A.
Scinax iquitorum	N.A.
Scinax oreites	N.A.
Scinax ruber s.s.	N.A
Scinax x-signatus Bahía	N.A.
UCSC	N.A.
UCSD	N.A.
UCSF	N.A.
UCSG	0.3
UCSR	1.4
UCSR-MTD-1	1.4
UCSR-MTD-2	N.A

Table S1-4. Continues.

B.

Taxon																			
Scinax iquitorum																			
Scinax ictericus	7.8																		
Scinax funereus	3.4	6.5																	
Scinax oreites	11.6	9.7	10.7																
CCS7	11.1	8.0	10.0	9.5															
Scinax chiquitanus	11.0	9.0	9.9	9.0	6.1														
Scinax x-signatus	14.2	15.7	13.8	16.3	16.7	16.2													
CCS1	12.3	11.5	10.5	12.5	12.2	12.7	12.8												
CCSX	12.2	11.5	10.1	12.4	12.0	12.2	13.1	1.6											
CCS2	13.2	12.2	11.4	12.1	11.1	11.5	13.6	9.7	9.6										
CCS5	13.3	12.7	11.6	13.0	11.7	12.2	14.7	9.2	9.2	1.7									
CCS6-C	13.8	12.8	12.0	12.6	12.0	12.4	13.8	10.5	10.4	1.2	2.2								
CCS6	13.6	12.4	11.6	12.5	11.3	12.1	14.2	9.4	9.3	1.0	1.2	1.4							
CCS3	12.7	12.0	11.3	13.3	12.2	12.2	15.5	9.6	9.9	5.1	5.2	6.1	5.0						
CCS4	12.7	13.2	13.2	13.5	12.1	12.2	17.2	10.9	10.9	7.7	8.5	8.3	7.7	8.0					
UCSC	17.2	16.0	15.1	14.7	14.6	14.2	19.6	12.0	11.3	9.3	9.8	10.4	9.4	10.7	7.9				
UCSF	13.6	13.7	13.5	13.4	12.8	11.8	17.2	9.8	9.8	8.5	8.4	9.0	8.3	8.5	4.7	1.8			
UCSG	13.8	11.6	12.3	12.6	11.5	11.2	18.4	9.7	9.3	8.5	8.0	8.8	7.7	8.3	6.8	4.7	3.7		
Scinax ruber s.s.	14.6	13.3	13.7	14.5	13.3	13.0	19.1	11.4	10.9	10.2	9.6	10.5	9.4	10.2	8.4	5.7	4.8	1.8	
UCSR	14.0	13.2	12.5	14.1	12.2	12.1	17.6	9.6	9.5	8.1	8.3	9.0	8.1	8.0	7.5	3.4	2.9	4.5	6.1

Table S1-4. Continues.

UCSR-MTD-1	13.7	13.7	12.4	12.8	12.2	12.3	17.9	9.6	9.3	8.3	8.5	9.0	8.3	8.3	7.6	3.6	2.8	4.2	6.2	1.8		
UCSR-MTD-2	16.4	16.1	14.6	15.3	14.9	14.9	20.6	13.0	12.3	11.9	11.9	12.4	11.8	11.7	10.8	7.7	6.9	9.1	10.6	6.2	4.4	
UCSD	16.3	14.9	14.5	15.3	14.2	19.5	19.5	11.6	11.7	10.3	10.5	10.9	10.2	10.8	9.1	5.4	5.4	7.1	8.9	5.5	5.8	9.0

2. Capítulo 2: The evolutionary lineages of "Scinax ruber" in the Magdalena Valley of Colombia: Towards an understanding and implications of cryptic species complexes in northern South America

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2.1 Abstract

Aim: “*Scinax ruber*” is one of the most widely distributed tree-frog species in the Neotropics*.* Despite its well-known status as a species complex, little research has been performed to discriminate the different lineages it includes, their characteristics and their relationships to habitats. Here we assess the diversity and distributions of the trans-Andean populations of what has been traditionally known as “*Scinax ruber”* and *“S. x-signatus*” in Colombia*,* comparing them to *S. caprarius*. We also identify a cis-Andean X-marked *Scinax* lineage closely related to them and the potential threats on survival of all the considered taxa.

Location: Cauca and Magdalena river valleys and norhteastern Andean piedmont of Colombia.

Methods: We performed an integrative analysis composed of the following: a) morphometric evaluation of 67 adult individuals, b) phylogenetic and phylogeographic analyses of 73 sequences of the 16S rRNA gene (23 provided in this study), and c) species distribution models (SDM) for each lineage identified here.

Results: Our analyses revealed three macro-sympatric and philopatric distinct evolutionary lineages in the Cauca-Magdalena valley of Colombia, morphologically allied to “Scinax ruber” s. l. and S. x-signatus but not necessarily related to them (e.g. S. caprarius). This allowed us to hypothesize on the origin of their actual distribution patterns and conservation caveats, as well as to reassess the taxonomy of the “S. ruber” species complex in the area.

Main conclusions: By recognizing the cryptic diversity of the studied groups, we identified three endemic, philopatric, and probably threatened evolutionary lineages instead of two wide-ranging nominal species. In addition, we confirmed that 1) S. x-signatus (as presently known) is neither present in the study area nor in Colombia; 2) S. blairi does not reach the northwestern piedmont of Colombia; and 3) the “X” or inverse “V” dorsal patterns traditionally used to diagnose and discriminate species within Scinax and especially to refer a given specimen to S. x-signatus are not of taxonomic and biogeographic relevance, while the colour pattern of hidden limbs is.

2.2 Introduction

Integrative taxonomy using phylogeographic approaches allows the discovery of evolutionarily divergent but morphologically similar populations, associated with historically shaped distributions. The outcome facilitates the proper description of the alpha diversity of a region (Schlick-Steiner et al., 2010). In the northern Andes ecoregion of South America this integrative approach is remarkably powerful. In the region with the greatest species richness of Earth (Hutter, Guayasamin, & Wiens, 2013), the taxonomically complex amphibians account for an important portion of the species richness (Jenkins et al., 2013). Besides high rates of species discovery per year (Fouquet, Gilles, et al., 2007; Funk et al., 2011) amphibians are also one of the most threatened groups of vertebrates (or the most) due to their physiological and ecological characteristics, which make them vulnerable to ecosystem fragmentation, global climate change, and pathogenic infections (Isaac, Redding, Meredith, & Safi, 2012; Stuart et al., 2004).

An important cause of the repeated underestimation of species richness, diversity, and threats to the amphibians of South America, especially lowland amphibians (Vacher et al., 2020), is the prevalence of cryptic species: multiple evolutionary lineages that are grouped under the same nominal taxon due to their (apparent) morphological similarity (Bickford et al., 2007). Ignoring the answer to the very basic question “What lives where?” makes it impossible to discover patterns and processes of diversification (Kim & Byrne, 2006) and to reveal actual, past, and potential distributional ranges and threats. Such issues are mandatory for improving conservation strategies that support conservation actions aimed at reducing the effects of habitat degradation.

This paper focusses on the hidden diversity, distribution, and conservation implications of the taxa currently subsumed under the names “*Scinax ruber*” (Laurenti, 1768) (Laurenti, 1768)and “*Scinax x-signatus*” (Spix, 1824) in the Magdalena region of Colombia (Hernández-Camacho et al., 1992) including the macro-sympatric *Scinax caprarius* (Acosta-Galvis, 2018). *Scinax ruber sensu lato* has been recorded for the entire distributional area of the genus *Scinax* (eastern Central America to northern Argentina), and beyond, often as an invasive species. Inside Colombia, it is present in the Caribbean region, the northern Chocó, the Cauca-Magdalena valley, the Orinoquia and the Amazonia regions, while records outside Colombia include Argentina, Brazil, Costa Rica, Ecuador, Guyana, Panamá, Perú, and Venezuela (Mendez-Narvaez, Ortiz-Navia, & Bolívar-G., 2014; Solís et al., 2010). As an invasive species, *Scinax ruber* has been recorded in Saint Lucia, Martinique, and Puerto Rico (Breuil & Ibéné, 2008; Joglar, 1998). In addition, previous molecular studies have revealed that "*S. ruber*" is a complex of cryptic species throughout its entire distributional range (W. E. Duellman et al., 2016; Ferrão et al., 2016; Fouquet, Vences, et al., 2007; Guarnizo, Paz, Muñoz, Flechas, & Crawford, 2015).

Scinax x-signatus (Spix, 1824) is still mentioned as a member of the Amphibian fauna of Colombia (Acosta-Galvis, 2021), despite the recent designation of a neotype (Araujo-Vieira et al., 2020) for populations in Brazil and Guiana, which, on that basis, regards the Colombian records as doubtful (no sequences from Colombia were available to the authors). On the other hand, *Scinax caprarius* (Acosta-Galvis, 2018) was confused with "*Scinax ruber*" until 1996, when Nieto-Castro (Nieto-Castro, 1996) identified and diagnosed a species of Scinax with finely spiculate dorsum, central vocal sac, a particular colour pattern on flanks and hidden hindlimbs, and several unique osteological characters. She called that taxon "Scinax sp. A," in her bachelor's degree unpublished manuscript, but it was not properly described until Acosta-Galvis's (2018) paper, in which he provided further morphological and acoustic evidence to diagnose the species. Confusion remains, however, and the pictures in the later paper suggest that there were records identified as S. caprarius and S. ruber which should require further examination.

In the present study we integrate and analyse genetic evidence together with morphological data and species distribution models (SDMs), aiming at assessing the real diversity and distributions of the "*Scinax ruber*-like" species complex in our study area, including *S. caprarius* and *S. x-signatus*. We also aim to clarify the taxonomy of the "*Scinax ruber*" lineages from the Cauca and Magdalena valleys of Colombia and to evaluate their use as biodiversity indicators, which has been a common omission in the Environmental Impact Assessments (EIA) carried in Colombia during the 21st century (i.e. ANLA, 2016). Consequently, we discuss the current distributions of those lineages under an actual conservation perspective, stressing the risks of further degradation of their habitats. Extensive livestock farming, extensive oil palm plantations, and hydrocarbon mining have been threats for at least three decades, but the actual "pilot research projects" (PPI, because of their initials in Spanish) of unconventional hydrocarbon exploitation ("fracking") and the still persistent civil war in the deep territories are the most actual and worrying hazards.

2.3 Methods

2.3.1 Study area

Our study area corresponded to low and middle elevations of the Cauca-Magdalena inter-Andean valleys of Colombia. The Cauca and Magdalena river basins constitute the most important drainage systems of the Andean region in the country. They are home for 80% of the Colombian human population, and most of the thermal and hydroelectric energy of the country is produced there (Cormagdalena, 2007). The Magdalena river runs from the Páramo de las Papas in Huila department --ca. 3700 meters above sea level (masl) in the southwestern Andes-- to its mouth in the Caribbean Sea, along a 1528 km course (Sarmiento, Puentes, & Sierra, 2015). The river passes through a variety of ecosystems including humid and tropical dry forests in the Andean foothills, wetlands, swamps, tropical dry forests, tropical rainforests, sub-xerophytic and xerophytic formations, and coastal savannas in the inter-Andean and coastal sections. That diversity provides a wide variety of habitat for frogs in general and for *Scinax* in particular. Nevertheless, all the river course is deeply affected by human impacts, such as colonization, degradation of forest for livestock, extensive plantations including cereals, cotton, sugar cane, and oil palm, among others (Garzón & Gutiérrez, 2013), and more recently, coal, oil, and mining for precious minerals (Güiza Suárez & Aristizabal, 2013; Veiga & Marshall, 2019).

2.3.2 Morphological and morphometric data

In the face of obvious morphological differences between the sampled populations, external morphological traits of specimens from different localities were described and measured (**Table S2-1**). Our review of specimens and occurrence records was based on material from the following collections: Museo de Historia Natural ANDES-Universidad de los Andes (ANDES-A), Colección Zoológica de Prácticas de Docencia-Universidad del Valle (CPZ-UV), Colección de Anfibios-Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH-Am), and Colección de Anfibios del Instituto de Ciencias Naturales-

Universidad Nacional de Colombia (ICN-Anfibios), plus still uncatalogued specimens housed in the Banco de Tejidos de la Biodiversidad Colombiana, Instituto de Genética-Universidad Nacional de Colombia (BTBC-IGUN). Permits for newly collected specimens were provided by the Resolution 0255/2014 of the Autoridad Nacional de Licencias Ambientales (ANLA), Ministerio de Ambiente y Desarrollo Sostenible de Colombia.

The following measurements according to W. E. Duellman & Wiens (1993) were taken: SVL (snout-vent length), TL (tibia length), FL (femur length), HL (head length), HW (head width), ED (eye diameter), TD (tympanum diameter), E-N (eye-nostril distance), E-S (eye-snout distance), IOD (interorbital distance), and FING (maximum width of terminal disc of the third finger). Sex and age for every individual were determined based on the presence of vocal sacs, vocal slits, and direct examination of testes for males and the oviductal state and the presence of eggs for females, when sex assignment based on external morphology was unclear. These measurements were taken to the nearest 0.01mm with an Ubermann digital calliper.

A Principal Component Analysis (PCA) and a permutational multivariate analysis of variance (PERMANOVA) were performed for adult individuals using PAST v 3.24 (Hammer, Harper, & Ryan, 2001) in order to identify the sources of variation among the dimensions and to assess the significance of morphometric differentiation among the populations considered ($p<0.005$). A possible morphometric dimorphism was considered by running independent analyses for each sex. Given that SVL is correlated with most of the other body dimensions (i.e. ED/HL, ES/HL, IOD/HW, TD/ED, TD/HL, and TD/HW), covariance was controlled by using the ratios of those measures to SVL.

2.3.3 Laboratory procedures, mitochondrial DNA amplification and sequencing

Our molecular sampling is based on a 570 bp fragment of the 16S ribosomal RNA gene (16S rRNA) obtained from 23 specimens and 50 additional GenBank sequences (Table S2-1). The 16S rRNA gene has been widely used to barcode amphibians and it is a powerful tool for discriminating individual species (J. Köhler, Glaw, Pabijan, & Vences, 2015; Nogueira et al., 2016; Vences et al., 2005). DNA extractions of samples preserved in 95%

ethanol were performed with the UltraClean Tissue and Cells DNA Isolation Kit (MoBio), following manufacturer's instructions. The polymerase chain reaction (PCR) was performed with the 16S A-L (5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16S B-H (5' - CCG GTC TGA ACT CAG ATC ACG T - 3') primers (Palumbi et al., 1991) under the following conditions: 30 µl total volume composed 3.00 µl of 10X PCR buffer, 2.40 µl of MgCl2 solution 25 mM, 0.60 µl of dNTP solution 10mM, 0.80 µl of each primer at 10µM, 0.90 µl of BSA at 10mg/ml, 0.15 µl of Taq polymerase at 5 U/µl, and 1 µl of DNA at a 10-18 µg/µl concentration. The volume was completed with ultra-pure water. The PCR cycle initiated with a 5-min-long denaturation step at 95º C, followed by 37 cycles of denaturation (95º C x 30''), annealing (58º C x 1') and extension (72º C x 1'), plus a final extension step (72ºC x 8''). PCR products were purified with the ammonium acetate protocol of Bensch *et al.* (2000). Sequences were obtained by an ABI 3130XL Genetic Analyzer automatic sequencer (Applied Biosystems™) in the sequencing facilities of the Servicio de Secuenciación y Análisis Molecular, Instituto de Genética – Universidad Nacional de Colombia (SSIGMOL-IGUN).

2.3.4 Phylogeographic, phylogenetic and haplotype network analyses

Tissues for molecular analyses were obtained from BTBC-IGUN and CPZ-UV. We performed two phylogenetic analyses: the first one with the aim of identifying the relationships among the populations of the Magdalena Valley and their supposed closely related lineages; and the second one, to map the relations between the clusters identified by us inside a wider sampling, including representatives of all the species groups of *Scinax* currently known for Colombia. For the first analysis, we used 23 frog sequences obtained by us and 10 sequences from GenBank, three of which were initially selected as outgroups: the aquatic *Pseudis paradoxa,* sequences from Argentina (AY843740) and Brazil (MK293738), and the arboreal *Scinax boulengeri*, from Costa Rica (AY843755). For the second analysis, we included 10 sequences obtained by us and 40 sequences from GenBank. We also included sequences of *Dendropsophus microcephalus* (KP149423), *Phyllomedusa venusta* (MG030711) and *Sphaenorhynchus lacteus* (JF790144) as outgroups, in addition to the two *Pseudis paradoxa* sequences mentioned above.

The resulting chromatograms were explored using CHROMAS (http://www.technelysium.com.au/chromas.html). The sequences were then aligned with CLUSTALW as implemented in BIOEDIT (Hall, 2005), and visually inspected. Every alignment obtained was manually quality controlled. The best model of evolution was determined using JMODELTEST 2.1.7 (Darriba, Taboada, Doallo, & Posada, 2012) as GTR-G. A Maximum Likelihood analysis (ML) was performed with RAXMLGUI 2.0.0 (Edler, Klein, Antonelli, & Silvestro, 2021) using the thorough bootstrap and 1000 repetitions. A Bayesian analysis was conducted with MRBAYES 3.2.1(Ronquist et al., 2012), using 10,000,000 repetitions, a burning fraction of 0.25, four Markov chains and a tree sample frequency every 1000 generations.

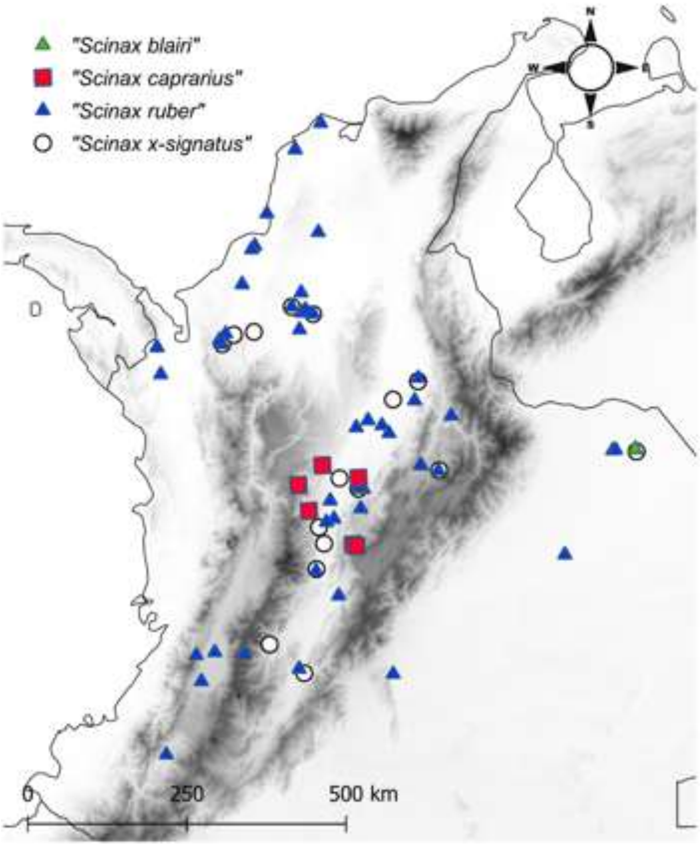
To quantify the genetic differentiation between and within the revealed monophyletic groups, uncorrected p distances for the16S rRNA gene fragment were calculated using MEGA 10.1.8 (Kumar et al., 2018). In these analyses, six sequences (EF217488 from French Guiana: Kourou, KDQF01000927 from Martinique, Diamant; KDQF01003307 from Brazil, Amapá, Macapá; KDQF01001470 from Brazil, Bahia, Una; KU495575 from Brazil, Pernambuco, Sanharó and KU495576 from Brazil, Bahia, Povoado) were downloaded from GenBank and incorporated by Araujo-Vieira *et al*. (2020) and recovered within the same clade, being the later the neotype of *S. x-signatus* . Additionally, a haplotype network was drawn using TCS 1.21 (Clement et al., 2000). Mutational steps among distantly related haplogroups were determined with DNASP (Rozas et al., 2017).

2.3.5 Integrative taxonomy and delimitation of species

Considering conspicuous morphological traits according to the original descriptions of *Scinax blairi*, *S. caprarius*, *S. ruber*, and *S. x-signatus*, as well as the variation among the specimens identified as any of those, several adult morphotypes were established and incorporated into the analyses, even if they were collected outside our study area. During the mid-90s, an X-marked "*Scinax ruber*” was reported to occur in the northwestern Orinoquia area (Ruiz-Carranza, Ardila-Robayo, & Lynch, 1996) in a locality that was close to the records provided by Guarnizo *et al.* (2015) for a “*Scinax ruber*” lineage present on both northern slopes of the Cordillera Oriental. Conversely, tadpoles from Arauca in the vicinity of Caño Limón, consistent with those described by León (1975) for *S. x-signatus*, were reviewed and identified as such by Lynch & Suárez Mayorga (2011), precisely along the north-eastern slope of the Cordillera Oriental where *S. blairi* was also reported to occur. We included *S. blairi* in our comparisons simply because it was described as a member of the “*Hyla rubra*” species complex (Fouquette & Pyburn, 1972) with an open parentheses scapular/sacral dorsal pattern, which may resemble the X-signed pattern.

In our taxonomic description we included sequences from Andean Amazonia and from the nominal type locality of *Hyla x-signata Spix*, 1824, in order to clarify the presence of *Scinax x-signatus* (Spix 1824) in Colombia. A geographic account of our samples is shown in **Figure 2-1**, according to the taxonomic identifications in the hosting collections of the specimens.

Figure 1-19: Distribution in Colombia of the samples evaluated in this analysis with their *a priori* assigned identity.



We follow Vieites *et al.*, (2009) in assigning categories for candidate species based on the combination of different lines of evidence, such as uncorrected pairwise distances for the 16S rRNA fragment, morphology, distribution, and bioacoustics. Of special relevance for our study are confirmed candidate species (CCS), that means those that are distinct in morphology and bioacoustics and with high genetic divergences, because they likely present distinct undescribed species.

2.3.6 Species distribution models (SDM) and effects on conservation

For each CCS and *Scinax caprarius*, a SDM was built following the methods described in (Vargas-Ramírez et al., 2016). We incorporated geographic coordinates for primary occurrence records (Table S2-3) and WorldClim 2.1 data (Fick & Hijmans, 2017) and used ArcGis Pro 2.4.2 (ESRI, 2012) and the MaxENT algorithm (Phillips *et al.*, 2020) for inferring SDMs. Later we contrasted sample occurrence data with an oil production layer (Agencia Nacional de Hidrocarburos de Colombia geoportal, www.anh.org) to identify the risk of oil production-related activities (especially the deployment of oil wells and PPI) congruent to the habitats of every evolutionary lineage considered.

2.4 Results

2.4.1 Molecular, morphological, and morphometric data

All studied collection specimens are listed in **Table S2-1**. Catalogue/collection number, sex, age category, and geographic coordinates (when available) were recorded. The molecular information for each specimen and the GenBank sequences are summarized in Table **S2-2**. From the collection-recorded identities of our samples we defined four operational taxonomic groups: Scinax1 for the cis-Andean population, Scinax3 and ScinaxX for the inter-Andean populations according to their distinctive morphologies (Figure 2-2), and finally, *Scinax caprarius*.

Table 2-1 contains the morphological dimensions (ranges and mean) of the specimens measured and reviewed to perform the PCA, once assigned to an operational group. We assigned 9 individuals to S. caprarius, 4 to Scinax1, 32 to Scinax3, and 22 to ScinaxX (**Table 2-1**). A graphic representation of the PCA is shown in Figure 2-3 a, b; and from that outcome there seems to be a morphometrical overlapping among populations. Considering males only (**Figure 2-3**, a) the first principal component (PC1) explained 47.15% of the variance with longitudinal head dimensions (TD/ED = 0.90; ED/HL = 0.23) accounting as its main loadings. The second principal component (PC2) explained 29.57% of the variance of which transverse head dimensions (IOD/HW = 0.69; ES/HL = 0.44) were its main loadings. Nonetheless, the PERMANOVA results (Table 2a) indicated a significant morphometric difference ($p<0.05$) among the Magdalena Valley lineages but not among any of the trans-Andean groups and the cis-Andean Scinax1.

Figure 1-20: Evolutionary lineages of the "*Scinax ruber*-like" group in the Magdalena Valley of Colombia and the western Llanos. (a, b) Scinax1 (AFAG 473, Arauca, Cravo Norte). (c, d) Scinax3 (MAV 326, Antioquia, Yondó. (e, f) ScinaxX, (ICN 36617 Huila, Villavieja). (g, h) Scinax caprarius, (CPZ-UV 5990 Cundinamarca, Nocaima). Scale bar equal to 1 cm.

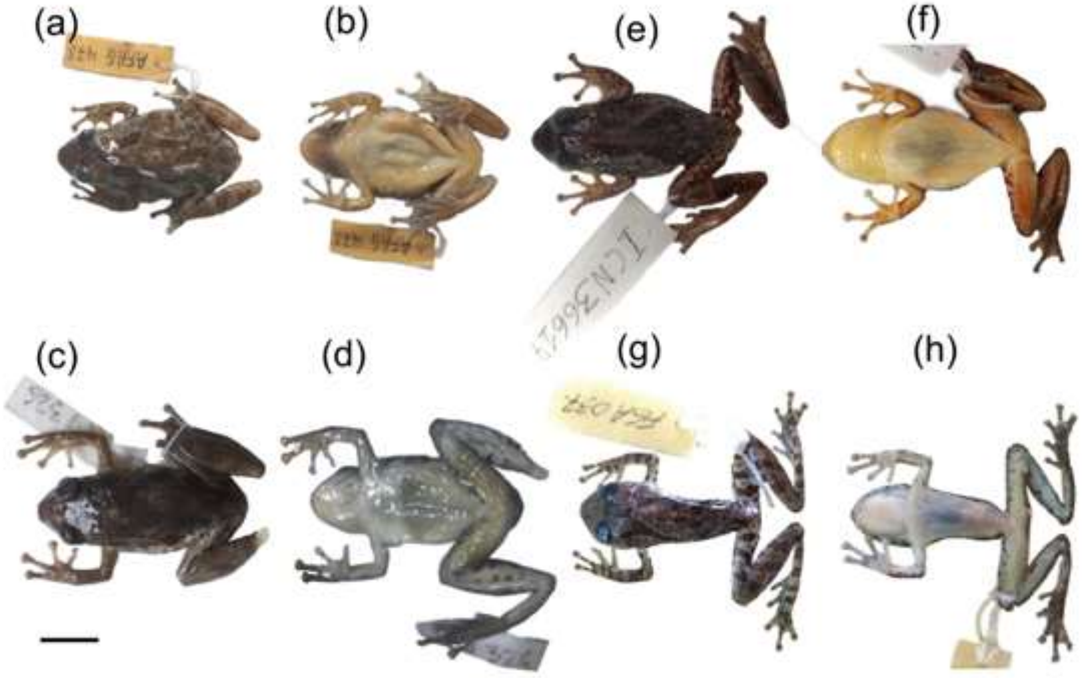


Table 2-5. Dimensions used in the morphometrical analysis (**Figure 2-3**). Values are expressed in mm and were taken for adult individuals. SVL=Snout-vent length, TL=Tibia length, FL=Femur length, TD=Tympanum diameter, ED=Eye diameter.

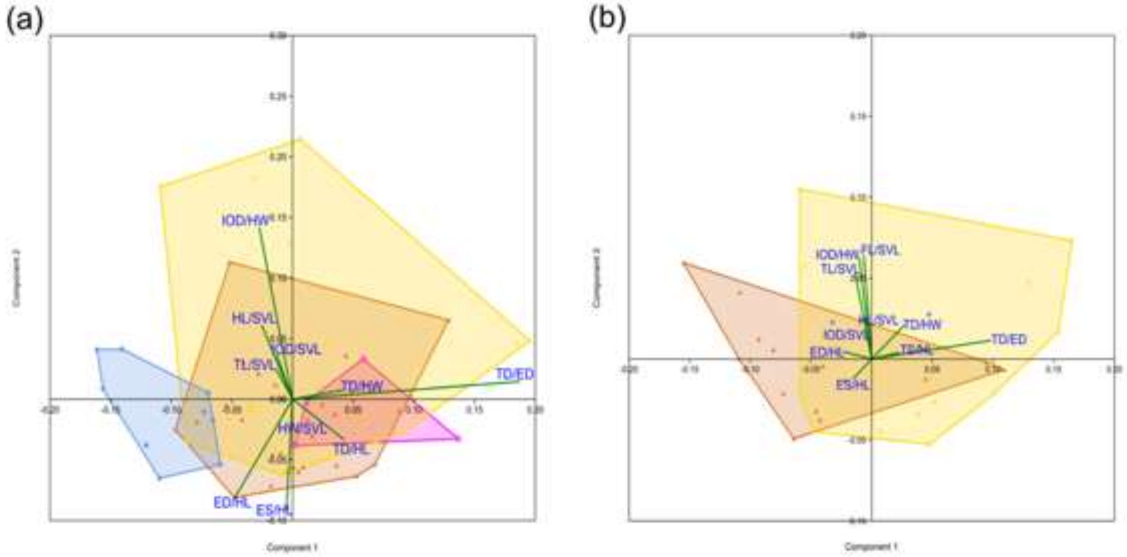
Group	Sex	N	ED	ES	HL	HW	IOD	SVL	TD
Scinax1	Male	4	3.73–4.53	5.04–6.18	10.35–12.67	9.74–12.04	3.20–4.07	32.96–39.70	1.83–2.40
Scinax3	Male	32	3.85–5.52	4.40–7.00	10.65–14.15	9.56–14.00	2.99–4.65	34.06–42.00	1.85–2.74
Scinax3	Female	16	4.06–5.58	5.70–7.33	11.50–15.95	11.14–14.89	3.04–4.83	33.56–46.84	1.80–2,93
ScinaxX	Male	22	3.10–5.20	4.20–6.20	9.40–13.35	9.00–12.70	2.76–4.20	27.70–38.83	1.55–2.88
ScinaxX	Female	16	3.41–5.04	4.80-6.57	10.42–13.27	8.80–12.32	2.80-4.00	28.10–38.55	2.06–2.68
S. caprarius	Male	9	3.50–4.67	4.40–5.65	9.83–11.88	8.80–11.19	2.80–3.65	25.40–31.56	1.20–1.92
S. caprarius	Female	2	4.23–5.22	5.74–6.70	11.60–14.27	11.52–13.93	3.83–3.88	34.17–40.29	2.37–2.51

Figure 2-3, b represents graphically the PCA of the measurements of female-only individuals; no adult female specimen of Scinax1 was available for comparison. We assigned 16 females to Scinax3, 16 to ScinaxX, and two to *S. caprarius*. The PC1 explained 62.83% of the variance, but in this group the entire head (TD/ED = 0.89; TD/HW =0.25) accounted for the main loadings. The PC2 explained 13.86% of the variance and its principal loadings were FL/SVL (0.58) and IOD/HW (0.57). The combination of longitudinal and transverse measurements in the loadings of females suggested an apparently evident difference in size that was statistically significant between Scinax3 and ScinaxX (**Table 2-2, b**).

2.4.2 Phylogeographic, phylogenetic and haplotype network analyses

Both tree-building approaches confirmed the presence of two monophyletic and robustly supported lineages inside the nominal “*Scinax ruber*” for the Cauca-Magdalena valleys of Colombia (one of which was traditionally confused with *S. x-signatus*), plus *S. caprarius* (**Figures 2-4, 2-5**):

Figure 1-21: Morphometric analysis of 105 specimens of the different evolutionary linages genetically identified. (a) PCA of size-corrected morphometric variables for male individuals. (b) PCA of size-corrected morphometric variables for female individuals. Colour codes: blue = *Scinax caprarius*, brown = *ScinaxSCINAX3*, pink = *Scinax1*, yellow = *ScinaxX*,



a) ***Scinax*X** contained individuals identified as *Scinax ruber* and *S. x-signatus*, which morphologically were similar to the latter but were genetically distinct (**Figure 2-4**). The following localities have been genetically confirmed with tissues and voucher samples: Caldas (La Dorada); Córdoba (Ayapel), Cundinamarca (Nilo), Santander (Sabana de Torres and Los Santos), Tolima (Guayabal), and Valle del Cauca (Cali). All of those localities except for the last one were inside the Magdalena Valley and the Caribbean plains of Colombia, while Cali is located in the Cauca Valley (Morrone, 2014). The Cauca and the Magdalena valleys have been connected since their origins (Mora et al., 2018), and their ecosystems are generally similar.

b) **Scinax3** corresponded to samples identified as S. ruber and S. x-signatus in the very humid portion of the Magdalena Valley of Colombia: Antioquia (Yondó), Córdoba (Ayapel and Pueblo Nuevo), Santander (Barrancabermeja, Sabana de Torres, and San Vicente de Chucurí) and Sucre (San Benito).

Table 2-6. **a.** PERMANOVA results for the morphometric differences between the evaluated groups using males only. Significantly different values (p<0.05) are indicated with an asterisk (see **Figure 2-3a**). **b.** PERMANOVA results for the morphometric differences between the evaluated groups using females. Significantly different values (p<0.05) are indicated by an asterisk (see **Figure 2-3b**).

a.

Group	Scinax1	Scinax3	ScinaxX	*S. caprarius*
Scinax1		0.0586	0.2288	0.0008*
Scinax3	0.0586		0.0428*	0.0001*
ScinaxX	0.2288	0.0428*		0.0002*
S. caprarius	0.0008*	0.0001*	0.0002*	

b.

Group	Scinax3	ScinaxX	*S. caprarius*
Scinax3		0.0174*	0.5275
ScinaxX	0.0174*		0.6143
S. caprarius	0.5275	0.6143	

c) ***Scinax caprarius*****:** In our initial analyses (inside the "*Scinax ruber*" group, **Figure 2-4**) this was the sister clade of a GenBank sequence of *S. x-signatus* from Bahia, Brazil (the type locality of the species). However, considering the very particular morphology of this taxon --externally it resembles *Scinax sateremawe* (Sturaro & Peloso, 2014) for which molecular information in GenBank is lacking-- we decided to compare it to other *Scinax* recorded for Colombia, thus running a more extensive analysis (see the Methods section). In the resulting topology and in every subsequent analysis with a wider sampling, this taxon was the sister clade of *S. elaeochroa* **Figure 2-5**).

ScinaxX was always recovered as the sister species of the cis-Andean *Scinax1* from the northern Orinoquia piedmont and plains, and it is phylogenetically well separated from *Scinax3*. *Scinax caprarius* is not phylogenetically related to any newly identified lineage.

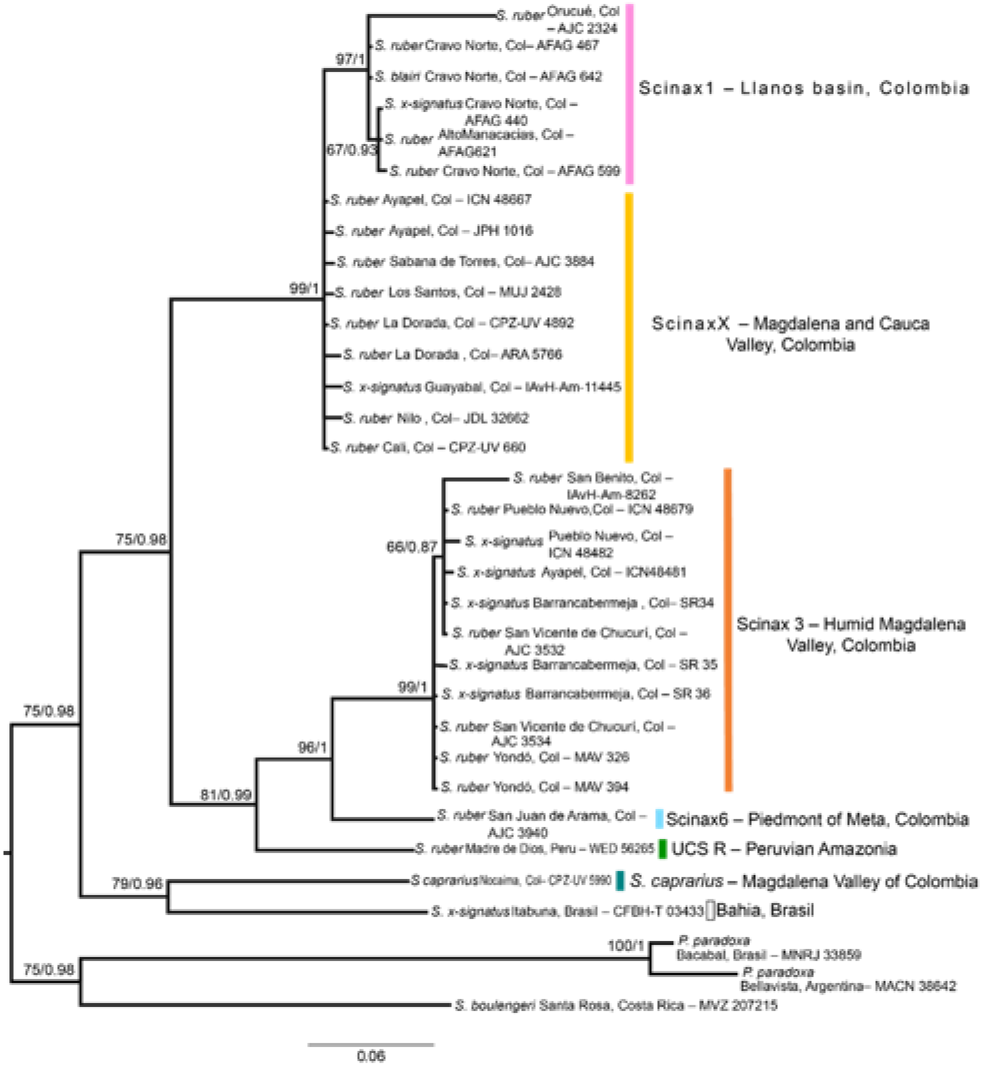
The uncorrected genetic *p*-distances in the 16S rRNA fragment between and within groups are presented in **Table 2-3**. Distances between our anticipated Magdalena Valley groups were larger than 3% —10.0% minimum— but within groups they were smaller than 0.5%. Therefore, we are confident in regarding those lineages as evolutionarily different candidate species. Scinax1 and ScinaxX were separated by a between-groups uncorrected *p* distance of only 1.7%, which was well below the traditional 3% threshold. However, integrative evidence suggested and supported the existence of two different CCS inside this complex and the SDMs complied with that assignment.

ScinaxX was always recovered as the sister species of the cis-Andean Scinax1 from the northern Orinoquia piedmont and plains, and it is phylogenetically well separated from Scinax3. *Scinax caprarius* is not phylogenetically related to any newly identified lineage.

Table 2-7. Uncorrected *p* distances for the 16S rRNA fragment of the phylogenetic groups identified. Percentages shown on the diagonal in italics are within-group divergences; percentages below the diagonal are between-group divergences. Abbreviations follow Suárez-Mayorga et al. (2020): CCS6 is the lineage of the eastern piedmont of Colombia; UCSR is an Amazonian complex of species; cap and *elaeo* are *Scinax caprarius* (our samples) and S. *elaeochroa* from Costa Rica (EF376076 and AY843757), respectively. 03433 is CFBHT03433 (KU495577), former identified as *S. x-signatus* from Brazil, Bahia, Itabuna and later as *S. argyreornatus* by Araujo-Vieira *et al*. (2020); *xNeo* is what Araujo et al. (*ibidem*) defined as *Scinax x-signatus* (EF217488 from French Guiana, Kourou, KDQF01000927 from Martinique, Diamant, KDQF01003307 from Brazil, Amapá, Macapá, KDQF01001470 from Brazil, Bahia, Una, KU495575 from Brazil, Pernambuco, Sanharó and KU495576 from Brazil, Bahia, Povoado Senote).

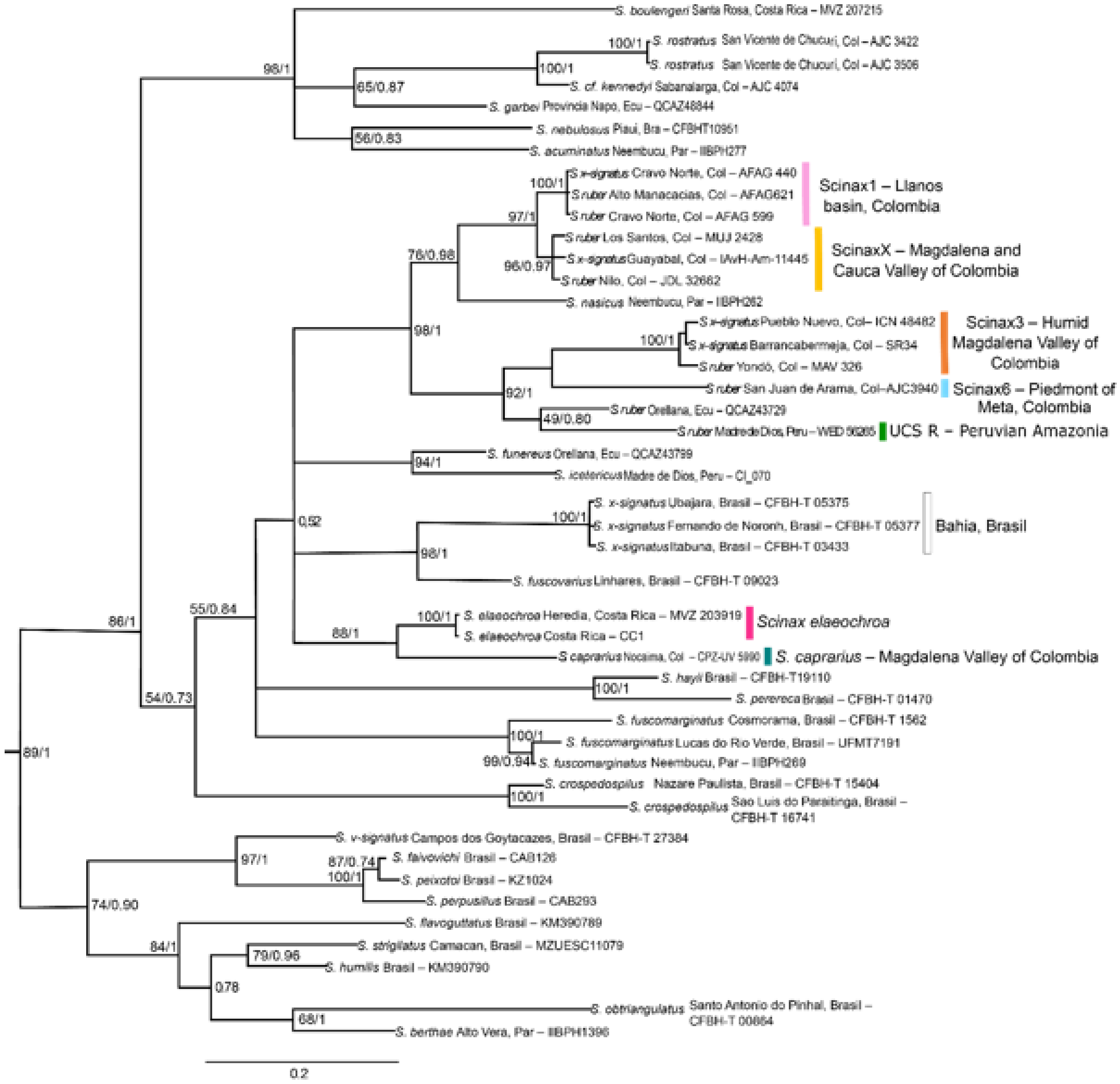
Taxa	N	CCS1	CCS3	CCS6	CCSX	UCS R	*cap*	*elaeo*	03433	*xNeo*
CCS1	6	*0.1*								
CCS3	11	10.1	*0.5*							
CCS6	1	9.8	6.7	-						
CCSX	9	1.7	10.0	10.4	*0.5*					
UCS R	1	9.7	8.0	8.7	9.3	-				
S. caprarius	1	13.3	13.8	14.9	12.6	13.5	-			
S. elaeochroa	2	10.1	13.0	13.6	9.4	11.6	7.9	0.0		
CFBHT03433	1	13.4	14.5	14.3	13.7	15.3	12.8	14.1	-	
S. x-signatus (Araujo-Vieira et al. *2020)*	6	12.4	14.1	13.7	12.9	14.4	12.0	12.7	0.8	*0.8*

Figure 1-22: 16S rRNA phylogeny of *Scinax caprarius, "S. ruber" and "S. x-signatus"* and their related lineages for the Magdalena valley of Colombia. Numbers adjacent to nodes are bootstrap and bayesian posterior probability (BPP) values, respectively. Names adjacent to tips are nominal species identified a priori, followed by its locality and its voucher ID. Clade names follow the nomenclature proposed by Suárez-Mayorga *et al*. (2021).



The uncorrected genetic *p*-distances in the 16S rRNA fragment between and within groups are presented in **Table 2-3**. Distances between our anticipated Magdalena Valley groups were larger than 3% —10.0% minimum— but within groups they were smaller than 0.5%. Therefore, we are confident in regarding those lineages as evolutionarily different candidate species. Scinax1 and ScinaxX were separated by a between-groups uncorrected *p* distance of only 1.7%, which was well below the traditional 3% threshold. However, integrative evidence suggested and supported the existence of two different CCS inside this complex and the SDMs complied with that assignment.

Figure 1-23: 16S rRNA phylogeny of clades identified in **Figure 2-2** within *Scinax* genus. Numbers adjacent to nodes are bootstrap and bayesian posterior probability (BPP) values, respectively. Names adjacent to tips are nominal species identified a priori, followed by its locality and its voucher ID. Clade names follow the nomenclature proposed by Suárez-Mayorga et al. (2021).

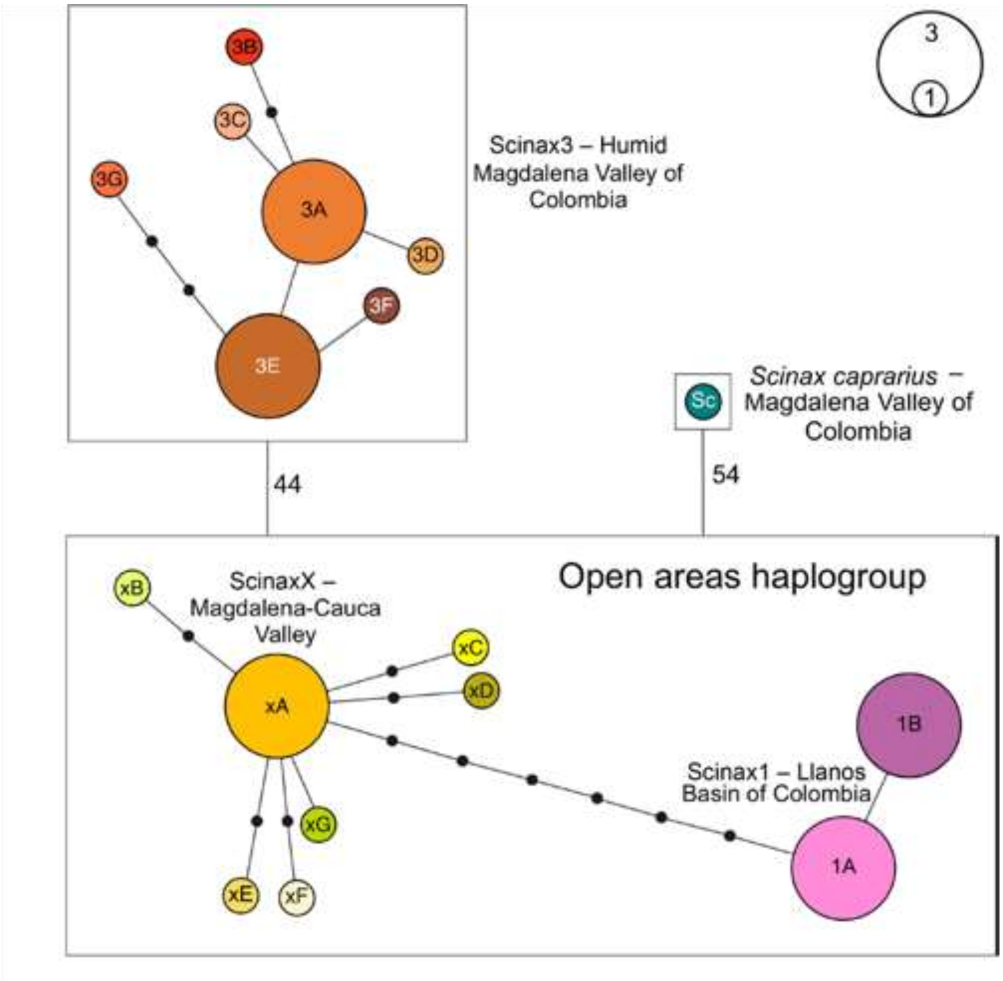


The resulting haplotype network for groups Scinax1, Scinax3, ScinaxX and *S. caprarius*, is presented in **Figure 2-6**, while **Figure 2-7** shows the georeferenced collection records included in the network. Scinax1 and ScinaxX haplotypes constituted an haplogroup distributed in open areas. Scinax1 had two haplotypes: 1A and 1 B. ScinaxX contained seven haplotypes (xA, xB, xC, xD, xE, xF, and xG), Scinax3 contained seven haplotypes as well (3A, 3B, 3C, 3D, 3E, 3F, and 3G) and S. caprarius was represented by the haplotype Sc. The identification of each of the georeferenced haplotype is presented in **Table S2-2**.

Haplotype 3A was shared between three samples from Córdoba (Ayapel), Santander (Barrancabermeja), and Sucre (San Benito). Haplotypes 3B and 3C were found in Córdoba (Pueblo Nuevo) but 3B was separated from 3A by two mutational steps, while 3C was separated from 3A only by one. Haplotype 3D was also from Santander (San Vicente de Chucurí) and diverged from 3A by a single mutational step. The same pattern was present between haplotypes 3E and 3A: a divergence of only one mutational step between samples from Antioquia (Yondó) and Santander (Barrancabermeja and San Vicente de Chucurí) located on the same ecosystem but from different margins of the Magdalena river. Haplotype 3F was from Barrancabermeja as well, and it was separated from 3E by one mutational step. Haplotypes 3E and 3G were found macro-sympatrically in Antioquia (Yondó), but they were separated by three mutational steps.

The group formed by haplotypes of Scinax1 and ScinaxX clades was separated from the group of Scinax3 haplotypes by 44 mutational steps. Haplotype xA was the only haplotype present in more than one locality as follows: Caldas (La Dorada), Córdoba (Ayapel), and Valle del Cauca (Cali). Haplotypes xB (Ayapel), xC (Santander, Sabana de Torres), xD (Santander, Los Santos), xE (La Dorada), and xF (Tolima, Guayabal) were separated from xA by two mutational steps, while haplotype xG, from Cundinamarca (Nilo) was separated from xA by only one mutational step. Regarding Scinax1 haplotypes, haplotype 1A was separated from haplotype xA by seven mutational steps. This differentiation is congruent with the actual isolation caused by the Cordillera Oriental of Colombia. On the other hand, *S. caprarius* was represented just by one sequence (haplotype Sc) that was 54 mutational steps distant from the Scinax1 and S. ScinaxX haplogroups. This pronounced divergence confirms *S. caprarius* as a deeply divergent evolutionary lineage, completely outside of the "*Scinax ruber*-like clades."

Figure 1-24: Haplotype network of “Scinax ruber-like” evolutionary lineages in the Magdalena-Cauca Valley and Northwestern Orinoquia, plus Scinax caprarius. The size of the circle represents frequency. Mutational steps are represented by lines separated by black dots. Black dots represent missing or unsampled haplotypes. Colour code follows previous figures.

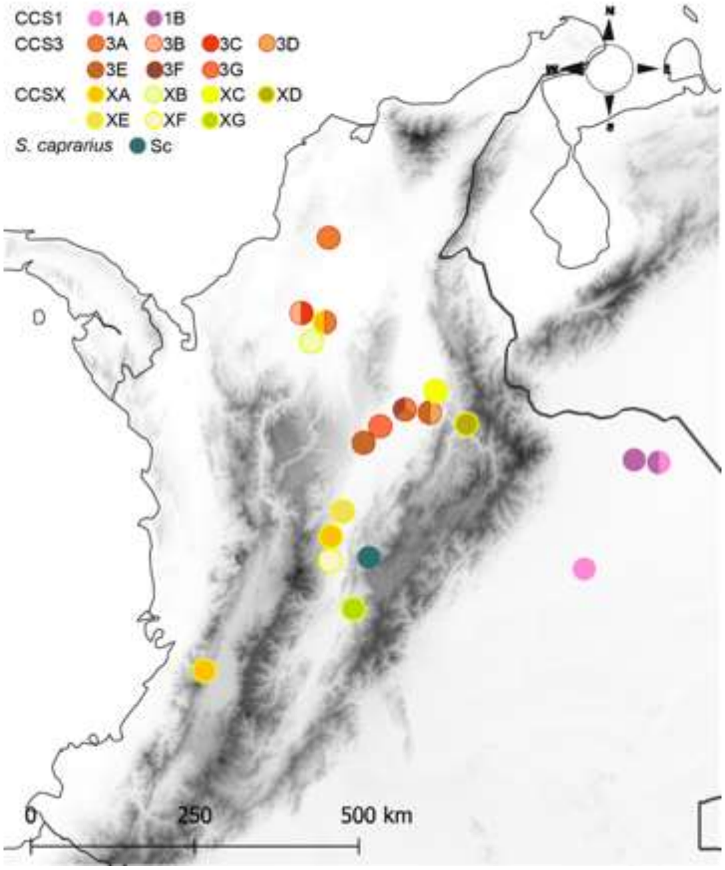


2.4.3 Integrative taxonomy and delimitation of candidate species

The three distinct evolutionary lineages identified in the present study are well supported as confirmed candidate species; hence they are being formally described for science. The macro-sympatric ScinaxX and Scinax3 are morphologically distinguishable based on size and colour pattern on the concealed thighs. Scinax1 is not easily distinguishable from

ScinaxX –except for the position of the vocal sac—but they live in different slopes of the Cordillera Oriental of Colombia, and they occupy different habitats.

Figure 1-25: Geographic location of the identified mitochondrial haplotypes for *Scinax caprarius*, *S. sp.* Scinax1, *S. sp.* Scinax3 and *S. sp.* ScinaxX inside Colombia. Colour code corresponds to that used in **Figure 2-4**.

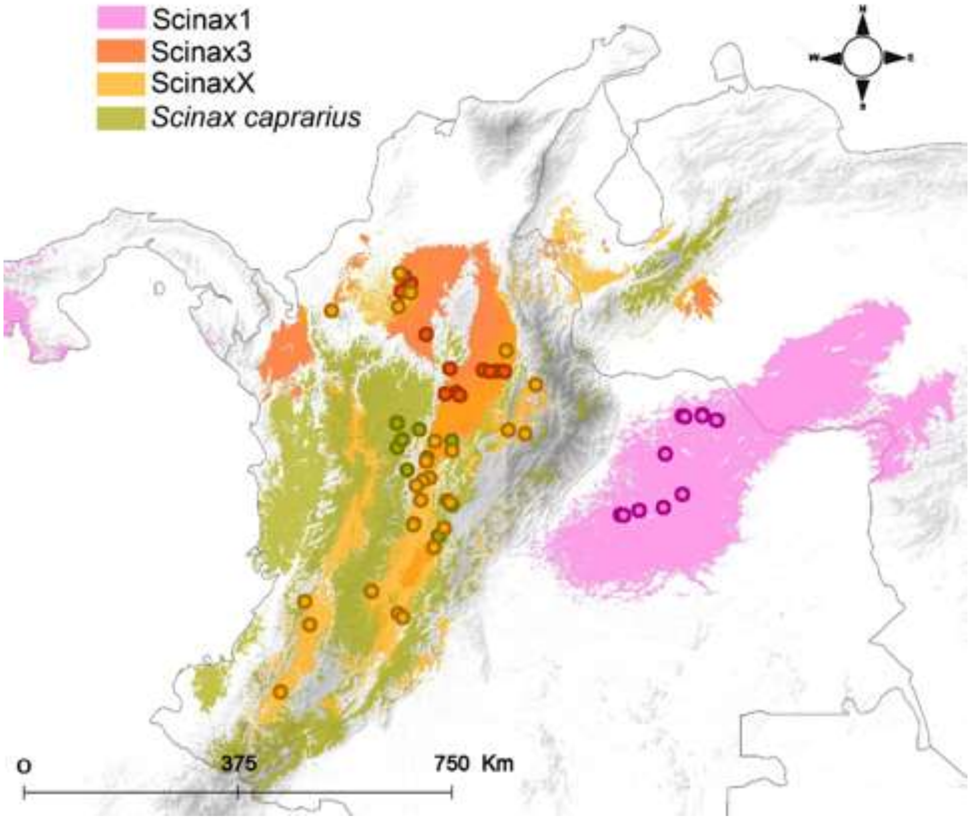


2.4.4 Species distribution models (SDMs) and effects on conservation

The SDMs for the taxa considered herein are presented in **Figure 2-8**. Scinax3, ScinaxX and *S. caprarius* were macrosympatric for the Magdalena Valley. However, the former two seemed to be restricted either to humid or to drier areas, respectively, underlining the ecological heterogeneity that characterizes this region. Both groups were mutually exclusive on their modelled distributions. Scinax3 was not restricted to the Magdalena

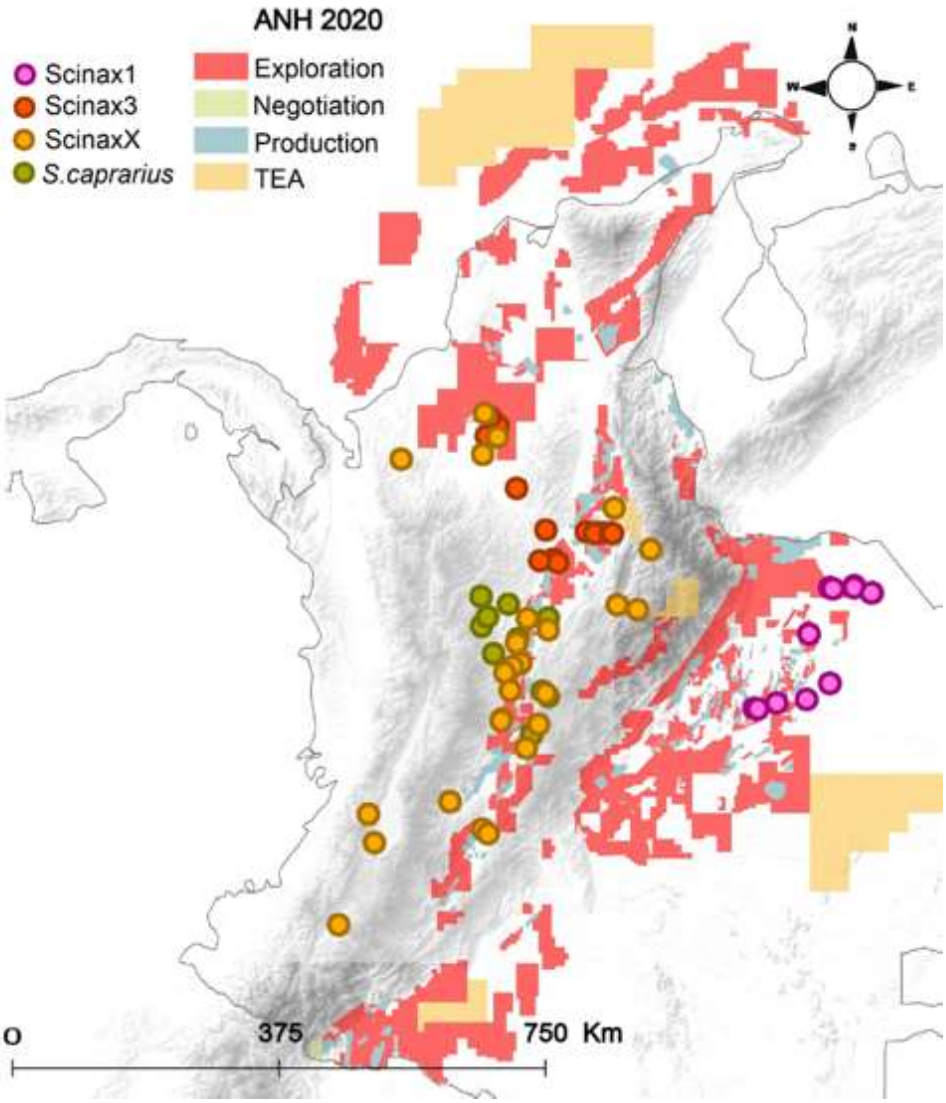
Valley but to the Chocó-Magdalena province defined by Hernández-Camacho *et al*. (1992), including the northern cis-Andean Pacific lowlands of the country. *Scinax caprarius* was distributed along the entire inner piedmonts of the Cordillera Central and Cordillera Oriental, reaching the lower border of the montane forest ecosystem (1800 masl). The distribution of ScinaxX reached almost the same extension in the Magdalena Valley and potentially, along the Cauca valley, but ScinaxX was restricted to the drier, lower (up to 800 m elevation), and more open areas.

Figure 1-26: Spatial Distribution Models of *Scinax1*, *Scinax3*, *ScinaxX* and *Scinax caprarius*. Overprediction was left in the map to indicate suitable habitat according to the climatic envelope.



It seems possible that several anthropogenic threats are affecting the newly identified CCSs, considering the history of human colonization and human intervention all along the Magdalena and Cauca valleys. Given that Colombia has produced oil and gas since the mid-20th Century, the maintenance of the economic balance and the self-supply of hydrocarbons have been priorities for every government. Nowadays, "*Proyectos Piloto de Invesigación*" (PPI in Spanish, or Pilot Research Projects in English) for the initiation of unconventional oil exploitation (fracking) are beginning, and a superficial look to our **Figure 2-9** (distribution records of each lineage over the map of oil blocks with present negotiation, exploration, or exploitation processes) will show the extensive overlap of these commercial activities with our newly defined lineages.

Figure 1-27: Occurrence data for *Scinax1*, *Scinax3*, *ScinaxX* and *Scinax caprarius* lineages contrasted with oil production activities in Colombia.



2.5 Discussion

Our results provide evidence for the existence of three genetically distinct and deeply differentiated evolutionary lineages of "*Scinax ruber" s. l.* for the Magdalena region (Hernández-Camacho *et al.*, 1992) of Colombia, but neither should be identified as *Scinax x-signatus* nor to be related to it, despite their morphological character of and X-marked dorsum or the original description of the species by Spix (1824). This is clarified in the manuscript presented in Chapter 4 (Addendum), where the identification of the Spix types, as well as other material remaining in the Zoologische Staatssamlung München (ZSM) is reviewed.

The identification of those lineages and their potential distributions illustrate the threats and opportunities of survival for populations and the ecosystems they inhabit. Regarding our sampling, the topology in **Figure 2-4** recovered two large clades, each of them with a particular ecological association. The clade comprised of the trans-Andean ScinaxX and the cis-Andean Scinax1 seems to prefer open habitats, while the clade composed by the trans-Andean Scinax3 and its sister cis-Andean relative is apparently linked to well-preserved forests.

According to the field notes that were available to us, Scinax1 inhabits mosaic savannas with estuaries, wetlands, and gallery forests, while ScinaxX occupies the dry lowlands of the Cauca and Magdalena valleys. Both lineages seem to flourish in a landscape of wetlands, pastures, and tropical dry forest galleries. Even though both are externally very similar (especially after preservation), Scinax1 seems to be slightly larger. Although the uncorrected *p*-distance of the16S rRNA fragment between them is well below the general 3% threshold for species distinctness (Vences *et al*., 2005), our data suggested the existence of a premating reproductive barrier due to their distributions and habitat preferences (**Figure 2-8**); this is supported by the presence of unique haplotypes in the mitochondrial (**Figure 2-6**) and the nuclear fragments (A. M. Suárez-Mayorga, Castillo-Rodríguez, Ernst, Fritz, & Vargas-Ramírez, 2021). Comparably low uncorrected *p*-distances in 16S rRNA between different evolutionary lineages have been also observed outside Hylidae, as in *Engystomops* (Funk *et al*., 2011) and *Physalaemus* (Lourenço *et al*., 2015).

There is extensive palynological, sedimentological, and paleontological evidence supporting the existence of several marine-lacustrine intrusions into northern South America during the Miocene (Boonstra, Ramos, Lammertsma, Antoine, & Hoorn, 2015; De la Parra, Pinzón, Rodríguez, Bedoya, & Benson, 2019; Rasanen, Linna, Santos, & Negri, 1995). The Llanos and western Amazonia were covered by a mega-wetland system during the Precursor (24 to 16 Ma), Pebas (16 to 11.3 Ma), and Acres phases (11.3 to 7 Ma) (Hoorn *et al.*, 2010) with at least two short events of marine intrusions (18.1-17.2 My) before the present (Jaramillo et al., 2017) that may have partitioned the distributions of terrestrial biota. Those environments might have been like the current ones in our study area, providing habitat continuity to an ancestor population that may have diversified both because of the rise of the Andes and the reduction of the availability of terrestrial lowland habitat. More recent micro-dispersals through mountain passes or the extreme (north and south) slopes of the Cordillera Oriental would also have been possible (Hernández-Camacho *et al*., 1992; Rodríguez-Muñoz, Montes, & Crawford, 2020), contributing to the exchange between cis-Andean and trans-Andean biotas. In fact, the SDMs in **Figure 2-8**, as well as the low uncorrected *p*-distance in the 16S rRNA fragment between lineages Scinax1 and ScinaxX, suggest that the northern Cordillera Oriental and Táchira depressions may have acted as permeable barriers.

On the other hand, the forest clade Scinax3 is well separated genetically and ecologically from the open areas group. The individuals collected by ASM and MAV years ago originated in the "El Silencio" Natural Reserve, a private initiative which constitutes one of the main forest corridors in the northern middle Magdalena Valley. It was established precisely to promote the reconversion of pastures to silvopastoral systems and the connection of forest patches along the strongly intervened landscape around the Ciénaga de Barbacoas. Our review of biological collections revealed this taxon as the Magdalena Valley protected areas representative: it is present along (1) the Serranía de las Quinchas Regional Natural Park in Boyacá, (2) the Cesar portion of the Sierra Nevada de Santa Marta National Natural Park, and (3) in the south-eastern portion of the Serranía del Perijá Regional Natural Park, as well as along the forest remnants of the Selva del Carare, little of which is left due to coal, oil and gas mining and extensive cattle farming. Despite the still wide distribution of the Scinax3 lineage, the little divergent haplotypes inside the network (**Figure 2-4**) suggest connected populations.

From a coarse scale perspective, Scinax3 and ScinaxX are macrosympatric in the Magdalena Valley, but they seem not to share microhabitats. Inside our sampling, both lineages were found in the same collecting locality at Córdoba, Ayapel, which is a municipality characterized by decades of human intervention (livestock farming) that replaced the original land cover (swamps, savannas, and the remains of the most threatened ecosystem in the country, the tropical dry forest) with pastures (Pizano *et al*., 2016). Our argument for this sort of arrangement is that each lineage takes up a different habitat, which is reflected in morphology and supported by the fieldnotes that were available to us (John Lynch's fieldnotes). Nonetheless, hybridization events should be likely due to the explosive reproductive biology of this species group.

The last taxon under scrutiny is *Scinax caprarius*. It is a well differentiated lineage, both genetically and morphometrically (**Tables 2-2 and 2-3**), and it is not allied to the "*Scinax ruber*-like" species group despite its decorated hidden thighs and groins (**Figures 2-2, 2-3**). Its "forest" colour pattern is well concordant with the SDM prediction: tropical to pre-montane humid forests in the piedmonts of the Cordillera Central and Cordillera Oriental (**Figure 2-8**), and it is also coherent with the known distribution of its sibling species (*S. elaeochroa*), which can inhabit tropical lowland forests, pre-montane areas and disturbed forest habitats in eastern Central America (Cope, 1975; IUCN SSC Amphibian Specialist Group, 2020; G. Köhler, 2011) and the nortwestern Colombia. A close relationship between both species was proposed many years ago by Nieto (1996), based on shared morphological characters such as the polymorphic dorsal spots and stripes and the lateral flash-mark pattern. She was never able to review live or recently preserved specimens of any of those species, otherwise she would have identified the presence of green bones in support of that sibling relationship.

2.5.1 Conservation implications

Scinax1, Scinax3 and ScinaxX should be considered as different evolutionary significant units (sensu Moritz, 1994) of conservation concern from now on, due to the following conditions:

a) Scinax1 is already restricted to one of the most transformed areas in the Colombian Orinoquia, where many oil wells have been in production for at least 50 years. Additionally, newly developed crops and livestock farming enterprises are being expanded in the "development locomotives" framework launched during the last decade, in order to expand the infrastructure for land transportation, agriculture, and mining. **Figure 2-9** exposes this panorama.

b) All the *Scinax* linages inhabiting our study area in the Magdalena Valley might be affected by oil/ gas extraction activities and habitat degradation, the same as many other water-dependent amphibian taxa in the area (*Dendropsophus, Leptodactylus*, *Scarthyla*, etc.) with similar ecological preferences. But in the present pandemic-recession scenario, the threats fall directly on ScinaxX and Scinax3 (**Figure 2-9**) because they live where people do. While the former is present in the most intervened and populated regions of Colombia, one could infer that it might be somehow favoured by human expansion. However, humans incorporate contamination, habitat degradation, and over-extraction into the equation. The apparent preference of Scinax3 for highly preserved forests is a threat in the current economic and social structure of the country since it implies degrading forest to accommodate people. The consequences of fracking for that region are unknown, but it potentially represents a risk of larger and faster ecosystem degradation and water contamination, which these species could not survive. A public policy focused on preserving and connecting the remaining forest fragments of the region, as well as the implementation of a precautionary principle, will facilitate additional knowledge and will guarantee the survival of this biodiversity.

2.5.2 Relevant taxonomic considerations on the use of some traditional characters

Acosta-Galvis (2018) defined *Scinax caprarius* by its moderate size (rather small: 28,5-31,7 mm), its colour in life (reddish-brown with dark brown irregular markings and pale-yellow armpits, groin and anterior, and posterior thighs irregularly marked with brown), the shape of its snout, green bones, the tuberculated dorsum but smooth flanks, and the eye colour.

Short descriptions of vocalizations (two specimens, two sampling localities) were also provided, as well as images of four different tadpoles, one of them with the characteristic body-shape of a member of the *Scinax rostratus* group. Based on that, we suspect that the tadpoles in his Figure 9 are not conspecific, and we could not assign them confidently to *S. caprarius*.

Acosta-Galvis (*ibidem*) remarked the importance of green bones for diagnosing that species. We find it interesting but tricky, because green coloration is a consequence of a high concentration of biliverdin, or chlorosis, a phenomenon that has evolved independently at least 41 times in anuran history (Taboada et al., 2017, 2020) and may be subject to fluctuations during ontogeny and from the stresses produced by diet, microhabitat, or season. Neither the causes and consequences of bones colour nor the intra-populational variation of this trait in some *Scinax ruber*-like populations (evident to us NCR and ASM) have been evaluated so far, nor is the apparent lack of relationship between Central American and Amazonian green-boned species (NCR unpublished data). Consequently, we highlight the importance of more research to better understand the meaning of this character.

The presence of *Scinax x-signatus* in Colombia was first proposed by Nieto-Castro (1999) after a morphological review of the material available at the ICN collection during the early 90’s. Nonetheless, our phylogenetic results showed that the species delimitation for x-marked *Scinax* previously identified as *S. ruber or S. x-signatus* was not correct: Every new clade defined here is comprised of samples identified independently and by several taxonomists, either in the Magdalena Valley or in our cis-Andean sampling. This suggests that the dorsal X markings are not only polymorphic but independently originated many times and they should not be used to diagnose or discriminate species inside the already polymorphic *Scinax* genus. This wide variety of dark chevrons to X-markings dorsal pattern occurs in a broad variety of frog genera and families, such as *Atelopus* (Lötters, La Marca, & Vences, 2004), *Eleutherodactylus* (Díaz & Fong, 2001) or *Leptodactylus* (Rodríguez-Cabrera, García-Padrón, Acosta Galvis, de Sá, & Alonso Bosch, 2018).

2.5.3 Modifications on the *Scinax* species list for Colombia and South America:

Based on the present discussion, we must reject *Scinax x-signatus* as a member of the amphibian fauna of Colombia, confirming the opinion of Araujo-Vieira *et al.* (2020). However, they did not need to designate a neotype because the holotype seems to exist (see Chapter 4 of this document). We want also to call the attention on *Scinax blairi*, another potentially chevron marked *Scinax* of the eastern piedmont of the Andes. It was described by Fouquette & Pyburn (1972) from “near junction of Río Guaviare and Río Ariari, Vaupés” in the north-western Amazon region of Colombia, and later recorded by Ruiz-Carranza *et al.* (1996) in the northern piedmont of Arauca. One of the sequences assigned to Scinax1 comes from a specimen initially identified as *S. blairi* (AFAG 642) from Cravo Norte, Arauca, but genetically and morphologically consistent with Scinax1. Considering the apparent habitat specificity of these taxa, we hereby discard the presence of *S. blairi* for that area.

2.6 Conclusions

The outcome of integrating genetic information (one mitochondrial gene), alongside distributional and morphological data, illustrates the cryptic diversity of “*Scinax ruber*” *sensu lato* in the inter-Andean valleys of Colombia. We identified three macro-sympatric, endemic and threatened evolutionary lineages (Scinax3, ScinaxX and *S. caprarius*) that were previously confused under two names (*S. ruber* and *S. x-signatus*) which actually do not occur in Colombia. Neither was considered to deserve conservation actions, nor have they presented policy or management implications, but in the current socio-political context of the country and its ecosystems, and adequate acknowledgement of their diversity is vital to support accurate decisions.

Scinax3 is the unique trans-Andean representative of a forest associated clade that seems to be highly diverse in the Amazon and Orinoco basins of Colombia –and in the entire Amazonia. It shows an ecological preference for humid forest ecosystems, thus inhabiting forest relicts along the Magdalena basin previously known as the “Carare-Opón Forest”. It is of conservation concern because of the accelerated degradation and loss of its habitat, especially with the possibility of developing fracking pilot wells throughout its distribution range; that would add a new threat to that historically affected territory.

ScinaxX forms an open areas clade with Scinax1, and their distributions are dissected by the Cordillera Oriental. Both inhabit wetlands and flooding savannas ecosystems in a different versant of the Cordillera Oriental where livestock farming and urbanization, extensive monocultures and many productive oil and gas wells have been established and remain. Natural ecosystems of both regions are clearly threatened by accelerated land transformation, hence both lineages might be already threatened with extinction.

Scinax caprarius inhabits tropical to pre-montane humid forests in Magdalena valley, the most highly populated area of the country. Its survival may depend on the conservation of the forest it apparently prefers, which has been affected by continuous urbanization projects in the last 10 years. It might not be threatened now –but we cannot assure that, because our knowledge of its population status and trends is inexistent. However, based on the IUCN Red List Categories and Criteria (IUCN, 2012) it should be categorized at least as Near Threatened (NT) or Vulnerable (VU) under the Criteria A3 c, instead of the Low Concern classification proposed within its description.

After discarding the presence of *S. blairi* in Arauca and *S. x-signatus* in Colombia, and identifying new endemic, apparently threatened, and for long time confused *Scinax* lineages, we want to underline the importance of integrative (or well supported) taxonomy for deciding on conservation objects and threats. Providing multiple lines of evidence beyond morphology or molecules, such as vocalizations and tadpole morphology will allow us to complete our new species descriptions, which we will try to gather as long as they are available. But we hope that the categorization of evolutionary significant units (ESU) for these three lineages helps from this moment to provide accurate conservation policies.

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COMPETING INTERESTS

The authors have declared there are no competing interests.

DATA RESOURCES

In addition to the data generated by us, crucial data from GBIF and GenBank were used.

Table S1-1: Voucher specimens measured for the morphometric analysis of the populations sampled in the northern South America. Unless juvenile specimens were measured, their measurements were not considered for comparison purposes. All sampling locatiosn are in Colombia.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
AFAG440	AFAG440	Scinax1	Arauca, Cravo Norte, vereda Cumare, Finca Don Abel	6.3605556	-70.4169306	Male	Adult	39.70	*Scinax x-signatus*
AFAG441	AFAG441	Scinax1	Arauca, Cravo Norte, vereda Cumare, Finca Don Abel	6.3605556	-70.4169306	Male	Adult	37.13	*Scinax x-signatus*
AFAG442	AFAG442	Scinax1	Arauca, Cravo Norte, vereda Cumare, Finca Don Abel	6.3605556	-70.4169306	Male	Adult	38.67	*Scinax x-signatus*
AFAG467	AFAG467	Scinax1	Arauca, Cravo Norte, vereda Campo Abierto, Finca Santa Marta	6.3896667	-70.4317222	Male	Juvenile	22.37	*Scinax ruber*
AFAG473	AFAG473	Scinax1	Arauca, Cravo Norte	6.3896667	-70.4317222	Male	Adult	32.96	*Scinax ruber*
AFAG599	AFAG599	Scinax1	Arauca, Cravo Norte	6.3841389	-70.7135556	Male	Juvenile	30.47	*Scinax ruber*
AFAG621	AFAG621	Scinax1	Arauca, Cravo Norte	6.3779722	-70.7523139	Male	Juvenile	27.84	*Scinax ruber*
AFAG642	AFAG642	Scinax1	Arauca, Costa Río Cravo Norte	6.3993889	-70.4358611	Male	Juvenile	27.16	*Scinax blairi*
ANDES-A 1449	AJC 3534	Scinax3	Santander, Sabana de Torres	7.0796	-73.548	Male	Adult	42.00	*Scinax ruber*
ANDES-A 1810	AJC 3884	ScinaxX	Santander, Sabana de Torres	7.3496	-73.4981	Male	Adult	31.00	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
ANDES-A 1834	AJC 3562	ScinaxX	Tolima, Mariquita	5.2627	-74.8894	Male	Adult	31.20	*Scinax ruber*
ANDES-A 1835	AJC 3554	ScinaxX	Tolima, Mariquita	5.2627	-74.8894	Male	Adult	33.60	*Scinax ruber*
CPZ-UV 5990	FGA037	*Scinax caprarius*	Cundinamarca, Nocaima, vereda la Florida, Predio Tesoros de Colombia	5.0555556	-74.4176111	Male	Juvenile	31.20	*Scinax caprarius*
CPZ-UV3200	WB1983	*Scinax caprarius*	Caldas, Florencia	5.5245	-75.0403056	Male	Adult	25.40	*Scinax caprarius*
CPZ-UV3784		Scinax3	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.3673889	-74.7935	Male	Adult	32.00	*Scinax ruber*
CPZ-UV3822	EB30	ScinaxX	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.3673889	-74.7935	Male	Adult	32.50	*Scinax ruber*
CPZ-UV3823	EB031	ScinaxX	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.3673889	-74.7935	Male	Adult	31.80	*Scinax ruber*
CPZ-UV3842		ScinaxX	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.3673889	-74.7935	Male	Adult	30.90	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
CPZ-UV4892	JKT022	ScinaxX	Caldas, La Dorada, vereda la Atarraya, Hacienda los Ingleses	5.67	-74.7399997	Female	Adult	28.10	*Scinax ruber*
CPZ-UV660	WB1408	ScinaxX	Valle del Cauca, Santiago de Cali	3.4889722	-76.6307222	Female	Adult	33.10	*Scinax ruber*
IAvH-Am-11360	ARA6062	*Scinax caprarius*	Cundinamarca, carretera entre La Vega y Villeta, 4 km al occ del caso urbano de la Vega, Centro ecológico y de vacaciones San Felipe	5.0290556	-74.3693611	Male	Adult	29.79	*Scinax caprarius*
IAvH-Am-11362	ARA6028	*Scinax caprarius*	Cundinamarca, carretera entre La Vega y Villeta, 4 km al occ del caso urbano de la Vega, Centro ecológico y de vacaciones San Felipe	5.0290556	-74.3693611	Male	Adult	31.56	*Scinax caprarius*
IAvH-Am-11363	ARA6029	*Scinax caprarius*	Cundinamarca, carretera entre La Vega y Villeta, 4 km al occ del caso urbano de la Vega, Centro ecológico y de vacaciones San Felipe	5.0290556	-74.3693611	Male	Adult	29.89	*Scinax caprarius*
IAvH-Am-14418	ARA7376	*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.8888056	-75.1883333	Male	Adult	29.87	*Scinax caprarius*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
IAvH-Am-14419		*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.8888056	-75.1883333	Male	Adult	31.39	*Scinax caprarius*
IAvH-Am-14420		*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.8888056	-75.1883333	Male	Adult	31.53	*Scinax caprarius*
IAvH-Am-14421	ARA7411	*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.8888056	-75.1883333	Male	Adult	29.43	*Scinax caprarius*
IAvH-Am-14422	ARA7477	*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.8888056	-75.1883333	Male	Adult	29.50	*Scinax caprarius*
IAvH-Am-16480		*Scinax caprarius*	Boyacá, Otanché, vereda Quinchas, lote terreno Chorro seco, nacimiento vereda los Ángeles	5.98975	-74.3415278	Female	Adult	40.29	*Scinax caprarius*
ICN00308		Scinax3	Santander, Campamento forestal Capote, región Cararé Opón	6.6182222	-73.9116389	Female	Adult	41.78	*Scinax ruber*
ICN00932		Scinax3	Santander, Bolívar, Campo Capote, 3.5 Km al SE de las Montoyas	6.7293889	-74.0094722	Male	Adult	37.34	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
ICN11771	PR6708	ScinaxX	Huila, Villavieja, Quebrada La Batea y 10 Km de la carretera Villavieja-La Victoria	3.3007778	-75.1784444	Male	Adult	33.67	*Scinax ruber*
ICN11803	PR6740	ScinaxX	Huila, Villavieja, Quebrada La Batea y 10 Km de la carretera Villavieja-La Victoria	3.3007778	-75.1784444	Female	Adult	35.29	*Scinax ruber*
ICN22516		ScinaxX	Tolima, Chaparral, Cuira, Los Chorritos	3.6452778	-75.5941667	Male	Adult	33.98	*Scinax x-signatus*
ICN22523		ScinaxX	Tolima, Chaparral, Cuira, Los Chorritos	3.6452778	-75.5941667	Male	Adult	35.22	*Scinax x-signatus*
ICN36617		ScinaxX	Huila, Villavieja, vereda Los Hoyos	3.2392222	-75.1066111	Male	Adult	35.09	*Scinax x-signatus*
ICN37071	PR16511	Scinax3	Cundinamarca, Yacopí, inspección de policía Gudualito	5.5586111	-74.3088889	Male	Adult	37.69	*Scinax ruber*
ICN37113	LR002	Scinax3	Chocó, Río Sucio, corregimiento La Honda Vda La Balsa	7.4474167	-77.1311111	Female	Adult	35.63	*Scinax ruber*
ICN37114	LR004	Scinax3	Chocó, Río Sucio, corregimiento La Honda Vda La Balsa	7.4474167	-77.1311111	Female	Adult	42.02	*Scinax ruber*
ICN37116	JRO25	Scinax3	Chocó, Río Sucio, corregimiento La Honda Vda La Balsa	7.4474167	-77.1311111	Male	Adult	38.40	*Scinax ruber*
ICN37225	CMC473	ScinaxX	Antioquia, Puerto Triunfo, corregimiento Las Mercedes	5.9836111	-74.6077778	Male	Adult	29.23	*Scinax x-signatus*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
ICN37230	MOM407	ScinaxX	Córdoba, Santa Cruz de Lorica	9.2570278	-75.8029167	Male	Juvenile	31.60	*Scinax ruber*
ICN37352	MC8176	ScinaxX	Boyacá, Puerto Boyacá, Puerto Romero, Campamento Techint de Ecopetrol	5.8374444	-74.3439444	Female	Adult	33.83	*Scinax x-signatus*
ICN37354	MC8253	ScinaxX	Boyacá, Puerto Boyacá, Puerto Romero, Campamento Techint de Ecopetrol	5.8374444	-74.3439444	Male	Adult	35.23	*Scinax x-signatus*
ICN37364	PR17252	ScinaxX	Boyacá, Puerto Boyacá	5.8374444	-74.3439444	Male	Adult	29.70	*Scinax x-signatus*
ICN37445	MC8247	Scinax3	Boyacá, Puerto Boyacá, Inspección Puerto Romero, serranía Las Quinchas	5.8339167	-74.2716667	Male	Adult	36.91	*Scinax ruber*
ICN38535	MC8808	Scinax3	Boyacá, Puerto Boyacá, Inspección Puerto Romero, vereda La Fiebre, quebrada La Fiebre, en los alrededores de la Finca La Barrilera	5.8823611	-74.3502222	Female	Adult	46.84	*Scinax ruber*
ICN39198	CMV670	Scinax3	Córdoba, Tierralta, Quebrada La Mina	7.8704167	-76.2623056	Male	Adult	37.31	*Scinax ruber*
ICN39199	CHV671	Scinax3	Córdoba, Tierralta, Quebrada La Mina	7.8704167	-76.2623056	Male	Adult	36.20	*Scinax ruber*
ICN39202	CMV698	Scinax3	Córdoba, Tierralta, Quebrada La Mina	7.8704167	-76.2623056	Male	Adult	37.75	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
ICN41321	JDL21646	ScinaxX	Córdoba, Urrá	7.9348611	-76.3028333	Male	Adult	30.69	*Scinax ruber*
ICN41754	RR17183	ScinaxX	Atlántico, Barranquilla	10.9877222	-74.8752778	Female	Adult	36.70	*Scinax ruber*
ICN42297	JC 011	ScinaxX	Sucre, San Onofre, Finca los Morros	9.7127222	-75.6334167	Male	Adult	34.91	*Scinax ruber*
ICN42767	CB2018	ScinaxX	Tolima, San Sebastián de Mariquita, Cañon del rio Guarino, margen derecha. Tierra Roja, 2 Km. Antes del caserio Puerto Rico	5.2933333	-74.8980556	Male	Adult	32.89	*Scinax x-signatus*
ICN43164	JDL21694	ScinaxX	Tolima, Venadillo, Finca Paloballo, Km. 40 via Alvarado-Venadillo	4.7036111	-74.9327778	Female	Adult	32.70	*Scinax x-signatus*
ICN43268	46	ScinaxX	Tolima, Chaparral, vereda Providencia, Instituto Agricola CaMale Angarita	3.6452778	-75.5941667	Female	Adult	36.45	*Scinax x-signatus*
ICN43425		ScinaxX	Córdoba, Tierralta, Urrá I	8.0177778	-76.2147222	Male	Adult	34.44	*Scinax ruber*
ICN43437		ScinaxX	Córdoba, Tierralta, Urrá I	8.0177778	-76.2147222	Male	Adult	34.45	*Scinax ruber*
ICN43441		ScinaxX	Córdoba, Tierralta, Urrá I	8.0177778	-76.2147222	Male	Juvenile	32.68	*Scinax ruber*
ICN43448		Scinax3	Córdoba, Tierralta, Urrá I	8.0177778	-76.2147222	Female	Juvenile	28.48	*Scinax ruber*
ICN43449		Scinax3	Córdoba, Tierralta, Urrá I	8.0177778	-76.2147222	Female	Adult	43.71	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
ICN44387	JDL22130	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Male	Adult	36.70	*Scinax ruber*
ICN44389	JDL22132	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Male	Adult	38.83	*Scinax ruber*
ICN44392	JDL22135	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	37.71	*Scinax ruber*
ICN44394	JDL22137	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	38.01	*Scinax ruber*
ICN44399	JDL22142	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	38.55	*Scinax ruber*
ICN44403	JDL22146	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	38.53	*Scinax ruber*
ICN44406	JDL22146	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	36.87	*Scinax ruber*
ICN44407	JDL22150	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.6166667	-75.2333333	Female	Adult	37.93	*Scinax ruber*
ICN44592		Scinax3	Boyacá, Puerto Boyacá, vereda La Fiebre, Finca Los Guayabales, quebrada la Fiebre	5.8536111	-74.3180556	Male	Adult	34.06	*Scinax ruber*
ICN44687	GWL022	ScinaxX	Boyacá, Puerto Boyacá, Inspección de policía Puerto Romero	5.8371111	-74.3424444	Female	Adult	30.01	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
ICN45353	MC11159	Scinax3	Boyacá, Puerto Boyacá, vereda La Fiebre, Km. 34-35 carretera a Otanche, quebrada la Fiebrecita alta y baja y alrededores	5.8561111	-74.3241667	Male	Adult	40.95	*Scinax ruber*
ICN47103	JVR142	Scinax3	Chocó, PNN Los Katios, Hacienda Santatá	7.8261111	-77.1830556	Female	Adult	43.83	*Scinax ruber*
ICN47105	JVR144	Scinax3	Chocó, PNN Los Katios, Hacienda Santatá	7.8261111	-77.1830556	Female	Adult	44.78	*Scinax ruber*
ICN47108	JVR203	Scinax3	Chocó, PNN Los Katios, Hacienda Santatá	7.8261111	-77.1830556	Female	Adult	39.25	*Scinax ruber*
ICN48481	JDL26136	Scinax3	Córdoba, Ayapel, Hacienda Quibranche	8.2963889	-74.9794444	Female	Adult	34.06	*Scinax x-signatus*
ICN48482	JDL26812	Scinax3	Córdoba, Pueblo Nuevo, Hacienda Toronto	8.4	-75.2833333	Male	Adult	31.27	*Scinax x-signatus*
ICN48655	JDL25760	Scinax3	Córdoba, Montería, Hacienda El Diluvio	8.716666	-75.98331	Male	Adult	34.64	*Scinax ruber*
ICN48658	JDL25763	Scinax3	Córdoba, Montería, Hacienda El Diluvio	8.716666	-75.98332	Female	Adult	41.19	*Scinax ruber*
ICN48660	JDL25765	Scinax3	Córdoba, Montería, Hacienda El Diluvio	8.716666	-75.98331	Female	Adult	33.70	*Scinax ruber*
ICN48661	JDL25766	Scinax3	Córdoba, Montería, Hacienda El Diluvio	8.716666	-75.98333	Female	Adult	32.60	*Scinax ruber*
ICN48665	JDL26570	Scinax3	Córdoba, Montería, Hacienda El Diluvio	8.716666	-75.98331	Female	Adult	33.56	*Scinax ruber*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
ICN48667	JDL26135	ScinaxX	Córdoba, Ayapel, Hacienda Quibranche	8.3	-74.9833	Female	Adult	30.37	*Scinax ruber*
ICN48668	JDL26138	Scinax3	Córdoba, Ayapel, casco urbano	8.3161111	-75.1416667	Female	Adult	41.01	*Scinax ruber*
ICN48680	JDL26774	Scinax3	Córdoba, Pueblo Nuevo, Hacienda Toronto	8.4	-75.2833333	Female	Adult	38.74	*Scinax ruber*
ICN48685	JDL26842	ScinaxX	Córdoba, Pueblo Nuevo	8.4	-75.2833333	Male	Adult	31.70	*Scinax ruber*
ICN48769	JDL26773	Scinax3	Córdoba, Pueblo Nuevo, Hacienda Toronto	8.4	-75.2833333	Female	Adult	36.99	*Scinax ruber*
ICN49166	JDL24557	ScinaxX	Sucre, San Marcos, vereda La Florida, Finca Crocodylia	8.6	-75.15	Male	Adult	27.15	*Scinax ruber*
ICN49169	JDL24660	ScinaxX	Sucre, San Marcos, vereda La Florida, Finca Crocodylia	8.36	-75.09	Male	Adult	28.20	*Scinax ruber*
ICN49171	JDL24832	ScinaxX	Sucre, San Marcos, vereda La Florida, Finca Crocodylia	8.36	-75.09	Male	Juvenile	21.52	*Scinax ruber*
ICN52124	JDL21724	ScinaxX	Tolima, Venadillo, 4 Km. Al S de Venadillo	4.6766667	-74.9380556	Female	Adult	30.32	*Scinax ruber*
ICN52451	MOM1078	*Scinax caprarius*	Antioquia, San Carlos, carretera a Patio Bonito, Hacienda Las Vegas	6.1666667	-74.85	Female	Adult	34.17	*Scinax ruber*
ICN54567	JDL27940	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	34.07	*Scinax x-signatus*

Table S2-1: Continues.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
ICN54570	JDL27943	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	40.10	*Scinax x-signatus*
ICN54571	JDL27944	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.35	*Scinax x-signatus*
ICN54574	JDL27947	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.90	*Scinax x-signatus*
ICN54577	JDL27950	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	35.92	*Scinax x-signatus*
ICN54579	JDL27952	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.30	*Scinax x-signatus*
ICN54582	JDL27955	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	39.14	*Scinax x-signatus*
ICN54585	JDL27958	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	41.11	*Scinax x-signatus*
ICN54586	JDL27965	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	35.88	*Scinax x-signatus*
ICN54587	JDL27960	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.34	*Scinax x-signatus*
ICN54588	JDL27961	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.30	*Scinax x-signatus*
ICN54589	JDL27962	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	34.25	*Scinax x-signatus*

Table S2-1: Continues.

Catalog No.	Collection ID	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	SVL (mm)	Prior identity
ICN54590	JDL27963	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	39.66	*Scinax x-signatus*
ICN54591	JDL27964	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	38.51	*Scinax x-signatus*
ICN54593	JDL27966	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	37.24	*Scinax x-signatus*
ICN54594	JDL27967	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	35.30	*Scinax x-signatus*
ICN54596	JDL27969	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	36.29	*Scinax x-signatus*
ICN54597	JDL27970	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	38.13	*Scinax x-signatus*
ICN54599	JDL27990	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	36.49	*Scinax x-signatus*
ICN54602	JDL27993	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	36.23	*Scinax x-signatus*
ICN54603	JDL27994	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35	-73.5	Male	Adult	36.04	*Scinax x-signatus*
ICN55180	HRM367	ScinaxX	Córdoba, Tierralta, corregimiento El Loro, vereda La Oscurana, Finca Onomas	8.0048611	-76.1005556	Male	Adult	33.61	*Scinax x-signatus*

Table S2-1: Continues.

Catalog No.	**Collection ID**	**Proposed group**	**Sampling location**	**Latitude**	**Longitude**	**Sex**	**Age**	**SVL (mm)**	**Prior identity**
ICN55181	HRM368	ScinaxX	Córdoba, Tierralta, corregimiento El Loro, vereda La Oscurana, Finca Onomas	8.0048611	-76.1005556	Male	Adult	27.70	*Scinax x-signatus*
MAV326	MAV326	Scinax3	Antioquia, Yondó, Reserva el Silencio, vereda Barbacoas, Casa principal	6.80059	-074.20643	Male	Adult	37.10	*Scinax ruber*
MAV394	MAV394	Scinax3	Antioquia, Yondó, Finca Pampas	6.69981	-74.37271	Male	Adult	34.14	*Scinax ruber*

Table S1-2: Taxonomic identities, collection IDs and GenBank accession numbers of 16S rRNA sequences used in the phylogenetic and phylogeographic analyses. Haplotypes 1A and 1B correspond to lineage Scinax1; 3A, 3B, 3C, 3D, 3E and 3F to Scinax3; xA, xB, xC, xD, xE, xF and xG to ScinaxX.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
AF2378	*Scinax x-signatus*	Martinique: Diamant	KDQF01000927	–	J.-P. Vacher *et al.*, 2020
AF451	*Scinax x-signatus*	Brazil: Bahia, Una	KDQF01001470	–	J.-P. Vacher *et al.*, 2020
AFAG440	*Scinax x-signatus*	Colombia: Arauca, Cravo Norte, Vda. Cumare, Fca Don Abel	–	1B	This publication
AFAG467	*Scinax ruber*	Colombia: Arauca, Cravo Norte, Vda. Campo Abierto, Fca. Santa Marta. Sabana Inundable	–	1ª	This publication
AFAG599	*Scinax ruber*	Colombia: Arauca, Cravo Norte, Sabana con potrero	–	1B	This publication
AFAG621	*Scinax ruber*	Colombia: Arauca, Cravo Norte, Estero	–	1B	This publication
AFAG642	*Scinax ruber*	Colombia: Arauca, Costa Río Cravo Norte, Bosque de Galería	–	1ª	This publication
AJC2324	*Scinax ruber*	Colombia: Casanare, Municipio Orucue, Reserva Wisirare	KP149491	1A	Guarnizo *et al.*, 2015
AJC3422	*Scinax rostratus*	Colombia: Santander, San Vicente de Chucuri, vereda las Margaritas, Reserva el arboretum	KP149284	–	Guarnizo *et al.*, 2015
AJC3506	*Scinax rostratus*	Colombia: Santander, San Vicente de Chucuri, vereda las Margaritas, Reserva el arboretum, Cerro de las Margaritas	KP149435	–	Guarnizo *et al.*, 2015

Table S2-2: Continues.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
AJC3532	*Scinax ruber*	Colombia: Santander, San Vicente de Chucuri,vereda las Margaritas, Reserva el arboretum	KP149347	3D	Guarnizo *et al.*, 2015
AJC3534	*Scinax ruber*	Colombia: Santander, San Vicente de Chucuri,vereda las Margaritas, Reserva el arboretum	KP149295	3E	Guarnizo *et al.*, 2015
AJC3884	*Scinax ruber*	Colombia: Santander, Sabana de Torres, Sabana de Torres, Electrificadora	KP149330	xC	Guarnizo *et al.*, 2015
AJC3887	*Dendropsophus microcephalus*	Colombia: Santander, Sabana de Torres, Sabana de Torres, Finca carretera. Sobre junco en pantano	KP149423	–	Guarnizo *et al.*, 2015
AJC3940	*Scinax ruber*	Colombia: Meta, San Juan de Arama, Caserío Mira flores, vereda la valastrera, Finca los almendros	KP149320	–	Guarnizo *et al.*, 2015
AJC4074	*Scinax cf. Kennedyi*	Colombia: Casanare, Sabanalarga, Sabanalarga	KP149463	–	Guarnizo *et al.*, 2015
ARA5766	*Scinax ruber*	Colombia: Caldas, Municipio la Dorada, Hacienda La Española, Márgen derecha río Purrio	–	xA	This publication
CAB126	*Scinax faivovichi*	Brazil	JN100003	–	Bell *et al.,* 2012
CAB293	*Scinax perpusillus*	Brazil	JN100016	–	Bell *et al.,* 2012
CC1	*Scinax elaeochroa*	Costa Rica	EF376076	–	Salducci *et al*., 2005
CFBH27384	*Scinax v-signatus*	Brazil: State of Rio de Janeiro, Campos dos Goytacazes	MK266761	–	Araujo-Vieira *et al*., 2019
CFBHT00864	*Scinax obtriangulatus*	Brazil: Sao Paulo, Santo Antonio do Pinhal	KU495562	–	Lyra *et al.*, 2017

Table S2-2: Continues.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
CFBHT01470	*Scinax perereca*	Brazil	KU306387	–	Sousa *et al.*, 2015. Direct submission.
CFBHT01562	*Scinax fuscomarginatus*	Brazil: Sao Paulo, Cosmorama	KU495548	–	Lyra *et al.*, 2017
CFBHT03433	*Scinax x-signatus*	Brazil: Bahia, Itabuna	KU495577	–	Lyra *et al.*, 2017
CFBHT05375	*Scinax x-signatus*	Brazil: Ceara, Ubajara	KU495574	–	Lyra *et al.*, 2017
CFBHT05377	*Scinax x-signatus*	Brazil: Ceara, Ubajara	KU495579	–	Lyra *et al.*, 2017
CFBHT09136	*Scinax x-signatus*	Brazil: Bahia, Caetité, Povoado Senote	KU495576	–	Lyra *et al.*, 2017
CFBHT09023	*Scinax fuscovarius*	Brazil: Espirito Santo, Linhares	KU495559	–	Lyra *et al.*, 2017
CFBHT09433	*Scinax x-signatus*	Brazil: Pernambuco, Sanharó	KU495575	–	Lyra *et al.*, 2017
CFBHT10951	*Scinax nebulosus*	Brazil: Baixa Grande, Piaui	KJ004190	–	Brusquetti *et al.*, 2014
CFBHT15404	*Scinax crospedospilus*	Brazil: Sao Paulo, Nazare Paulista, Lagora e banhado na casa do Senhor Aparicio	KU495542	–	Lyra *et al.*, 2017
CFBHT16741	*Scinax crospedospilus*	Brazil: Sao Paulo, Sao Luis do Paraitinga, PESM Nucleo Santa Virginia	KU495541	–	Lyra *et al.*, 2017
CFBHT19110	*Scinax hayii*	Brazil	MH206251	–	de Rezende *et al.*, 2018 via GenBank.
CI_070	*Scinax ictericus*	Peru: Los Amigos Biological Station, Provincia Manu, Madre de Dios	MN172527	–	von May *et al.*, 2019
CPZ-UV4892	*Scinax ruber*	Colombia: Caldas, Municipio la Dorada, vereda la Atarraya, Hacienda los Ingleses	–	xE	This publication

Table S2-2: Continues.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
CPZ-UV5990	*Scinax caprarius*	Colombia: Cundinamarca, Nocaima, Predio Tesoros de Colombia.	–	Sc	This publication
CPZ-UV660	*Scinax ruber*	Colombia: Valle del Cauca, Santiago de Cali	–	xA	This publication
IAvH-Am-11445	*Scinax x-signatus*	Colombia: Tolima, Guayabal, corregimiento Méndez, Reserva de La Sociedad Civil, Hacienda Jabirú	–	xF	This publication
IAvH-Am-8262	*Scinax ruber*	Colombia: Sucre, San Benito, vereda La Caimanera, Sector de la Caimanera	–	3A	This publication
ICN48482	*Scinax x-signatus*	Colombia: Córdoba, Pueblo Nuevo, Hacienda Toronto	–	3B	This publication
ICN48679	*Scinax ruber*	Colombia: Córdoba, Pueblo Nuevo, Hacienda Toronto	–	3C	This publication
ICN48481	*Scinax ruber*	Colombia: Córdoba, Ayapel, Hacienda Quibranche	–	3A	This publication
ICN48667	*Scinax ruber*	Colombia: Córdoba, Ayapel, Hda. Quibrache	–	xA	This publication
IGUN Sx 34	*Scinax x-signatus*	Colombia: Barrancabermeja	–	3A	This publication
IGUN Sx 35	*Scinax x-signatus*	Colombia: Santander, Barrancabermeja	–	3E	This publication
IGUN Sx 36	*Scinax x-signatus*	Colombia: Santander, Barrancabermeja	–	3F	This publication
IIBPH1396	*Scinax berthae*	Paraguay: Alto Vera, Itapua	KJ004191	–	Brusquetti *et al.*, 2014
IIBPH262	*Scinax nasicus*	Paraguay: Estancia San Jose, Neembucu	KJ004188	–	Brusquetti *et al.*, 2014
IIBPH269	*Scinax fuscomarginatus*	Paraguay: Estancia San Jose, Neembucu	KJ004182	–	Brusquetti *et al.*, 2014

Table S2-2: Continues.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
IIBPH277	*Scinax Acuminatus*	Paraguay: Estancia San Jose, Neembucu	KJ004189	–	Brusquetti *et al.*, 2014
JDL32662	*Scinax ruber*	Colombia: Cundinamarca, Nilo	–	xG	This publication
JPH1016	*Scinax ruber*	Colombia: Córdoba, Ayapel, Hacienda la Candelaria UdeA	–	xB	This publication
JSM176	*Phyllomedusa venusta*	Colombia: Bolívar, Zambrano	MG030711	–	Cruz-Piedrahita *et al.*, 2018
KM390789	*Scinax flavoguttatus*	Brazil	KM390789	–	Chaves *et al.*, 2014 via GenBank
KM390790	*Scinax humilis*	Brazil	KM390790	–	Chaves *et al.*, 2014 via GenBank
KZ1024	*Scinax peixotoi*	Brazil	JN100005	–	Bell *et al.,* 2012
MACN38642	*Pseudis paradoxa*	Argentina: Corrientes, Dto. Bellavista, Camino San Roque-Bellavista	AY843740	–	Faivovich *et al.,* 2005
MAV326	*Scinax ruber*	Colombia: Antioquia, Yondó, Reserva el Silencio, vereda Barbacoas, Casa principal	–	3F	This publication
MAV394	*Scinax ruber*	Colombia: Antioquia, Yondó, Finca Pampas	–	3E	This publication
MNK:A:9387	*Sphaenorhynchus lacteus*	Bolivia	JF790144	–	Jansen *et al.*, 2011
MNRJ33859	*Pseudis paradoxa*	Brazil: Bacabal Municipality, Maranhao	MK293738	–	Aguiar *et al.*, 2007
MTR13988	*Scinax x-signatus*	Brazil: Amapá, Macapá	KDQF01003307	–	J.-P. Vacher *et al.*, 2020
MUJ 2428	*Scinax ruber*	Colombia: Santander, Los Santos, vereda la granja, Hacienda el Roble	–	xD	This publication

Table S2-2: Continues.

Collection ID	**Recorded identity**	**Sampling location**	**GenBank accession no.**	**Haplotype**	**Source**
MVZ203919	*Scinax elaeochroa*	Costa Rica: Heredia, Starkey's Woods, 1.5-3.0 km E Rio Frio Rd., at 1 km NW entrance Estacion Biologica La Selva	AY843757	–	Faivovich *et al.,* 2005
MVZ207215	*Scinax boulengeri*	Costa Rica: Guanacaste, ca. 0.2 km W Costa Rica Hwy. 1 on first paved road 10.0 km N entrance Santa Rosa National Park along Hwy. 1	AY843755	–	Faivovich *et al.,* 2005
MZUESC11079	*Scinax strigilatus*	Brazil: Camacan, Bahia	KT438895	–	Nogueira *et al.*, 2016
QCAZ43729	*Scinax ruber*	Ecuador: Provincia Orellana, 5 km S Puerto Francisco de Orellana (Coca), near Rio Napo	MH662500	–	S. R. Ron *et al.*, 2018
QCAZ43799	*Scinax funereus*	Ecuador: Provincia Orellana, El Descanso, La Primavera, Rio Napo	MH662481	–	S. R. Ron *et al.*, 2018
QCAZ48844	*Scinax garbei*	Ecuador: Provincia Napo, Reserva Ecologica Yachana	MH662483	–	S. R. Ron *et al.*, 2018
UFMT7191	*Scinax fuscomarginatus*	Brazil: Lucas do Rio Verde, Mato Grosso	KJ004185	–	Brusquetti *et al.*, 2014
WED56365	*Scinax ruber*	Peru: Madre de Dios; Cusco Amazonico	AY326034	–	Darst & Cannatella, 2004
1BM	*Scinax ruber*	French Guiana: Kourou	EF217488	–	Fouquet, Vences, *et al.*, 2007

Table S1-3: Records used for Species Distribution Models with their collection IDs and taxonomic identities. All sampling locations are inside Colombia.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
AFAG440	AFAG440	Scinax1	Arauca, Cravo Norte, vereda Cumare, Finca Don Abel	6.36056	-70.41693	Male	Adult	*Scinax x-signatus*
AFAG467	AFAG467	Scinax1	Arauca, Cravo Norte, vereda Campo Abierto, Finca Santa Marta	6.38967	-70.43172	Male	Juvenile	*Scinax ruber*
AFAG599	AFAG599	Scinax1	Arauca, Cravo Norte	6.38414	-70.71356	Male	Juvenile	*Scinax ruber*
AFAG621	AFAG621	Scinax1	Arauca, Cravo Norte	6.37797	-70.75231	Male	Juvenile	*Scinax ruber*
AFAG642	AFAG642	Scinax1	Arauca, Costa Río Cravo Norte	6.39939	-70.43586	Male	Juvenile	*Scinax blairi*
ANDES-A 1449	AJC 3534	Scinax3	Santander, Sabana de torres	7.07960	-73.54800	Male	Adult	*Scinax ruber*
ANDES-A 1810	AJC 3884	ScinaxX	Santander, Sabana de torres	7.34960	-73.49810	Male	Adult	*Scinax ruber*
ANDES-A 1834	AJC 3562	ScinaxX	Tolima, Mariquita	5.26270	-74.88940	Male	Adult	*Scinax ruber*
CPZ-UV 5990	FGA037	*Scinax caprarius*	Cundinamarca, Nocaima, vereda la Florida, Predio Tesoros de Colombia	5.05556	-74.41761	Male	Juvenile	*Scinax caprarius*
CPZ-UV3200	WB1983	*Scinax caprarius*	Caldas, Florencia	5.52450	-75.04031	Male	Adult	*Scinax caprarius*
CPZ-UV3784		Scinax3	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.36739	-74.79350	Male	Adult	*Scinax ruber*

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
CPZ-UV3822	EB30	ScinaxX	Caldas, La victoria, corregimiento Guarinocito, vereda el Gigante Hacienda la Española	5.36739	-74.79350	Male	Adult	*Scinax ruber*
CPZ-UV4120		ScinaxX	Caldas, La Dorada, corregimiento Guarinocito, Cereda Purnio	5.41450	-74.68639	Male	Adult	*Scinax ruber*
CPZ-UV4892	JKT022	ScinaxX	Caldas, La Dorada, vereda la Atarraya, Hacienda los Ingleses	5.67000	-74.74000	Female	Adult	*Scinax ruber*
CPZ-UV660	WB1408	ScinaxX	Valle del Cauca, Santiago de Cali	3.48897	-76.63072	Female	Adult	*Scinax ruber*
IAvH-Am-11360	ARA6062	*Scinax caprarius*	Cundinamarca, carretera entre La Vega y Villeta, 4 km al occ del caso urbano de la Vega, Centro ecológico y de vacaciones San Felipe	5.02906	-74.36936	Male	Adult	*Scinax caprarius*
IAvH-Am-14418	ARA7376	*Scinax caprarius*	Antioquia, El Carmen de Viboral, vereda El Porvenir, caserío Escuela El Porvenir	5.88881	-75.18833	Male	Adult	*Scinax caprarius*
IAvH-Am-16480		*Scinax caprarius*	Boyacá, Otanché, vereda Quinchas, lote terreno Chorro seco, nacimiento vereda los Ángeles	5.98975	-74.34153	Female	Adult	*Scinax caprarius*

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
ICN00308		Scinax3	Santander, Campamento forestal Capote, región Cararé Opón	6.61822	-73.91164	Female	Adult	*Scinax ruber*
ICN00932		Scinax3	Santander, Bolívar, Campo Capote, 3.5 Km al SE de las Montoyas	6.72939	-74.00947	Male	Adult	*Scinax ruber*
ICN11771	PR6708	ScinaxX	Huila, Villavieja, Quebrada La Batea y 10 Km de la carretera Villavieja-La Victoria	3.30078	-75.17844	Male	Adult	*Scinax ruber*
ICN22516		ScinaxX	Tolima, Chaparral, Cuira, Los Chorritos	3.64528	-75.59417	Male	Adult	*Scinax x-signatus*
ICN36617		ScinaxX	Huila, Villavieja, vereda Los Hoyos	3.23922	-75.10661	Male	Adult	*Scinax x-signatus*
ICN37071	PR16511	Scinax3	Cundinamarca, Yacopí, inspección de policía Gudualito	5.55861	-74.30889	Male	Adult	*Scinax ruber*
ICN37113	LR002	Scinax3	Chocó, Río Sucio, corregimiento La Honda Vda La Balsa	7.44742	-77.13111	Female	Adult	*Scinax ruber*
ICN37225	CMC473	ScinaxX	Antioquia, Puerto Triunfo, corregimiento Las Mercedes	5.98361	-74.60778	Male	Adult	*Scinax x-signatus*
ICN37230	MOM407	ScinaxX	Córdoba, Santa Cruz de Lorica	9.25703	-75.80292	Male	Juvenile	*Scinax ruber*
ICN37352	MC8176	ScinaxX	Boyacá, Puerto Boyacá, Puerto Romero, Campamento Techint de Ecopetrol	5.83744	-74.34394	Female	Adult	*Scinax x-signatus*

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
ICN37445	MC8247	Scinax3	Boyacá, Puerto Boyacá, Inspección Puerto Romero, serranía Las Quinchas	5.83392	-74.27167	Male	Adult	*Scinax ruber*
ICN38535	MC8808	Scinax3	Boyacá, Puerto Boyacá, Inspección Puerto Romero, vereda La Fiebre, quebrada La Fiebre, en los alrededores de la Finca La Barrilera	5.88236	-74.35022	Female	Adult	*Scinax ruber*
ICN39198	CMV670	Scinax3	Córdoba, Tierralta, Quebrada La Mina	7.87042	-76.26231	Male	Adult	*Scinax ruber*
ICN41321	JDL21646	ScinaxX	Córdoba, Urrá	7.93486	-76.30283	Male	Adult	*Scinax ruber*
ICN41754	RR17183	ScinaxX	Atlántico, Barranquilla	10.98772	-74.87528	Female	Adult	*Scinax ruber*
ICN42297	JC 011	ScinaxX	Sucre, San Onofre, Finca los Morros	9.71272	-75.63342	Male	Adult	*Scinax ruber*
ICN42767	CB2018	ScinaxX	Tolima, San Sebastián de Mariquita, Cañon del rio Guarino, margen derecha. Tierra Roja, 2 Km. Antes del caserio Puerto Rico	5.29333	-74.89806	Male	Adult	*Scinax x-signatus*
ICN43164	JDL21694	ScinaxX	Tolima, Venadillo, Finca Paloballo, Km. 40 via Alvarado-Venadillo	4.70361	-74.93278	Female	Adult	*Scinax x-signatus*
ICN43425		ScinaxX	Córdoba, Tierralta, Urrá I	8.01778	-76.21472	Male	Adult	*Scinax ruber*
ICN43448		Scinax3	Córdoba, Tierralta, Urrá I	8.01778	-76.21472	Female	Juvenile	*Scinax ruber*
ICN44387	JDL22130	ScinaxX	Bolívar, Santa Catalina, Hacienda El Ceibal	10.61667	-75.23333	Male	Adult	*Scinax ruber*

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
ICN44592		Scinax3	Boyacá, Puerto Boyacá, vereda La Fiebre, Finca Los Guayabales, quebrada la Fiebre	5.85361	-74.31806	Male	Adult	*Scinax ruber*
ICN44687	GWL022	ScinaxX	Boyacá, Puerto Boyacá, Inspección de policía Puerto Romero	5.83711	-74.34244	Female	Adult	*Scinax ruber*
ICN45353	MC11159	Scinax3	Boyacá, Puerto Boyacá, vereda La Fiebre, Km. 34-35 carretera a Otanche, quebrada la Fiebrecita alta y baja y alrededores	5.85611	-74.32417	Male	Adult	*Scinax ruber*
ICN47103	JVR142	Scinax3	Chocó, PNN Los Katios, Hacienda Santatá	7.82611	-77.18306	Female	Adult	*Scinax ruber*
ICN48481	JDL26136	Scinax3	Córdoba, Ayapel, Hacienda Quibranche	8.29639	-74.97944	Female	Adult	*Scinax x-signatus*
ICN48482	JDL26812	Scinax3	Córdoba, Pueblo Nuevo, Hacienda Toronto	8.40000	-75.28333	Male	Adult	*Scinax x-signatus*
ICN48661	JDL25766	Scinax3	Córdoba, Montería, Hacienda El diluvio	8.71667	-75.98333	Female	Adult	*Scinax ruber*
ICN48667	JDL26135	ScinaxX	Córdoba, Ayapel, Hacienda Quibranche	8.30000	-74.98330	Female	Adult	*Scinax ruber*
ICN48668	JDL26138	Scinax3	Córdoba, Ayapel, Casco urbano	8.31611	-75.14167	Female	Adult	*Scinax ruber*
ICN48685	JDL26842	ScinaxX	Córdoba, Pueblo Nuevo	8.40000	-75.28333	Male	Adult	*Scinax ruber*

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
ICN49166	JDL24557	ScinaxX	Sucre, San Marcos, vereda La Florida, Finca Crocodylia	8.60000	-75.15000	Male	Adult	*Scinax ruber*
ICN49169	JDL24660	ScinaxX	Sucre, San Marcos, vereda La Florida, Finca Crocodylia	8.36000	-75.09000	Male	Adult	*Scinax ruber*
ICN52124	JDL21724	ScinaxX	Tolima, Venadillo, 4 Km. Al S de Venadillo	4.67667	-74.93806	Female	Adult	*Scinax ruber*
ICN52451	MOM1078	*Scinax caprarius*	Antioquia, San Carlos, carretera a Patio Bonito, Hacienda Las Vegas	6.16667	-74.85000	Female	Adult	*Scinax ruber*
ICN54567	JDL27940	Scinax3	Santander, Sabana de Torres, vereda Agua Bonita ca 5 km	7.35000	-73.50000	Male	Adult	*Scinax x-signatus*
ICN55180	HRM367	ScinaxX	Córdoba, Tierralta, corregimiento El Loro, vereda La Oscurana, Finca Onomas	8.00486	-76.10056	Male	Adult	*Scinax x-signatus*
https://colombia.inaturalist.org/observations/67065921		*Scinax caprarius*	Cundinamarca, La Vega	4.95593	-74.381656	Not identified	Juvenile	*Scinax caprarius*
https://colombia.inaturalist.org/observations/60492211		*Scinax caprarius*	Antioquia, Maceo	6.45524	-74.79123	Not identified	Adult	*Scinax caprarius*
https://colombia.inaturalist.org/observations/60491733		*Scinax caprarius*	Antioquia, San Carlos	6.251955	-75.01498	Female		*Scinax caprarius*
https://colombia.inaturalist.org/observations/39559		*Scinax caprarius*	Antioquia, San Carlos	6.190349	-74.990181	Male		*Scinax caprarius*
https://colombia.inaturalist.org/observations/67066590		*Scinax caprarius*	Cundinamarca, La Vega	4.955702	-74.381455			*Scinax caprarius*
IGUN-BATB 3365	MAV326	Scinax3	Antioquia, Yondó, Reserva el Silencio,	6.80059	-74.20643	Male	Adult	*Scinax ruber*

			vereda Barbacoas, Casa principal					

Table S2-3: Continues.

Catalogue No.	Collection No.	Proposed group	Sampling location	Latitude	Longitude	Sex	Age	Prior identification
MAV394	MAV394	Scinax3	Antioquia, Yondó, Finca Pampas	6.69981	-74.37271	Male	Adult	*Scinax ruber*
MHUA-A 5807		*Scinax caprarius*	Antioquia, Gómez Plata, Vegas de la Clara	6.581619	-75.195383			*Scinax caprarius*
MHUA-A 5968		*Scinax caprarius*	Antioquia, Gómez Plata, Vegas de la Clara	7.051483	-75.395189		Juvenile	*Scinax caprarius*

3. Capítulo 3: Diversification of lowland tree frogs along the Pebas-Eastern Andes system of Colombia: At the end, molecules say [almost] everything

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3.1 Abstract

The Andean uplift has been mentioned repeatedly as the ultimate cause of diversification of plants and animals in the Northern South America, from the Eocene to the middle Holocene. Whether and when the mountain chain configured a barrier, promoted a new source of barriers (i.e., rivers and river basins) or a brand-new unoccupied set of habitats for the lowland biotas is an unresolved question, and its resolution depends on the biology of the organisms and the integration of multiple lines of evidence under a critical view.

The dated phylogeny of four distantly related clades of tree frogs, co-distributed along the Caribbean coast, the Llanos of Colombia and Venezuela and the northwestern Amazon, revealed a shared diversification pattern which recalls the known history of the habitats. The combined examination of distributions, molecular data, natural history, and external morphology produced a systematic discovery of a variety of cryptic evolutionary independent lineages, often delimited by rivers or river basins. The evolution of the clades is hypothesized to occur in a south-eastern to north-western gradient, according to the pulsed Andean uplift, the marine incursions that formed the Pebas system and the subsequent structuring of the Orinoco and Amazon basins and tributary rivers.

The taxonomy inside those clades, however, has been traditionally misunderstood, because individuals present little or not evident morphological differences. The amphibian diversity of the eastern lowlands of Colombia and vicinities is, again, underestimated.

3.2 Introduction

The Andean uplift undoubtedly changed the configuration of the geographic and ecological space available for the lowland biotas of the Northern South America (C Hoorn *et al*., 2010). Beyond a physical barrier that may have broken the ancestral wide distribution of many lowland organisms, thus generating the Magdalena river and inter-Andean valleys of Colombia, the rising of the Andes also produced substantial changes in climate (Insel, Poulsen, & Ehlers, 2010), sediment transport and soil configurations (Ehlers & Poulsen, 2009), which favored the diversification of the extant biota, especially from the late Miocene

(12 Mya) to the present (Jaramillo, 2019). The Eastern Cordillera (Cordillera Oriental) of Colombia, and the marine incursions during the early (18.2-17.2 Mya) and the middle (16.1 to 12-4 Mya) Miocene, which formed the Pebas wetlands system, were responsible not only for the swampy environment which lasted some 4-5 My in the north-western portion of the Amazon versant of the continent, but for the radical change in vegetation that took place in along the entire Amazon basin (Jaramillo et al., 2017): a forest dominated landscape changed towards a succession of grasslands and gallery forests, and the depositional savannas extended eastwards and southwards as a consequence of the slight elevation of the Cordillera and a more arid climate (Carina Hoorn et al., 2017).

Despite the inherent difficulties of estimating how impacting would those changes be for non-flying and philopatric organisms such as the frogs we know today, their life histories and ecological conditions allow the supposition that range-restricted species should have been severely affected for such steady but dramatic situation. These impacts could have been beneficial for the accounting of the always underestimated Neotropical biodiversity (Vacher et al., 2020), but every impact has consequences. Hence, and with the help of five widespread species of tree frogs (family Hylidae) which do not live particularly on trees, in this paper we try to infer those consequences, understood as the patterns and paces of diversification of water-dependent frogs in the north-eastern territories of South America. At the same time, we expect to evaluate the extent of the underestimation of biodiversity richness that Colombia and the Orinoco-Amazon border represent in the global, the regional, and the institutional knowledge, which has been remarked in several publications during the last five years (Caminer *et al.,* 2017; Fouquet *et al.*, 2021; Rojas, Fouquet, Ron, *et al*., 2018; Vacher *et al.*, 2020, 2017).

3.3 Materials and methods

3.3.1 The model species

The selected models are three supposedly semi-aquatic (*Scarthyla goinorum*, *S. vigilans* and *Sphaenorhynchus lacteus*) and two supposedly terrestrial (*Scinax rostratus* and *S. wandae*) nominal species of tree frogs, which have been phylogenetically related (with poor evidence) in different contexts (Frost, 2021). *Scinax* has been considered monophyletic for a long time, and its evolutionary relationships to other clades of Hylidae are apparently clear except for *Scarthyla*: every mention of the genus *Scarthyla* includes changes on the known phylogenetic relationships for a different set of siblings. Those include *Dendropsophus* (similar adult shapes and habitats), *Scinax* (similar adult and larval aspects), *Sphaenorhynchus* (similar adult colors, larval mouths and patterns) or *Pseudis* (similar larval morphology and habits).

To explore the phylogenetic relationships of these genera and species, and to provide calibration points for the fossil-dated phylogeny, a larger set of taxa was selected and studied, including phylogenetically distant outgroups. Unless four out of five of these species are said to be common when present in a given inventory, very little is known about their reproductive biology, population trends, their taxonomy or morphology, which leads to constant confusions of names and distributions (see for example Acosta-Galvis, 2021; Medina-Rangel *et al*., 2019; Sturaro *et al*., 2010).

3.3.2 The study area

The geographic scope of this study comprises the distributions of the model species and their relatives, in the following areas below 1500 masl: the north-western Amazon (extreme northern Bolivia, northwest Brazil, Colombia, Ecuador and Peru); the Orinoco plains of Caribbean affinity in Colombia, Suriname, Venezuela and the Guianas, plus the northern Guiana shield north to Trinidad and Tobago; the Caribbean coast of those countries and the inter-Andean valleys of the Cauca and Magdalena rivers in Colombia. Provided that there is at least one morphology-based record of *Scinax rostratus* in the coastal Pará, Brazil, the Pará Center (P. Müller, 1973) will be tentatively included, as well as its dispersal centres Nos. 8-10, 19-22 and the north-western part of Müller's dispersal center No. 25 as modified by Morrone (2014) in his Figure 8.

3.3.3 The Data

Tissue samples of 63 individuals identified as *Scarthyla goinorum* (5), *S. vigilans* (4), *S. cruentomma* (2) or *S. sp. aff. cruentomma* (3), *Scinax garbei* (7) or *S. sp. aff. garbei* (1), *S. kennedyi* (2) or *S. sp. aff. kennedyi* (3), *S. nebulosus* (1), *S. pedromedinae* (4), *S. rostratus*

(9), "*S. ruber*" (1), *S. sp. gr. ruber* Scinax3 (1), *S. sugillatus* (1), *S. wandae* (7) or *S. sp. aff. wandae* (3) *Sphaenorhynchus carne*us (1), *Sphaenorhynchus dorisae* (2) or *Sphaenorhynchus lacteus* (6) were obtained with the unvaluable help of the following biological collections and their curators or administrators: "Banco de ADN y Tejidos de la Biodiversidad Colombiana" (BTBC) [Biodiversity Tissues Bank] of the Instituto de Genética, Universidad Nacional de Colombia; the Colección de Anfibios [Amphibian Collection] of the Instituto de Ciencias Naturales (ICN-Anfibios), Universidad Nacional de Colombia; Senckenberg Naturhistorische Sammlungen Dresden (MTD) [Museum of Zoology], Germany and the Museo Nacional de Ciencias Naturales (MNCN) [Museum of Natural Sciences] Madrid, Spain (**Table S3-1**).

In addition, 79 GenBank accessions of 38 specimens were used to strengthen the geographical sampling, for calibrating the dated phylogeny, and for comparison purposes in the phylogenetic analysis. The taxa with a known and datable fossil record comprised *Dryophytes versicolor* (Hylinae), *Litoria lesueurii*, (Pelodryadiade) and *Pseudacris crucifer* (Acridinae), which were considered together with their living relatives: Dendropsophinae (*Dendropsophus leucophyllatus*, *D. minutus*) and Phyllomedusidae (*Cruziohyla calcarifer*, *Pithecopus hypochondrialis*). Furthermore, two other unrelated taxa were selected as the outgroup: a dendrobatid (*Dendrobates auratus*), and *Boana calcarata* (Hylidae, Cophomantinae) (**Table S3-1**). Ages of the fossils were taken from Fossilworks (Behrensmeyer & Turner, 2013).

All the tissue samples were processed for obtaining sequences of three mitochondrial (12S rRNA, 16S rRNA and Cytochrome Oxidase I - COI) and two nuclear (Rho – Rhodopsin and Tyr - Tyrosinase) gene fragments, unless not all the fragments could be amplified for all the samples (See Appendix 1 and 2). DNA extractions of the tissues from the European collections were performed in the Molecular Genetics Laboratory of the State Museum of Zoology, Senckenberg Institute, Dresden (MTD) using the innuPREP DNA Mini Kit of Analytics Jenna. DNA extractions for the Colombian samples were performed with equivalent kits (Smobio™ and ThermoScientific™) in the Molecular Ecology Lab of the Instituto de Genética, Universidad Nacional de Colombia. All extractions were PCR-amplified using the primers L25195 fwd and H2916 rev for the 12S fragment (Vences et al., 2000), 16S A and 16S B for 16S fragment (Vences *et al.*, 2005), dgLCO1490 and dgHCO2198 for the COI gene fragment (Vences *et al.*, 2005), Rho1A and Rho1C primers for the nuclear Rho gene fragment (Bossuyt & Milinkovitch, 2000) and TyrC and TyrG for the nuclear Tyr gene fragment (Bossuyt & Milinkovitch, *ibidem*). PCRs were performed using an initial denaturation step at 95°C for 5 min, followed by 37 cycles of denaturation at 95ºC for 30 sec, annealing at 58ºC for 1 min and extension at 72ºC for 1 min and a final extension step at 72ºC for 8 min. All PCR products were cleaned with the ExoSap-IT PCR product cleaning agent (ThermoFisher Scientific™) and sequenced in the respective sequencing facilities of the Senckenberg Molecular Genetics lab and the Servicio de Secuenciación y Análisis Molecular [Sequencing and Molecular Analysis Service] - SSIGMOL of the Instituto de Genética, Universidad Nacional de Colombia.

3.3.4 Data analyses and settings

The sequences obtained were edited using BioEdit (Hall, 2005), aligned iteratively with the ClustalW algorithm as implemented in the same software and adjusted by visual inspection. The protein-coding DNA fragments were checked for the correct reading framework through the ExPASy web portal (Artimo et al., 2012). The best combination of partition scheme and substitution models for the entire alignment was obtained using PartitionFinder *(Lanfear et al*., 2017) under the corrected Akaike Information Criterion (AIC; **Table S3-2**). A general Bayesian phylogenetic analysis of the full set of sequences was performed with MrBayes (Ronquist et al., 2012) in order to identify the best supported phylogeny. For obtaining diversification dates and the 95% credibility intervals, four different analyses, each one with two independent runs (**Table S2-3**), were performed under the Fossilized Birth-Death model as implemented in Beast (Bouckaert *et al*., 2019), following the recommendations and default settings proposed by Barido-Sottani *et al*. (2018). As a secondary calibration we used the estimated time of the most recent common ancestor (MRCA) between *Scinax* and *Pseudinae* clades (45.32 Mya), proposed by Feng *et al.* (2017) based on a large set of genomic data and fossils.

In MrBayes, convergence was checked with the average standard deviation of split frequencies (<0.01) and with the minimum and average estimated sample size (ESS) provided by the program, and graphically, with the plots of the generation (x-axis) versus the log probability of observing the data (y-axis). In Beast, the results were checked for convergence, and the posterior age distributions were analyzed using Tracer v1.8 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018).The trees resulting from the analyses were edited using FigTree (Rambaut, 2018), Power Point™ and Inkscape (GPL license, version 0.92.5) at www.inkscape.org. Provided that in any case the topologies recovered the same number of clades, diversification was assessed by calculating within-group and between-group *p*- uncorrected distance matrices as implemented in Mega X (Kumar et al., 2018), for the 16S fragment of all the sequences to which the fragment was available.

Nuclear alignments were phased with the Phase algorithm as implemented in DNAsp (Rozas et al., 2017) and the phased haplotypes were used to build statistical parsimony networks as implemented in PopArt (Leigh & Briant, 2015). The final edition of the network and labelling were performed using Inkscape (GPL license, version 0.92.5) at www.inkscape.org.

3.4 Results

3.4.1 Phylogenetic analyses

The global Bayesian phylogeny is presented in **Figure 3-1**, while the global Beast dated phylogeny can be seen in **Figure 3-2**. 95% HPD intervals were too wide because the nuclear information was scarce for several taxa. Hence, node labels in **Figure 3-2** are the calculated average raw heights. In **Figure 3-3**, however, there is an overview of the other topologies produced by the different conditions and priors of the analyses.

Both MrBayes and Beast topologies showed almost the same distribution of related taxa (**Figures 3-1, 3-2**), with a clear and uncommon predominance of perfectly diverged monophyletic clades, despite the extensively commented cryptic diversity and the known life history traits of the nominal species selected. Four small but noticeable differences related to those nominal species call our attention: 1) the position of sample KU 215423 form Perú, Madre de Dios, Cusco Amazónico, which unless identified as *Scartyla goinorum*, it lies in the *S. vigilans* clade in the MrBayes topology (**Figure 3-1**). In the dated phylogeny (**Figure 3-2**) it is recovered as the sister taxon of *Scarthyla goinorum*. In that approach, *S. vigilans* is restricted to the Maracaibo-Caribbean region and *S. goinorum* is strictly Amazonian. 2) The position of the clade *Scinax kennedyi*, which was recovered in the MrBayes phylogeny as a member of the *S. rostratus* clade –thus regarding *S. rostratus* as currently understood paraphyletic, **Figure 3-1**–, while in the dated phylogeny it is the reciprocally monophyletic sister clade of the entire *S. rostratus* clade (**Figure 3-2**). 3) The position of *Scinax nebulosus*, which in the MrBayes topology is the sister species of *Scinax acuminatus*, while in the dated phylogeny it is the sister species of the *S. pedromedinae* clade. Lastly, 4) the topology of the *S. boulengeri-S. sugillatus* clade, which is a polytomy in the MrBayes phylogeny (**Figure 3-1**), while in the dated phylogeny the Colombian sample (JDL 31508, B45) is the sister lineage of the *S. boulengeri-sugillatus* samples, which are almost identical despite the fact that *S. boulengeri* is regarded as the Central American representative of the *S. rostratus* group (sample from Costa Rica) and *S. sugillatus* is the Chocoan representative (nearly topotypic sample).

3.4.2 General description of the recovered clades

a) **Pseudinae**: the second clade in the phylogeny (after the outgroups and the taxa used for calibration) contains a monophyletic *Pseudis paradoxa* clade, which is the sister clade of *Scarthyla.* This genus is composed of a monophyletic *Scarthyla vigilans* and a structured *Scarhyla goinorum*, according to the uncorrected 16S *p*-distances (**Table S4-A1**). The two samples from Perú are certainly divergent (*p*-distance above 10% between them) and separated from the other *S. goinorum* samples. The *p*-distance between the KU 215423 sample to *Scarthyla vigilans* is much lower (below 3%) than the distance to the Amazonian clade.

b) The ***Scinax*** mega-clade is also clearly monophyletic and comprised of *Scinax acuminatus* and *Scinax uruguayus* as the sister taxa of the rest of the phylogeny. In the dated phylogeny, however, there is the sample B16 (MAV 465), which was initially identified as, and morphologically consistent to *Scinax pedromedinae* (first reviewed by ASM) but falls outside the *Scinax rostratus* group, being the sister clade of *Scinax acuminatus* + *S. uruguayus* + the *S. rostratus* group. In both topologies, the *Scinax rostratus* group is comprised of six subclades:

    - The ***S. nebulosus*** clade, either related to *Scinax acuminatus* (**Figure 3-1**) or to *Scinax uruguayus* (**Figure 3-2**).

Figure 1-28: Bayesian inference of the phylogeny of *Scartyla*, *Scinax gr. rostratus*, *S. wandae* and *Sphaeonrhynchus lacteus* in a wide sampling in the north-western South America. A. Part 1: former two taxa. B. Part 2. Later two taxa.

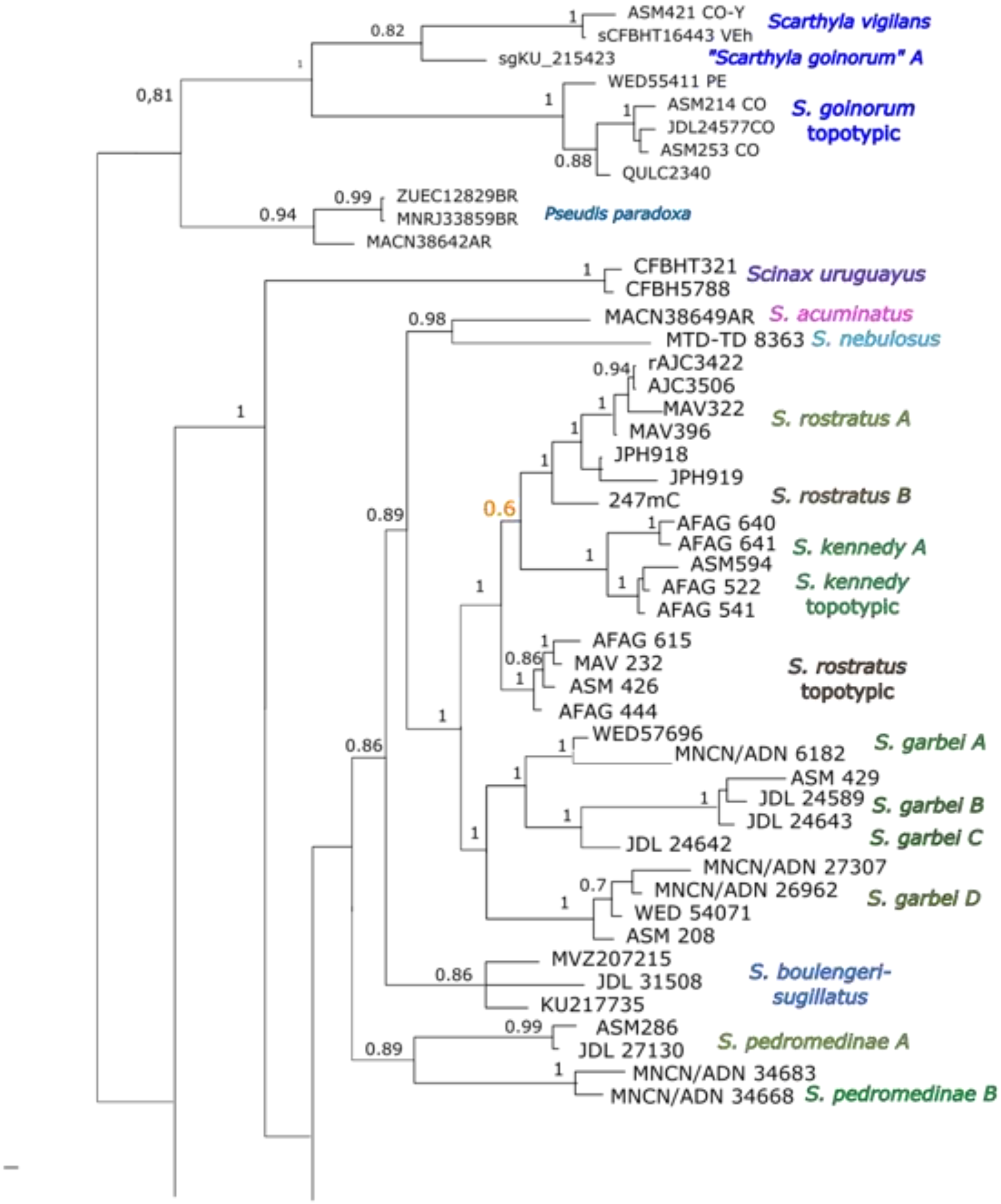
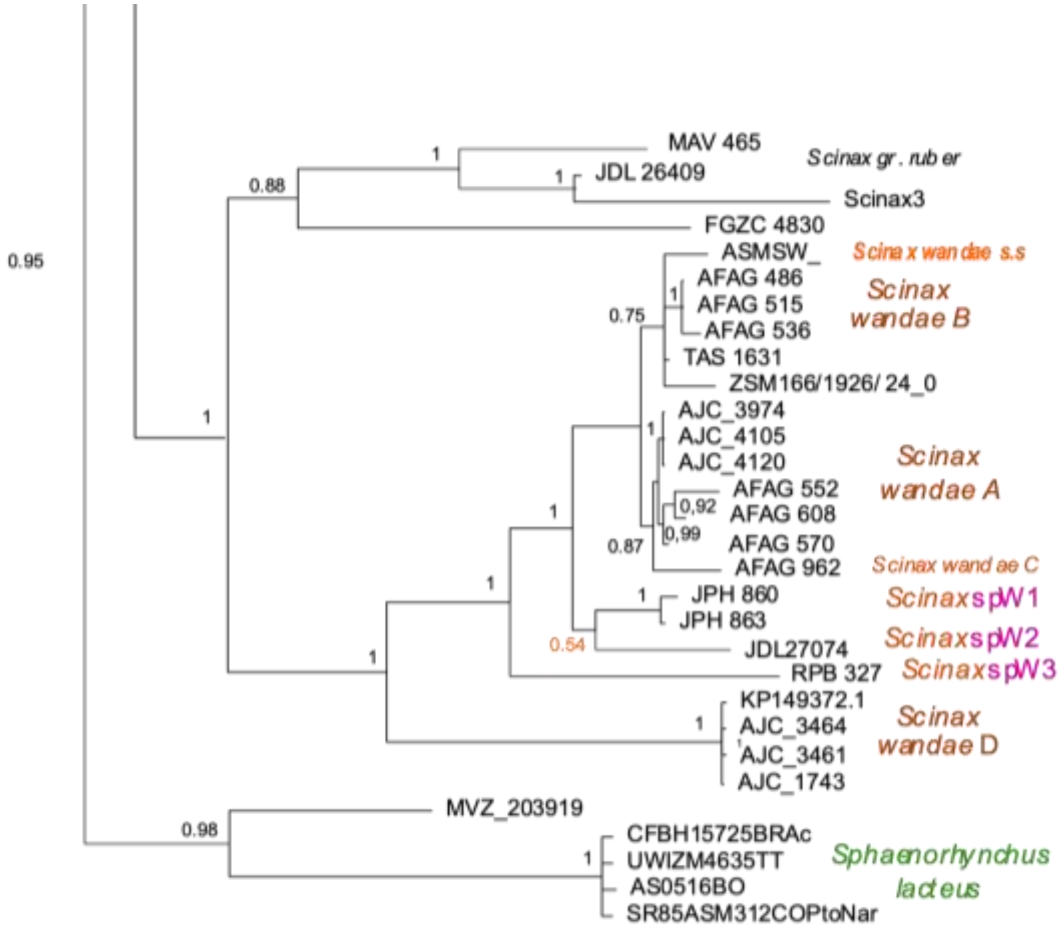


Figure 3-1 A.

Figure 3-1 B. (cont.)



- The ***S. pedromedinae*** clade is comprised in both topologies of two reciprocally monophyletic entities, structured south (Bolivia) to north.

- The ***S. boulengeri-sugillatus*** clade, comprised of the three samples identified above. In both MrBayes and dated phylogenies, it is related to forest lineages, i.e. *Scinax garbei.*

- The ***S. garbei*** clade, which is a strict forest clade comprised of a huge morphological crypticity. Four fully structured and divergent lineages can be identified inside the present sampling (distances between groups ranging from 2 to 19%, **Table S4B**):

  - *"Scinax garbei* A": comprised of samples from the southwestern portion of the Amazon (Bolivia and Perú).
  - *"Scinax garbei B":* this subclade includes the northernmost known sample of the nominal species in Colombia, in the Andean piedmont of Villavicencio, Meta. Its 16S sequence is nearly identical to those of the Leticia portion of the Colombian Amazon.
  - *"Scinax garbei C":* a single sample from the Leticia area but separated by an uncorrected *p*-distance of 6 to 16% from any other member of the subclade.
  - *"Scinax garbei* D": this subclade comprises the north-eastern Peruvian Amazon samples, including the one from the Colombia-Perú border in the Tipisca indigenous community. No morphological differences to other *S. garbei* lineages were noted when that sample was collected by ASM.

- The ***S. kennedyi*** clade: this is a Colombian endemic lineage normally confused with *Scinax rostratus,* yet there are evident morphological differences between both. The clade is comprised of two reciprocally monophyletic sets of samples: "*Scinax kennedyi* A", from the northern Llanos (Arauca) and "*Scinax kennedyi* topotypic" samples from the vicinities of the Serranía de la Macarena and the Guaviare river border, which are topotypic in fact: the recorded type locality of this species is "Alto de Menegua, Meta, Colombia" but according to Faivovich (pers. com. 2020) that locality is in error. The actual locality of that species is the Ariari river near the Serranía de la Macarena, which connects the two topotypic samples considered herein. The phylogenetic analyses are not competent to distinguish between a *rostratus*-linked (dated phylogeny) or a *rostratus* member (MrBayes phylogeny) position for this clade, but surely the haplotype networks can help with that task (see below).

- The ***S. rostratus*** clade, which is a strict open-areas clade in which morphological differences seem to be associated to snot shape and dorsal pattern. Three structured lineages can be identified inside the present sampling (distances between groups ranging from 2 to 19%):

  - *"Scinax rostratus* A*"* (Trans-Andean): comprised of samples from the western versant of the Eastern Cordillera of Colombia. Divergence of that clade to the cis-Andean ones ranges from 4.0 to 6.2% uncorrected p-distance.
  - *"Scinax rostratus* B*":* Guiana, a single sample. Uncorrected p-distances to the topotypic and to the A clade are equal (4.2%).
  - *Scinax rostratus* topotypic*:* composed of samples from the piedmont and northern llanos of Colombia. It is more distant from the Guiana sample than to the trans-Andean clade. Uncorrected p-distance to the B clade is 3.2%.

- The ***S. ruber*-like** clades were not part of the present paper –they were extensively discussed in the first and second chapters of the former author doctoral dissertation (Castillo-Rodríguez, Suárez-Mayorga, Gantiva, Fritz, & Vargas-Ramírez, 2020), but samples were included in order to track their position in the phylogenies and to obtain dating indications. It is strikingly surprising, however, that the clade in the MrBayes phylogeny is composed of three completely morphologically different samples, assigned to completely different identities: Scinax pedromedinae (MAV 465), Scinax wandae (JDL 26409) and Scinax sp. of the ruber group (MAV 326).

- The *S. ruber*-like clade in both topologies is the sister clade of the ***Scinax wandae*** megaclade. The nominal species was thought to be a Colombian Llanos endemic, but there were no doubts about its presence in Venezuela. During the last five years, an extensive sampling of Amazonian *Scinax* yielded a number of small orangish species with no color pattern on the posterior thighs (Ferrão et al., 2016, 2018), and Ferrao (*ibidem*) proposed, based on mitochondrial phylogenies, that they were related both to *S. cruentomma*, another common Amazon-associated taxon, and to *S. wandae*. This phylogeny confirms that association, and simply because the later species was described a year before the former, the clade will be referred as the *Scinax wandae* clade from now on. It is comprised of the following subclades:
  - *Scinax wandae s.s.:* the topotypic sample (ASMSW) considered herein was collected in the Restrepo-Cumaral border (the type locality is Restrepo, Meta, Colombia) and it is perfectly concordant morphologically with the original

description of the species. It has dorsolateral stripes, which are rarely present in other populations.

- *“Scinax wandae B”:* The position of sample TAS 1631 from San Martín, Meta, is not clear in any of the topologies, but it is likely the same taxon based on the distances (below 0.1%, Table S4C), as they are the other samples from the vicinity of that locality, referred to “S. wandae B”. This subclade is the sister clade of the one formed by “S. wandae A” plus “S. wandae C”.
- “*Scinax wandae* A”: this subclade is comprised of samples from Arauca and Casanare, and it is the sister clade of “Scinax wandae C” from Cumaribo, Vichada. There are noticeable morphological differences between Arauca and San Martín samples, related to the shape of snout and dorsal color pattern. However, uncorrected p-distances between the members of each clade are low (1.1%). The nuclear haplotype networks can be useful for deciding whether those lineages are diferent.
- “*Scinax wandae* C”: the samples from the extreme eastern Colombia in the Colombia-Venezuela border are also morphologically different: they have a rather tuberculated snout and their size is larger than the size of the topotypic ones. Besides, they have a typic dark dorsal color pattern and uncorrected p-distances to any of the other clades ranging from 1.1 (to the Arauca-Casanare *“S. wandae* C” clade) to 13.6% (“*S. wandae* D”).
- *“Scinax wandae* D*”:* that clade contains samples from the northern Andean piedmont in Casanare. Individuals within the clade are smaller than the topotypic ones and they are completely separated genetically, with uncorrected p-distances to any of the other groups ranging between 8 and 18% (Table S4C2).

- Finally, the ***Sphaenorhynchus*** clade is the sister clade of the entire *Scinax* and it is comprised of four samples with nearly identical molecular information. The uncorrected p-distances between all the *Sphaenorhynchus* samples considered herein are presented in Table S4D and it is evident that *Sphaenorhynchus lacteus* is genetically uniform along its entire distribution, with distances between the samples below 1% in any case. For comparison purposes, the last three samples in the table correspond to *S. carneus* (SR86) and S. *dorisae* (the remaining two),

two other and sympatric fully recognized *Sphaenorhynchus* species, to which uncorrected p-distances are higher than 7% in any case.

3.4.3 Phyogeography – haplotype networks

The two nuclear haplotype networks are presented in Figure 4 (Rho) and Figure 5 (Tyr). Only five Rho sequences of *Scarhyla* (2 of *S. goinorum* and 2 of *S. vigilans*) were obtained, so they are useless for comparison purpose, and they were not included in the networks. No nuclear sequences were neither obtained for S. sugillatus nor for any of the "*Scinax wandae* D" samples, hence they were not included in the networks either.

Despite the differences between the taxa considered, almost every single lineage is well separated and characterized in both nuclear haplotype networks and there are not shared haplotypes between geographically, morphologically, or molecularly (mitochondrial) close (uncorrected p- distances) lineages. Unique nuclear haplotypes in both networks (thus indicating evolutionary different lineages, or species (Suárez-Mayorga *et al*., 2021), are present for *S. nebulosus*, "*S. pedromedinae* A", "*S. pedromedinae* B", "*Scinax garbei*" A-D, "*S. kennedy* A", *S. rostratus* A", "*S. rostratus* topotypic", Scinax wandae *s.s.,* "*S. wandae* A", "*S. wandae* B" (TAS sample) and "*S. wandae* C".

3.4.4 Divergence times estimation

The topologies obtained varied in the estimation of divergence times according to the age selected as Origin: the more ancient that origin age, the more ancient the initial diversification process. However, the estimated age ranges for coherent cladogenesis (Figure 2, colors) were common among unrelated taxa, as follows:

- The most ancient event (**Figure 3-2**, dull turquoise column) corresponds to the Oligocene-Miocene border (ca. 20 Mya) when the sub-Andean rivers started to form after the basal rise of the Andes, and a main drainage to the north-west (the proto Magdalena river) was developed, separating the eastern and western sides of Colombia (Torrado, Carvajal-Arenas, Mann, & Bhattacharya, 2020). The northernmost areas of the continent were covered of Guianan sediment, and the Eastern Cordillera advanced eastwards, configuring the eastern and Caribbean alluvial planes (Torrado *et al., ibidem*). In the dated phylogeny (and concordant to the MrBayes topology) that event contributed to the diversification of the aquatic (*Pseudis*) and supposedly semi-aquatic lineages (*Scarthyla* and *Sphaenorhynchus*), which share a common ancestry to their sibling lineages (Hylinae and *Scinax*, respectively) around that time. With the evidence available it is not possible to decide on the areas of origin of *Sphaenorhynchus*. However, its distribution reaches the Caribbean coast, resembling that of Pseudis, and that genus was considered of Caribbean affinity since long ago (William Edward Duellman, 1979).

- The next common cladogenesis event (**Figure 3-2**, yellow column) is related to the first marine intrusion in South America during the early Miocene ca. 18Mya (Jaramillo *et al*., 2017), which had effects both in the Llanos and in the Solimões basins, but occurred first and longer in the former. That could have been the common origin of the only effectively widespread species inside the present sampling: *Pseudis paradoxa* and *Sphaenorhynchus lacteus*, and it could have been also related to the effective separation of *Scinax elaeochroa*, the only true Central American species in the sampling.

- The second marine intrusion during the Middle Miocene (around 12 Mya) and the Pebas system are considered the ultimate cause of most diversification events in the Northern South America. In the dated phylogeny (**Figure 3-2**, turquoise column) gave rise to a separation between forest and non-forest species, and that is evident in the strict separation of *Pseudis* from *Scarthyla* and the splitting of *Scinax* in the *Scinax ruber-Scinax wandae* clades, of supposed Guianese or Llanos origin, from the *Scinax rostratus* group, whose members are nowadays fundamentally forest species, with some exceptions. The dating does not allow to understand whether the non-forest/forest species of those groups moved outside their original environments (to the forest in the case of *Scarthyla*, and to the Llanos in the case of *Scinax rostratus*), but the new habitat availability provided by the Pebas system should have undoubtedly provide connection means for those water-dependent species to migrate and colonize new habitats, to which they seem to be attached.

Figure 1-29: Dated phylogeny of *Scartyla*, *Scinax* gr. *rostratus*, *S. wandae* and *Sphaeonrhynchus lacteus* in a wide sampling in the north-western South America. Node labels are raw dates (Mya) on the inverse axes. The phylogeny was calibrated with fossils and the origin of Scinax in 31.2 Mya according to Smith *et al.* (2017). Color bars indicate relevant events in the diversification process of the studied taxa: Opaque turquoise: Oligocene-Miocene border; yellow: first marine intrusion during the Miocene; turquoise: second marine intrusion and extension of the Pebas System; green: Acre System and onset of the Amazon fan, including redistribution of humid forests in the eastern and trans-Andean lowlands due to the onset of the Eastern Cordillera (Cordillera Oriental) of Colombia around 10 Mya; brown: Pliocene diversification, concordant to the deformation of the Eastern Cordillera of Colombia and the most recent pulse of uplift (Ochoa *et al.*, 2012).

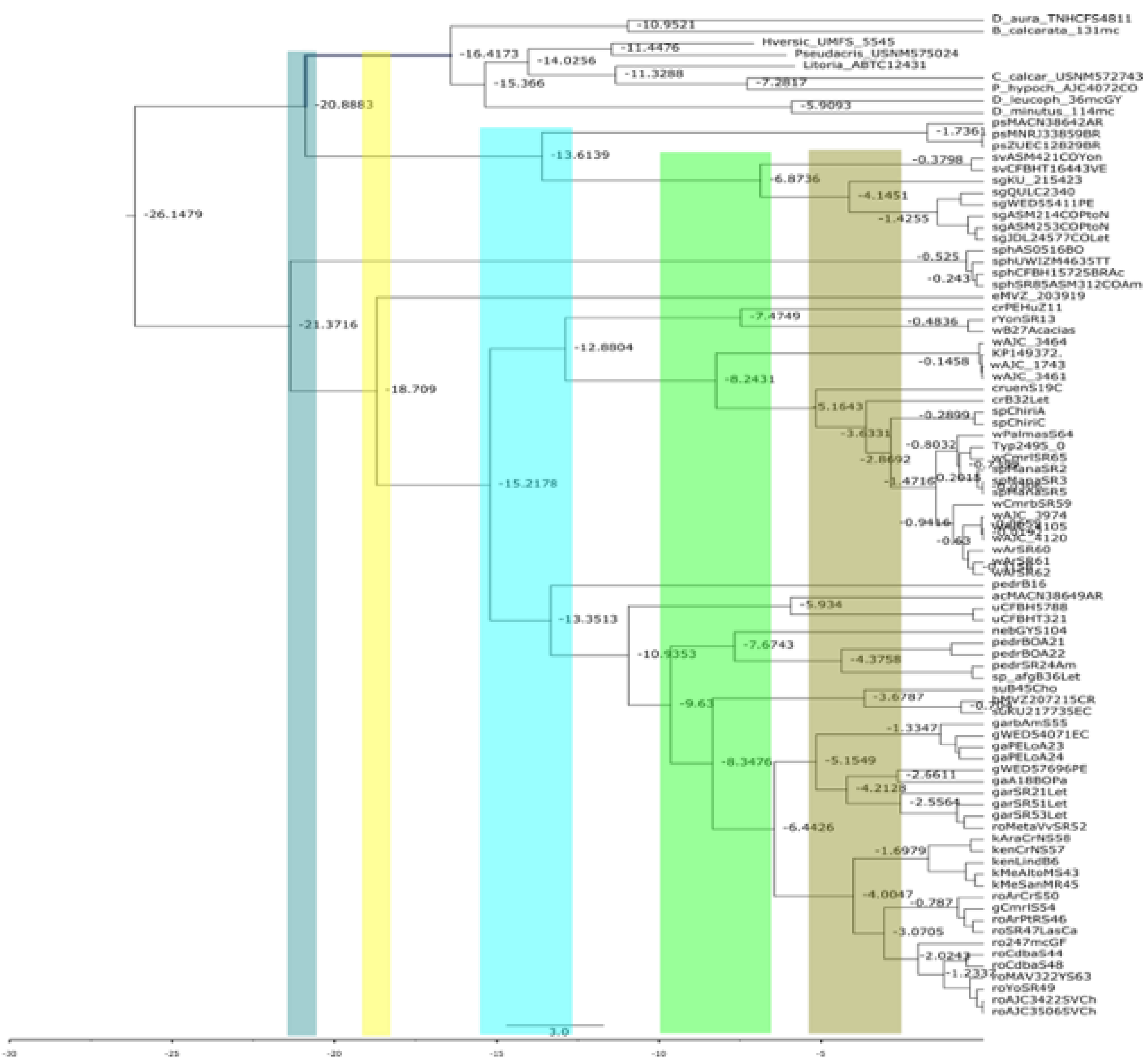
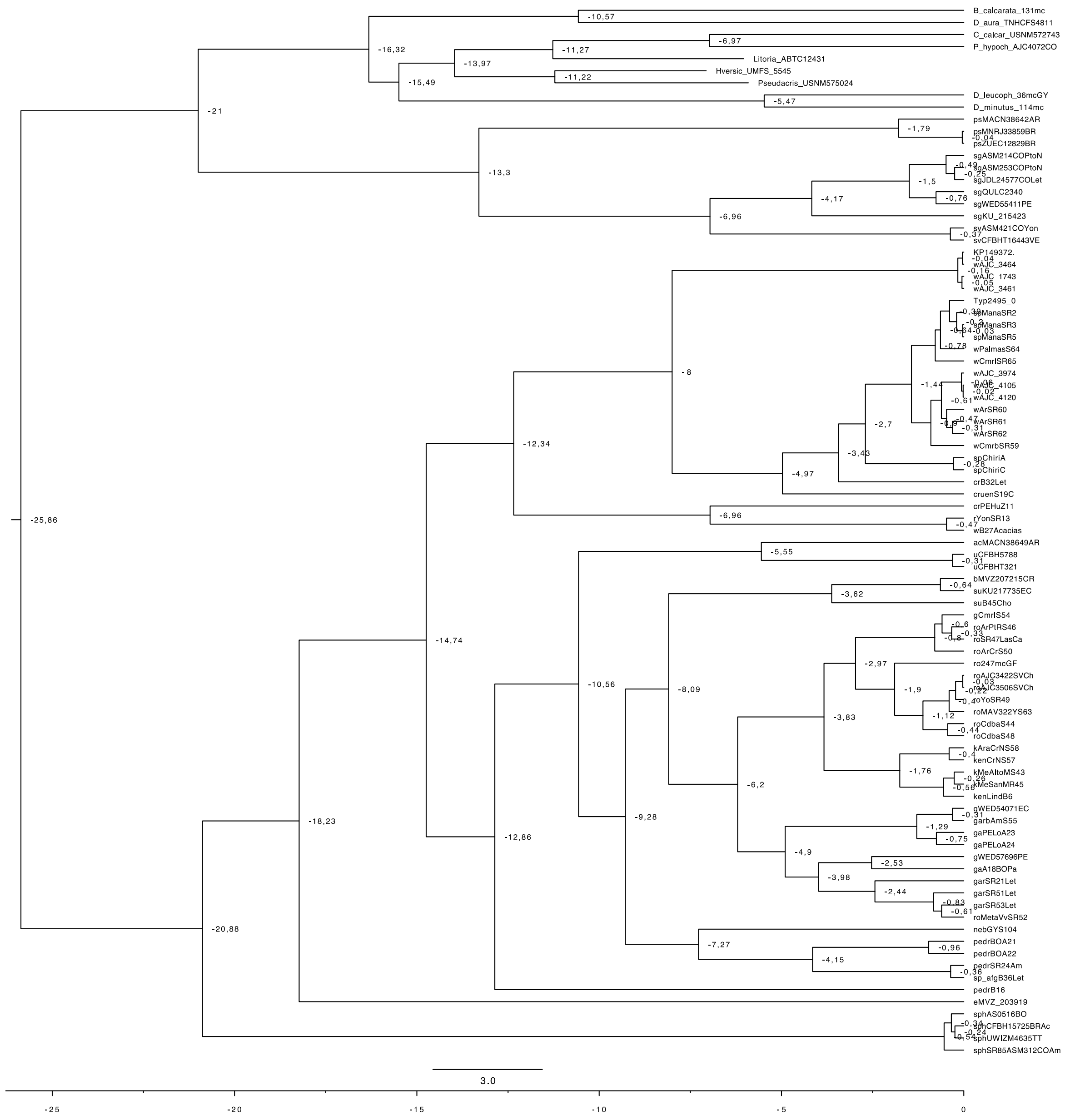
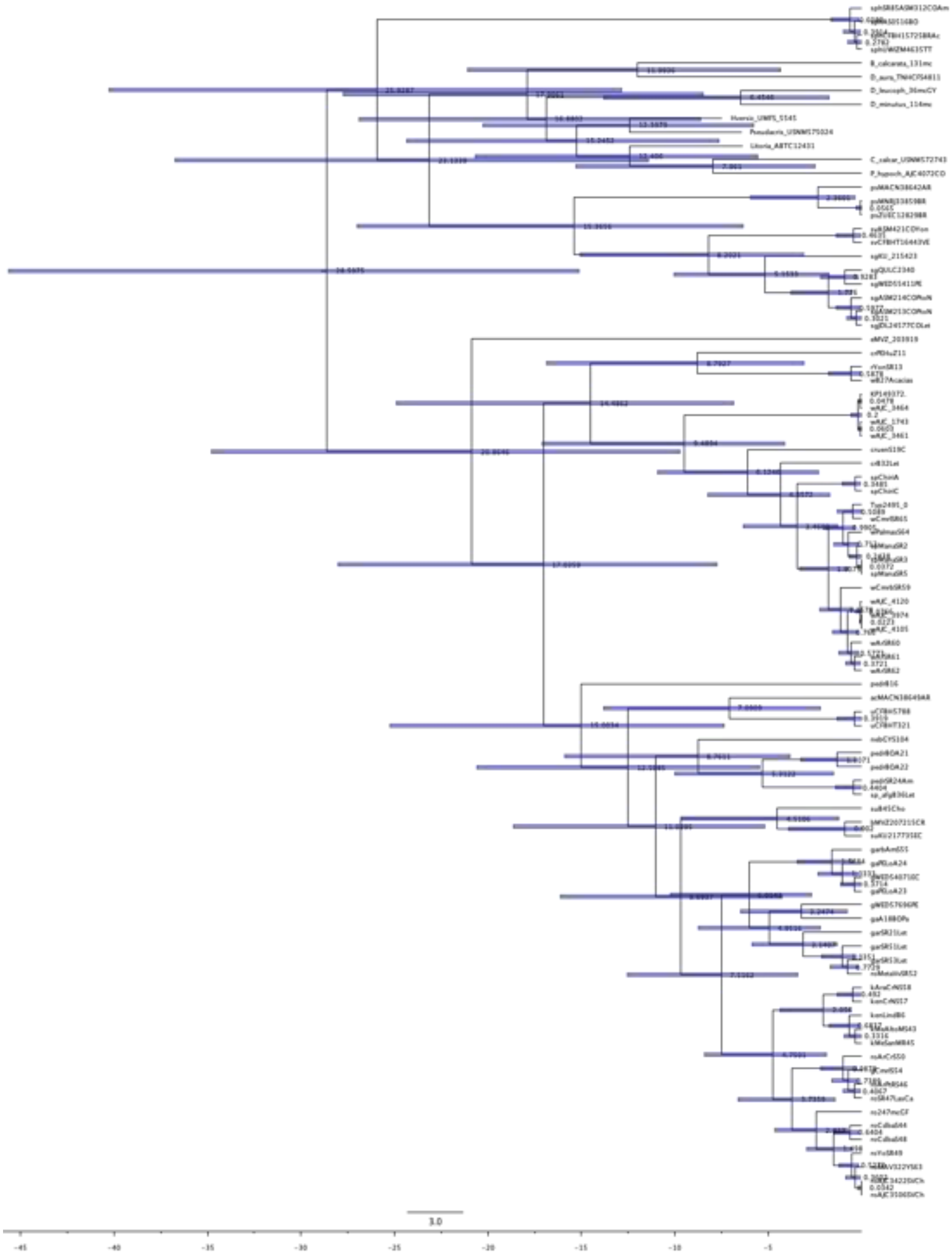


Figure 1-30: Overview of two different topologies produced with the same alignment under different priors and conditions of origin. A. Origin 54.8, first resulting node dated at 25.9 Mya B. Origin 61 Mya. First resulting node dated at 28.60 Mya.

A.



B.


B_calcarata_131mc
D_minutus_114mc
C_calcar_USNM572743
P_hypoch_AJC4072CD
sgKU_215423
eMVZ_203919
3.0
-45
-40
-35
-30
-25
-20
-15
-10
-5

- The re-distribution and paused uplift of the Eastern Cordillera, which took place approximately 9 Mya (C Hoorn *et al*., 2010; Jaramillo, 2019), could have influenced the diversification of the non-forest clades, while the slightly previous formation of the Vaupés Arch (10 Mya) could have separated forest populations which were previously widespread (Vargas-Ramírez *et al.*, 2020). The establishment of East-directed new drainages and allied deposition areas permitted new habitats available for colonization, but it could have acted initially as a barrier for gen flow too, thus promoting local diversification. That is evident in the dated phylogeny (**Figure 3-2**, green column) by the explosion of *Scinax* clades, separated by the Andes: *Scarthyla goinorum* separated from *S. vigilans* and the Chocoan-Magdalena *Scinax* of the *S. rostratus* group (*S. boulengeri-sugillatus*)" started to diverge from the Amazonian clade.

- Finally, the vertical surface uplift produced during the last 5 My in the Eastern Cordillera and influence areas (Torrado *et al*., 2020), along with the development of widespread terrace systems and the consequent restructuring of drainages (C Hoorn *et al.*, 2010; Ochoa *et al.*, 2012), produced changes in climate and microclimate (Ehlers & Poulsen, 2009), vegetation (Carina Hoorn et al., 2017; Jaramillo, 2019; Ochoa *et al.*, 2012) and ecosystems in general, which pushed the adaptation of the biotas to the new available environments, and consequent diversification. In the phylogeny that is consistent with the separation between the piedmont and Amazon clades inside "*Scinax wandae"*, the separation of the *Scinax kennedyi* lineages and the structure of *Scarthyla goinorum*.

3.5 Discussion

Diversification in the northern South America is undoubtedly related the Andes formation and setting (Gregory-Wodziki, 2000; Rull, 2011) but also to the development of drainages (Brandley, Guiher, Pyron, Winne, & Burbrink, 2010; Ferrão et al., 2016; Menezes et al., 2016; Ribas, Gaban-Lima, Miyaki, & Cracraft, 2005; Santorelli, Magnusson, & Deus, 2018). Either as barriers and consequent promotors of vicariance of lowland populations, or as vehicles of dispersal to new habitats provided by the uplifts or by floodings, both kinds of geographic and geological developments have interrelated to animal populations to structure the landscape that are seen nowadays.

Several studies have coincided in pointing to at least three geological events as responsible for shaping the actual diversity and distributions of the lowland faunas of the Northwestern South America: The onset and initial uplift of the Andes, followed by the proto-Magdalena river (late Oligocene to Early Miocene); The Pebas system (Middle Miocene, 12-16 Mya) and the Pleistocene changes in climate, vegetation and river courses which occurred thanks to the interaction of mountains and water (Jaramillo, 2019; Jaramillo et al., 2017).

The species and lineages considered herein are concordant to those events. Apparently, for the terrestrial and semi-aquatic species that succession of events was fundamental in providing wetlands, which they need at least for reproduction, and the alternance of forests/savannas in search of uncolonized habitats, especially from the Late Miocene to the Quaternary. On turn, for the aquatic species the combination of wet/dry flows produced semi-permanent connections along the territories, which favored gene flow through the river system, thus genetically unifying Pseudis and Sphaenorhynchus, which travel with the current or on top of floating vegetation from the southern Amazonian boundaries to the Caribbean. Similar histories have been told for the Platyrrhini monkeys (Kay, 2013), the toad Rhinella marina (Maciel, Collevatti, Colli, & Schwartz, 2010), Taygetis butterflies (Matos-Maraví, Peña, Willmott, Freitas, & Wahlberg, 2013), the bamboo rats (Upham, Ojala-Barbour, Brito M, Velazco, & Patterson, 2013) and more recently, the matamata turtle (Vargas-Ramírez *et al.,* 2020).

The extensive geographic and taxonomic sampling of this work, and the mandatory comparisons to previous approaches, produced also relevant taxonomic and phylogenetic updates:

1) As anticipated by previous reviews (Caminer *et al*., 2017; Rojas, Fouquet, Ron, *et al*., 2018; Vacher *et al.*, 2020), the species diversity of the Northern South America is not only underestimated, but poorly described. Except from *Scarthyla vigilans* and *Sphaenorhynchys lacteus*, every nominal species considered herein, and their sibling describe lineages, is composed of at least two different and divergent

evolutionary lineages. That is remarkably relevant in terms of conservation of biodiversity, because the conservation planning has been done without real data which support them.

Figure 1-31: Rho haplotype network for the entire alignment (except *Scarthyla*).

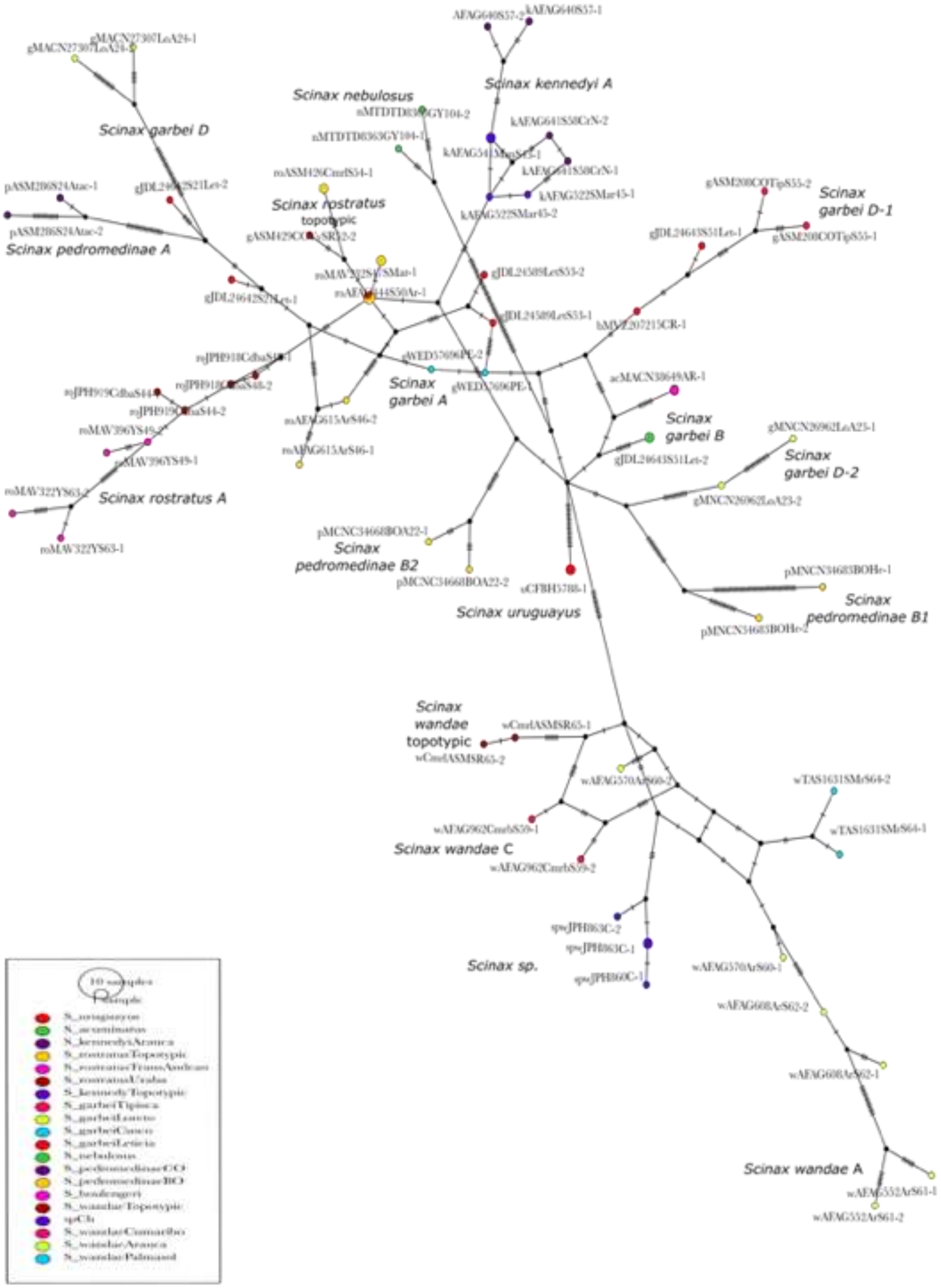
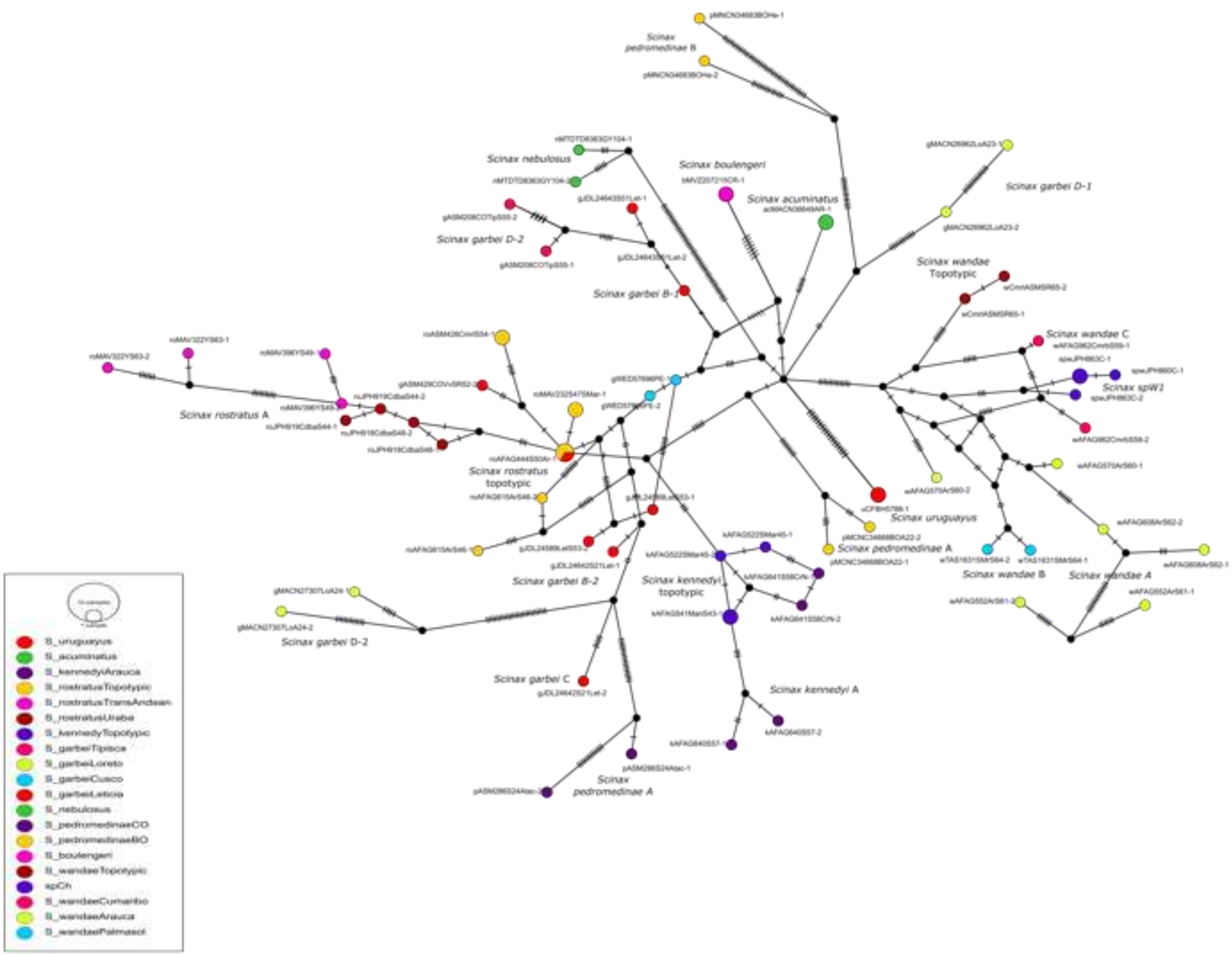


Figure 1-32: Tyr haplotype network for the entire alignment except *Scarthyla*



2) There are few widespread tree frog species in the study area, but the ones identified so far are related to the river courses. *Pseudis paradoxa, Scarthyla vigilans and Sphaenorhynchus lacteus* are, in fact, widely distributed, and their little genetic divergence suggests that they are able to use the river courses and the floating meadows as boats for dispersal. On the contrary, and considering the herein documented crypticity of the taxon, we doubt on the Guianan and Brazilian records of *Scinax rostratus* (Sturaro *et al.*, 2010)*,* which have given it the “widespread” status.

3) *Scinax wandae* is much more endemic than previously thought and restricted to the type locality. However, it forms a large clade with the frogs identified as *Scinax cruentomma* and allies, which have a morphological synapomorphy related to the extension of the vocal sac in males (J. Faivovich, com. pers. 2020). In Colombia only, it is comprised of five different evolutionary lineages, confirmed by the presence of unique nuclear haplotypes in both nuclear haplotype networks, though the uncorrected *p*-distances in the 16S rRNA fragment may be as low as 1%.

4) *Scinax rostratus* comprises in Colombia a forest-linked trans-Andean lineage and a cis-Andean open-areas lineage.

5) *Scinax garbei* is the most cryptic nominal species of the present sampling, comprising five different evolutionary lineages apparently structured south-eastern to northwestern.

6) Neither *Scinax* clade can be confirmed to have a latitudinal cline in size, simply because the comparison had been performed between different species.

3.6 Acknowledgements

No parts of this work could have been done without the unvaluable help of the different friends, colleagues and interested parties which contributed in feeding the biological collections, finding natural history data and new records of the species studied, and with their company and discussions for the last seven years. Ana María Saldarriaga and Sebastián Cuadrado helped the senior author to finally understand the Coalescent Bayesian Analysis and the tools for designing haplotype networks. Gilson Rivas was always trying to help with the topotypic material and important references. The professors Uwe Fritz, John D. Lynch, Ignacio de la Riva, Raffael Ernst and Frank Glaw were always open to share knowledge, specimens/tissues, and discussions to the senior author. The Instituto Sinchi and Dairon Cárdenas facilitated the fieldwork in Guaviare, while Catalina Cruz Piedrahita was responsible for the few specimens and experience in the Meta-Vichada border. Carolina Mayorga González and Germán Vargas Cuervo provided vital geologic information and references for giving the discussion its final shape.

Table S1-4: Tissue samples and GenBank sequences used for the molecular analyses, ordered by taxonomic identity. The “Recorded identity” corresponds to the identity recorded in the collection’s catalogue or in the documented database from which the sample was obtained. The column Tissue Code includes the GenBank codes for the sequences used, as well as the code for the herein provided sequences. Catalogue No. and Collector No. refer to the Darwin Core concepts Catalogue Number (for the unique identifier of the specimen catalogued in a biological collection) and Collection Number (for the code given to the specimen by its collector in the field).

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
AY843740.1 12S, 16S AY844727.1 Rho AY844167.1 Tyr	MACN 38642		*Pseudis paradoxa*	Argentina, Corrientes
MK293738.1	MNRJ:33859		*Pseudis paradoxa*	Brazil, Bacabal, Maranhao
MK293739.1 12S, 16S	ZUEC:12829		*Pseudis paradoxa*	Brazil, Bacabal, Maranhao
AY819521.1 16S AY819389.1 12S	KU 215423		*Scarthyla goinorum*	Perú, Madre de Dios, Cusco Amazónico
AY843752.1 12S, 16S AY844738.1 Rho	QULC 2340		*Scarthyla goinorum*	Brazil, Amazonas
AY326035.1	KU 205763	WED 55411	*Scarthyla goinorum*	Perú, Madre de Dios, Cusco Amazónico
XXXX_S76	IGUN-BATB 3283	ASM 214	*Scarthyla goinorum*	Colombia, Amazonas
XXXX_S77	IGUN-BATB 3284	ASM 216	*Scarthyla goinorum*	Colombia, Amazonas

GenBank No./ tissue code	**Catalogue No.**	**Collection No.**	**Recorded identity**	**Location**
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
XXXX_S78	IGUN-BATB 3287	ASM 253	*Scarthyla goinorum*	Colombia, Amazonas
XXXX_S79	IGUN-BATB 3302	ASM 310	*Scarthyla goinorum*	Colombia, Amazonas
XXXX_S73	IGUN-BATB 1028	JDL 24577	*Scarthyla goinorum*	Colombia, Amazonas
XXXX_S71	IGUN-BATB 3334	ASM 421	*Scarthyla vigilans*	Colombia, Antioquia, Yondó
XXXX_S72	IGUN-BATB 3333	ASM 417	*Scarthyla vigilans*	Colombia, Antioquia, Yondó
XXXX_S75	IGUN-BATB 3375	JPH 995	*Scarthyla vigilans*	Colombia, Córdoba
XXXX_S74	IGUN-BATB 3376	JPH 996	*Scarthyla vigilans*	Colombia, Córdoba
KU495532.1 16S KU494739.1 COI	CFBHT16443		*Scarthyla vigilans*	Venezuela, Táchira
AY843753.1 12S, 16S AY844739.1 Rho AY844176.1 Tyr	MACN38649		*Scinax acuminatus*	Argentina, Corrientes
AY843755.1 12S, 16S AY844741.1 Rho AY844177.1 Tyr	MVZ 207215	MVZFC14296	*Scinax boulengeri*	Costa Rica, Guanacaste
XXXXX_S19	IGUN-BATB 2337	RPB 327	*Scinax cruentomma*	Colombia, Caquetá
XXXXX_B32	IGUN-BATB 1163	JDL27074	*Scinax cruentomma*	Colombia, Amazonas
AY843757.1 12S y 16S AY844743.1 Rho AY844178.1 Tyr	MVZ 203919	MVZFC 14457	*Scinax elaeochroa*	Costa Rica, Heredia

GenBank No./ tissue code	**Catalogue No.**	**Collection No.**	**Recorded identity**	**Location**
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
XXXXX_S55	IGUN-BATB 3282	ASM 208	*Scinax garbei*	Colombia, Amazonas
AY326033.1 12S, 16S		WED 54071	*Scinax garbei*	Ecuador, Chimborazo
XXXXX_A23	gaPELoA23		*Scinax garbei*	Perú, Loreto
XXXX_A24	MNCN/ADN 27307		*Scinax garbei*	Perú, Loreto
DQ283030.1 12S, 16S DQ283759.1 Rho DQ282898.1 Tyr		WED 57696	*Scinax garbei*	Perú, Madre de Dios, Cusco Amazónico
XXXX_A18	MNCN/ADN 6182		*Scinax garbei*	Bolivia, Pando
XXXX_S21	IGUN-BATB 1043	JDL 24642	*Scinax garbei*	Colombia, Amazonas
XXXX_S51	IGUN-BATB 1044	JDL 24643	*Scinax garbei*	Colombia, Amazonas
XXXX_S53	IGUN-BATB 1030	JDL 24589	*Scinax garbei*	Colombia, Amazonas
XXXX_S45	IGUN-BATB 3841	AFAG 522	*Scinax kennedyi*	Colombia, Meta, Alto Manacacías
XXXX_B6	IGUN-BATB 6723	ASM 594	*Scinax kennedyi*	Colombia, Guaviare
XXXX_S104	MTD-TD 8363		*Scinax nebulusus*	Guyana, Iwokrama Forest
XXXX_A21	MNCN/ADN 34683		*Scinax pedromedinae*	Bolivia, La Paz

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
XXXX_A22	MNCN/ADN 34668		*Scinax pedromedinae*	Bolivia, La Paz
XXXX_S24	IGUN-BATB 3291	ASM 286	*Scinax pedromedinae*	Colombia, Amazonas
XXXX_B16		MAV 465	*Scinax pedromedinae*	Colombia, Amazonas
XXXX_S52	IGUN-BATB 3337	ASM 429	*Scinax rostratus*	Colombia, Meta, Villavicencio
XXXX_S50	IGUN-BATB 3794	AFAG 444	*Scinax rostratus*	Colombia, Arauca
XXXX_S54	IGUN-BATB 3794	ASM 426	*Scinax rostratus*	Colombia, Meta, Cumaral
XXXX_S46	IGUN-BATB 3917	AFAG 615	*Scinax rostratus*	Colombia, Arauca
XXXX_S47	IGUN-BATB 3364	MAV 232	*Scinax rostratus*	Colombia, Meta, San Martín
EF376145.1 Tyr EF376039.1 12S EF376071.1 16S	247mc		*Scinax rostratus*	French Guiana
XXXX_S48	IGUN-BATB 3373	JPH 918	*Scinax rostratus*	Colombia, Córdoba
XXXX_S44	IGUN-BATB 3374	JPH 919	*Scinax rostratus*	Colombia, Córdoba
XXXX_S63	IGUN-BATB 3371	MAV 322	*Scinax rostratus*	Colombia, Antioquia, Yondó
XXXX_S49	IGUN-BATB 3367	MAV 396	*Scinax rostratus*	Colombia, Antioquia, Yondó

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
KP149089.1 COI KP149284.1 16S		AJC 3422	*Scinax rostratus*	Colombia, 7.0796,-73.548
XXXX_TYP	ZSM166/1926/ 24		*Scinax ruber*	Venezuela
XXXX_Z11		FGZC 4830	*Scinax sp. aff.cruentomma*	Perú, Huánuco
XXXX_A	IGUN-BATB 2295	JPH 860	*Scinax sp. aff. cruentomma*	Colombia, Caquetá
XXXX_C	IGUN-BATB 2298	JPH 863	*Scinax sp. aff. cruentomma*	Colombia, Caquetá
XXXX_B36	IGUN-BATB 1197	JDL 27130	*Scinax sp. aff. garbei*	Colombia, Amazonas
XXXX_S57	IGUN-BATB 3932	AFAG 640	*Scinax aff. kennedyi*	Colombia, Arauca
XXXX_S58	IGUN-BATB 3933	AFAG 641	*Scinax aff. kennedyi*	Colombia, Arauca
XXXX_S43	IGUN-BATB 3855	AFAG 541	*Scinax aff. kennedyi*	Colombia, Meta, Alto Manacacías
XXXX_S2	IGUN-BATB 3851	AFAG 536	*Scinax sp. aff. wandae*	Colombia, Meta, Alto Manacacías
XXXX_S3	IGUN-BATB 3811	AFAG 486	*Scinax sp. aff. wandae*	Colombia, Meta, Alto Manacacías
XXXX_S5	IGUN-BATB 3834	AFAG 515	*Scinax sp. aff. wandae*	Colombia, Meta, Alto Manacacías
XXXX_S13	IGUN-BATB 3365	MAV 326	*Scinax sp. gr. ruber* Scinax3	Colombia, Antioquia, Yondó
AY819524.1 16S			*Scinax sugillatus*	Ecuador, Manabí

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
AY819392.1				
XXXX_B45	IGUN-BATB 2022	JDL 31508	*Scinax sugillatus*	Colombia, Chocó
AY843681.1 12S, 16S AY844674.1 Rho AY844123.1 Tyr	CFBH 5788		*Scinax uruguayus*	Brazil, Rio Grande do Sul
KU495568.1 16S KU494775.1 COI	CFBHT00321		*Scinax uruguayus*	Brazil, 29.064 S 50.055 W
XXXX_B27	IGUN-BATB 1094	JDL 26409	*Scinax wandae*	Colombia, Meta, Acacías
KP149250.1 COI KP149460.1 16S		AJC 3464	*Scinax wandae*	Colombia, Meta, S. J. Arama
KP149170.1 COI KP149372 16S		AJC 3942	*Scinax wandae*	Colombia, Meta, S. J. Arama
KP149225.1 COI KP149431.1 16S		AJC 3461	*Scinax wandae*	Colombia, Meta, S. J. Arama
KP149174.1 COI KP149376.1 16S		AJC 1743	*Scinax wandae*	Colombia, Meta, S. J. Arama
XXXX_S65		ASMSW	*Scinax wandae*	Colombia, Meta, Cumaral
XXXX_S64	IGUN-BATB 4864	TAS 1631	*Scinax wandae*	Colombia, Meta, San Martín
XXXX_S59	IGUN-BATB 4154	AFAG 962	*Scinax wandae*	Colombia, Vichada, Cumaribo
KP149127.1 COI KP149323.1 16S		AJC 3974	*Scinax wandae*	Colombia, Casanare
KP149179.1 COI KP149381.1 16S		AJC 4105	*Scinax wandae*	Colombia, Casanare
KP149123.1 COI KP149319.1 16S		AJC 4120	*Scinax wandae*	Colombia, Casanare

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
XXXX_S60	IGUN-BATB 3881	AFAG 570	*Scinax wandae*	Colombia, Arauca
XXXX_S61	IGUN-BATB 3866	AFAG 552	*Scinax wandae*	Colombia, Arauca
XXXX_S62	IGUN-BATB 3910	AFAG 608	*Scinax wandae*	Colombia, Arauca
XXXX_S86	IGUN-BATB 3304	ASM 313	*Sphaenorhynchus carneus*	Colombia, Amazonas
XXXX_S87	IGUN-BATB 3292	ASM 281	*Sphaenorhynchus dorisae*	Colombia, Amazonas
XXXX_S88	IGUN-BATB 3293	ASM 282	*Sphaenorhynchus dorisae*	Colombia, Amazonas
JF790143.1 16S		AS0516	*Sphaenorhynchus lacteus*	Bolivia 12.7720 S, 65.8109 W
JF790144.1 16S	MNK:A:9387	MJ1358	*Sphaenorhynchus lacteus*	Bolivia 12.7720 S, 65.8109 W
XXXX_E1	MNCN/ADN 2405		*Sphaenorhynchus lacteus*	Bolivia, Chalalán
XXXX_E5	MNCN/ADN 27047		*Sphaenorhynchus lacteus*	Perú, Loreto
XXXX_A19	MNCN/ADN 34638		*Sphaenorhynchus lacteus*	Perú, Loreto
XXXX_A20	MNCN/ADN 34636		*Sphaenorhynchus lacteus*	Perú, Loreto
MK266743.1 12S, 16S MK266655.1 Rho MK266684.1 Tyr	CFBH15725		*Sphaenorhynchus lacteus*	Brazil, Acre

GenBank No./ tissue code	Catalogue No.	Collection No.	Recorded identity	Location
EF376028.1 12S EF376064.1 16S EF376134.1 Tyr	131mc		*Boana calcarata*	French Guiana
FJ766565.1 COI	USNM:572743	KRL 0800	*Cruziohyla calcarifer*	Panama, Coclé
EU342656.1 12S, 16S HQ290917.1 Tyr	TNHCFS4811		*Dendrobates auratus*	Panama, Coclé
EF376023.1 12S EF376059.1 16S EF376129.1 Tyr	36mc		*Dendropsophus leucophyllatus*	French Guiana
EF376027.1 12S EF376063.1 16S EF376133.1 Tyr	114mc		*Dendropsophus minutus*	French Guiana
AY843682.1 12S, 16S AY844675.1 Rho AY844124.1 Tyr	UMFS 5545		*Dryophytes versicolor*	Germany, pet trade
FJ965899.1 12S FJ945401.1 16S	ABTC 12431		*Litoria lesueurii*	Australia
KP149489.1 16S KP149275.1 COI		AJC 4072	*Pithecopus hypochondrialis*	Colombia 4.773 N, 73.037 W
MN135395.1 16S MN135395.1 COI	USNM:Herp: 575024		*Pseudacris crucifer*	USA, 38.6181 N 77.5292 W
XXXX_S85	IGUN-BATB 3303	ASM 312	*Sphaenorhynchus lacteus*	Colombia, Amazonas
XXXX_S93	IGUN-BATB 1185	JDL 27107	*Sphaenorhynchus lacteus*	Colombia, Amazonas
EF217515.1 16S			*Sphaenorhynchus lacteus*	Guyana
MK266744.1	UWIZM4635		*Sphaenorhynchus lacteus*	Trinidad and Tobago

Table S1-5: Best partition schema and respective evolution model selected by PartitionFinder for the Bayesian (MisterBayes and Beast) dataset alignment (Lanfear *et al.*, 2017).

Scheme Name: 1_2_3_4_5_6_7_8_9_10_11
Scheme lnL: -22298.740112304688
Scheme AICc: 45196.6722776
Number of parameters: 266
Number of sites: 2381
Number of subsets: 11

Subset	**Best Model**	**# sites**	**subset id**	**Partition names**
1	GTR+I+G+X	565	7b298c837680186aae356daf4fcdbd48	Gene1
2	GTR+I+G+X	372	b25e33ae7f77bc432759d93ca0dbc4f9	Gene2
3	TRN+G+X	220	9450239c8d25c57c3bbb4391411be967	Gene3_pos1
4	TRN+I+G+X	220	6ed3984c885f9664a3ee025318acf950	Gene3_pos2
5	GTR+I+G+X	219	174457f91da1ced21b055304eb9756ed	Gene3_pos3
6	JC+I+G	104	59f5b8a29a144ff8e19b60dd7004700d	Gene4_pos1
7	GTR+I+G+X	104	18f9b65d9db56f969ab70e1e8fc8d4fa	Gene4_pos2
8	GTR+I+G+X	103	abd7d0bb31ea747c8f2a805926a13645	Gene4_pos3
9	HKY+I+X	158	d95bef83f7f5c056d00024d8fbe41924	Gene5_pos1
10	TRN+G+X	158	863a0456f436854973fe25e02d63e778	Gene5_pos2
11	GTR+G+X	158	2fe1f15c6b6cb12923114cb06979ec7f	Gene5_pos3

Table S1-6: Settings for the Beast exploratory analyses.

Output	TipDates Calibration	Condition	Origin MRCA (Mya)	Taxonomic Prior	Chain length	Store every	Pre Burnin
DatarFossils1	Mean ages of fossils: Litoria – 14.2 Pseudacris 6.81 Dryophytes 8.13	Origin Rho sampling	32.3	*Scinax* monophyletic *Dendropsophus* monophyletic	30.000.000	1000	2000
DatarFossils1	Mean ages of fossils: Litoria – 14.2 Pseudacris 6.81 Dryophytes 8.13	Origin Rho sampling	54.8	*Scinax* monophyletic *Dendropsophus* monophyletic	30.000.000	1000	2000
DatarFossilsFBD21	Age height: Litoria – 28.4 Pseudacris 13.6 Dryophytes 15.97	Origin Rho sampling	61.8	*Scinax* monophyletic *Dendropsophu*s monophyletic	30.000.000	1000	2000
DatarFossilsSinOG	Age height: Litoria – 28.4 Pseudacris 13.6	Root Rho sampling		*Scinax* monophyletic *Dendropsophus* monophyletic	100.000.000	-1	3000
DatarSecondary	Age height: Litoria – 28.4 Pseudacris 13.6 Dryophytes 15.97	Origin Rho sampling	45.32	*Scinax* monophyletic Pseudinae monophyletic	30.000.000	3000	

Table S1-7: Uncorrected *p*-distances for every clade (genus or species group) in the phylogeny. A. *Scarthyla*. 1: General; 2: Between groups in the phylogeny. B. *Scinax rostratus* group. 1: General; 2: Between groups in the phylogeny. C. *Scinax wandae* group. 1: General; 2: Between groups in the phylogeny. D. *Sphaenorhynchus* (general).

A1. Taxa and group assigned to each sample

svASM421COY_{S_vigilans_CO_MMV}		0.000	0.003	0.000	0.000	0.005	0.013	0.012	0.013	0.012	0.012	0.012	0.013
svASM417COYo_{S_vigilans_CO_MMV}	0.000		0.003	0.000	0.000	0.005	0.013	0.012	0.013	0.012	0.012	0.012	0.013
svCFBHT16443VETach_{S_vigilansTopo}	0.004	0.004		0.003	0.003	0.000	0.014	0.012	0.013	0.012	0.012	0.012	0.013
svJPH995COdba_{S_vigilans_ChoCO}	0.000	0.000	0.004		0.000	0.005	0.013	0.012	0.013	0.012	0.012	0.012	0.013
svJPH996COdba_{S_vigilans_ChoCO}	0.000	0.000	0.004	0.000		0.005	0.013	0.012	0.013	0.012	0.012	0.012	0.013
sgKU_215423PECusco_{S_vigilans_KU215423_PE}	0.017	0.017	0.000	0.017	0.017		0.014	0.013	0.013	0.013	0.013	0.013	0.013
sgWED55411PECusco_{S_vigilans_WED55411_PE}	0.102	0.102	0.101	0.102	0.102	0.101		0.006	0.005	0.006	0.006	0.006	0.000
sgASM214COPtoN_{S_goinorum_CO}	0.093	0.093	0.091	0.093	0.093	0.103	0.022		0.005	0.000	0.003	0.002	0.006
sgQULC2340BRAmaz_{S_goinorum_Topotypic}	0.096	0.096	0.094	0.096	0.096	0.094	0.016	0.011		0.005	0.005	0.005	0.005
sgJDL24577COLet_{S_goinorum_CO}	0.093	0.093	0.091	0.093	0.093	0.103	0.022	0.000	0.011		0.003	0.002	0.006
sgASM253COTip_{S_goinorum_CO}	0.095	0.095	0.093	0.095	0.095	0.105	0.022	0.004	0.015	0.004		0.003	0.006
sgASM310COPtoN_{S_goinorum_CO}	0.095	0.095	0.093	0.095	0.095	0.105	0.024	0.002	0.013	0.002	0.006		0.006
sgCI_065PEManu_{S_goinorum_PE_Manu}	0.095	0.095	0.094	0.095	0.095	0.107	0.000	0.020	0.015	0.020	0.020	0.022	

A2. Groups

VigilansMMV		0.003	0.000	0.005	0.012	0.012	0.012
VigilansTopo	0.004		0.003	0.000	0.013	0.012	0.012
VigilansChoCO	0.000	0.004		0.005	0.012	0.012	0.012
GoinorumKU215423	0.017	0.000	0.017		0.013	0.013	0.013
GoinorumPE	0.098	0.097	0.098	0.101		0.005	0.005
GoinorumCO-Topotypic	0.093	0.091	0.093	0.103	0.018		0.002

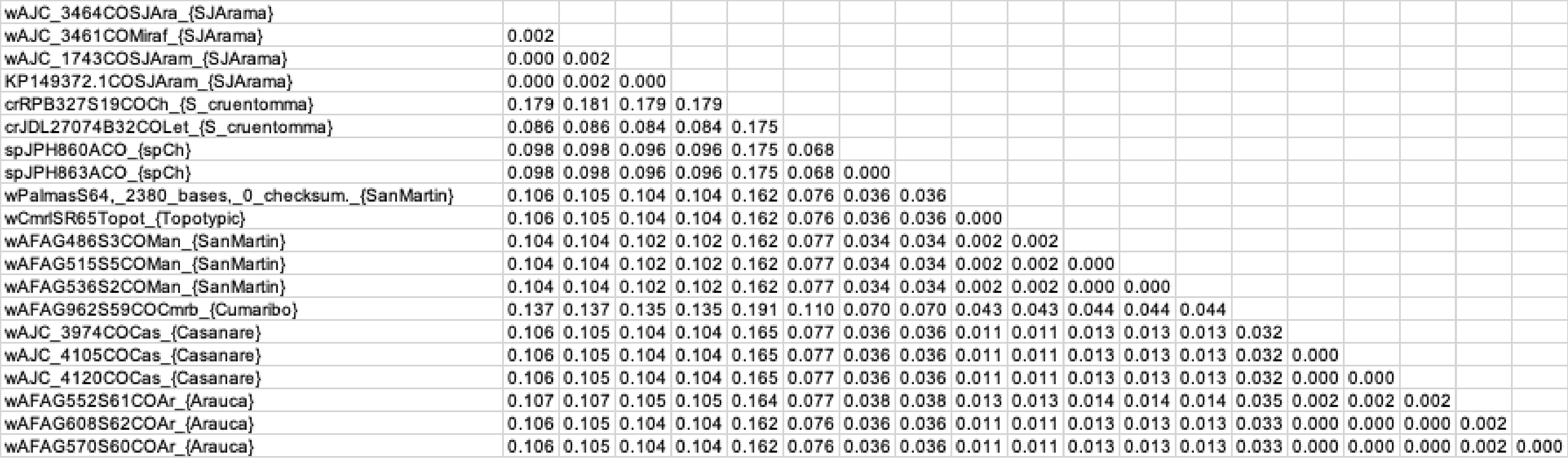
B1. Taxa and group assigned to each sample

peMAV465ArarB16_{PedroAra}		0.015	0.020	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.022	0.016	0.016	0.015	0.015	0.015	0.016	0.017	0.016	0.016	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.018	0.016	0.016	0.021	0.017	0.016	0.016	0.017	0.016	0.016
acMACN38649AR_{Acuminatus}	0.158		0.021	0.015	0.014	0.014	0.014	0.013	0.013	0.014	0.020	0.014	0.014	0.013	0.014	0.014	0.013	0.017	0.016	0.016	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.014	0.019	0.014	0.014	0.014	0.015	0.014	0.014
uCFBHT321_{Uruguayus}	0.199	0.203		0.003	0.021	0.020	0.020	0.021	0.021	0.023	0.104	0.022	0.020	0.020	0.020	0.020	0.021	0.022	0.022	0.022	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.024	0.020	0.021	0.020	0.021	0.021	0.021
uCFBH5788_{Uruguayus}	0.162	0.154	0.005		0.015	0.015	0.015	0.015	0.015	0.017	0.031	0.016	0.015	0.015	0.015	0.015	0.016	0.017	0.017	0.017	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.015	0.015	0.019	0.016	0.015	0.015	0.016	0.015	0.015
nMTD-TD8363GYS104_{Nebulosus}	0.169	0.122	0.219	0.172		0.014	0.014	0.014	0.014	0.014	0.015	0.014	0.014	0.013	0.013	0.013	0.013	0.017	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.015	0.014	0.015	0.014	0.014	0.019	0.015	0.014	0.013	0.015	0.014	0.014
peMACN/ADN34683BOA21_{PedromedinaeB}	0.162	0.122	0.206	0.159	0.119		0.000	0.008	0.008	0.013	0.012	0.014	0.014	0.013	0.013	0.013	0.013	0.016	0.015	0.016	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.015	0.013	0.013	0.019	0.015	0.014	0.014	0.015	0.014	0.014
peMACN/ADN34668BOA22_{PedromedinaeB}	0.163	0.123	0.206	0.160	0.120	0.000		0.008	0.008	0.013	0.012	0.014	0.014	0.013	0.013	0.013	0.013	0.016	0.015	0.016	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.015	0.013	0.013	0.019	0.015	0.014	0.014	0.015	0.014	0.014
peASM286S24COAtac_{PedromedinaeA}	0.163	0.114	0.199	0.156	0.123	0.043	0.044		0.000	0.013	0.008	0.013	0.014	0.013	0.013	0.013	0.013	0.016	0.016	0.016	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.015	0.013	0.013	0.018	0.014	0.013	0.013	0.015	0.014	0.014
peJDL27130B36COLet_{PedromedinaeA}	0.163	0.112	0.200	0.156	0.124	0.042	0.042	0.000		0.013	0.008	0.013	0.014	0.013	0.013	0.013	0.013	0.017	0.016	0.016	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.015	0.013	0.013	0.018	0.014	0.013	0.013	0.015	0.014	0.014
suJDL31508B45ChoCO_{Boulengeri_sugillatus}	0.167	0.135	0.238	0.182	0.132	0.117	0.118	0.114	0.112		0.000	0.010	0.015	0.013	0.013	0.014	0.014	0.016	0.016	0.016	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.014	0.020	0.015	0.014	0.014	0.016	0.015	0.015
suKU217735EC_{Boulengeri_sugillatus}	0.060	0.055	0.300	0.127	0.026	0.017	0.018	0.009	0.009	0.000		0.000	0.021	0.013	0.013	0.013	0.013	0.022	0.022	0.022	0.008	0.014	0.014	0.014	0.014	0.014	0.014	0.047	0.014	0.014	NC	0.018	0.014	0.016	0.031	0.017	0.017
bMVZ207215CR_{Boulengeri_sugillatus}	0.171	0.129	0.224	0.166	0.139	0.133	0.134	0.111	0.112	0.061	0.000		0.014	0.013	0.014	0.014	0.013	0.017	0.016	0.017	0.014	0.014	0.014	0.015	0.015	0.015	0.014	0.016	0.014	0.014	0.019	0.014	0.014	0.014	0.015	0.014	0.014
gWED54071EC_{GarbeiD}	0.181	0.125	0.199	0.150	0.127	0.121	0.121	0.116	0.116	0.128	0.041	0.122		0.003	0.004	0.003	0.011	0.016	0.015	0.015	0.011	0.014	0.014	0.013	0.014	0.013	0.014	0.014	0.014	0.014	0.017	0.014	0.013	0.014	0.014	0.014	0.014
gASM208S55COTip_{GarbeiD}	0.167	0.124	0.197	0.151	0.115	0.110	0.111	0.107	0.107	0.115	0.026	0.114	0.004		0.002	0.000	0.010	0.015	0.014	0.015	0.010	0.013	0.013	0.012	0.012	0.012	0.013	0.014	0.013	0.013	0.016	0.013	0.012	0.013	0.014	0.013	0.013
gMNCN/ADN27307A24PELo_{GarbeiD}	0.167	0.126	0.194	0.151	0.115	0.110	0.111	0.107	0.107	0.115	0.026	0.114	0.008	0.004		0.002	0.010	0.015	0.014	0.015	0.010	0.013	0.013	0.012	0.012	0.012	0.013	0.014	0.013	0.012	0.016	0.013	0.012	0.013	0.014	0.013	0.013
gMNCN/ADN26962A24PELo_{GarbeiD}	0.167	0.124	0.197	0.151	0.115	0.110	0.111	0.107	0.107	0.115	0.026	0.114	0.004	0.000	0.004		0.010	0.015	0.014	0.015	0.010	0.013	0.013	0.012	0.013	0.012	0.013	0.014	0.013	0.013	0.016	0.013	0.012	0.013	0.014	0.013	0.013
gWED57696PE_{GarbeiA}	0.175	0.113	0.198	0.148	0.117	0.109	0.110	0.112	0.112	0.122	0.019	0.111	0.070	0.063	0.063	0.063		0.015	0.015	0.015	0.009	0.012	0.012	0.012	0.012	0.012	0.012	0.013	0.012	0.012	0.017	0.013	0.012	0.012	0.013	0.012	0.012
gJDL24589S53COLet_{GarbeiB}	0.203	0.196	0.249	0.206	0.193	0.185	0.187	0.186	0.184	0.187	0.057	0.198	0.185	0.171	0.171	0.171	0.162		0.004	0.004	0.014	0.016	0.016	0.016	0.016	0.016	0.016	0.018	0.016	0.016	0.021	0.016	0.016	0.016	0.017	0.016	0.016
gJDL24643S51COLet_{GarbeiB}	0.189	0.184	0.242	0.192	0.180	0.174	0.176	0.175	0.174	0.178	0.060	0.188	0.171	0.159	0.159	0.159	0.152	0.008		0.003	0.014	0.016	0.015	0.016	0.016	0.016	0.015	0.017	0.015	0.015	0.020	0.016	0.016	0.015	0.017	0.016	0.016
gASM429SR52COVv_{GarbeiB}	0.191	0.190	0.242	0.194	0.182	0.178	0.180	0.179	0.178	0.182	0.061	0.192	0.174	0.162	0.162	0.162	0.153	0.010	0.004		0.014	0.016	0.016	0.016	0.016	0.016	0.015	0.018	0.016	0.015	0.021	0.016	0.016	0.015	0.017	0.016	0.016
gJDL24642S21COLet_{GarbeiC}	0.168	0.128	0.217	0.166	0.127	0.114	0.115	0.111	0.109	0.123	0.009	0.124	0.070	0.061	0.061	0.061	0.046	0.155	0.148	0.151		0.013	0.013	0.013	0.013	0.013	0.012	0.014	0.012	0.012	0.017	0.013	0.013	0.013	0.014	0.013	0.013
kAFAG640S57COArCrN_{KennedyA}	0.181	0.133	0.228	0.172	0.138	0.116	0.117	0.113	0.111	0.123	0.026	0.139	0.113	0.103	0.103	0.103	0.102	0.195	0.184	0.186	0.101		0.002	0.006	0.006	0.006	0.009	0.010	0.008	0.008	0.013	0.010	0.009	0.009	0.010	0.009	0.009
kAFAG641S57COArCrN_{KennedyA}	0.182	0.135	0.230	0.174	0.138	0.118	0.118	0.115	0.113	0.125	0.026	0.140	0.111	0.101	0.101	0.101	0.100	0.193	0.182	0.184	0.101	0.002		0.006	0.005	0.006	0.009	0.010	0.008	0.008	0.013	0.010	0.009	0.009	0.010	0.009	0.009
kASM594B6COTopotypic_{KennedyTopotypic}	0.173	0.131	0.230	0.173	0.132	0.117	0.118	0.118	0.118	0.130	0.026	0.137	0.107	0.097	0.097	0.097	0.089	0.193	0.184	0.186	0.101	0.022	0.020		0.003	0.002	0.009	0.009	0.008	0.008	0.012	0.010	0.009	0.009	0.010	0.009	0.009
kAFAG522S45COSMr_{KennedyTopotypic}	0.173	0.133	0.230	0.173	0.136	0.119	0.120	0.120	0.120	0.130	0.026	0.140	0.111	0.101	0.101	0.101	0.092	0.195	0.186	0.188	0.105	0.018	0.016	0.004		0.002	0.009	0.010	0.008	0.008	0.012	0.009	0.009	0.009	0.010	0.009	0.009
kAFAG541S43COMan_{KennedyTopotypic}	0.171	0.130	0.228	0.172	0.136	0.118	0.118	0.118	0.118	0.128	0.026	0.138	0.107	0.097	0.097	0.097	0.089	0.194	0.184	0.186	0.101	0.022	0.020	0.004	0.004		0.009	0.010	0.008	0.008	0.012	0.010	0.009	0.009	0.010	0.009	0.009
roAFAG615S46COAr_{RostratusTopotypic}	0.179	0.134	0.203	0.158	0.124	0.125	0.126	0.121	0.121	0.127	0.027	0.128	0.101	0.091	0.091	0.091	0.084	0.175	0.166	0.171	0.086	0.050	0.049	0.043	0.047	0.045		0.004	0.004	0.004	0.011	0.008	0.008	0.008	0.009	0.008	0.008
roAFAG444S50COArCrN_{RostratusTopotypic}	0.208	0.140	0.209	0.170	0.140	0.138	0.138	0.137	0.137	0.148	0.105	0.144	0.106	0.104	0.104	0.104	0.089	0.211	0.198	0.202	0.102	0.053	0.051	0.042	0.047	0.047	0.009		0.004	0.004	0.009	0.009	0.009	0.009	0.009	0.009	0.009
roMAV232SR47COSMr_{RostratusTopotypic}	0.180	0.134	0.214	0.164	0.126	0.119	0.119	0.117	0.118	0.128	0.026	0.128	0.102	0.093	0.093	0.093	0.086	0.184	0.173	0.177	0.091	0.040	0.038	0.033	0.036	0.036	0.009	0.006		0.000	0.009	0.008	0.008	0.008	0.009	0.008	0.008
roASM426S54COCmrl_{RostratusTopotypic}	0.178	0.133	0.214	0.162	0.125	0.117	0.118	0.116	0.116	0.126	0.026	0.127	0.101	0.092	0.092	0.092	0.085	0.183	0.173	0.176	0.090	0.040	0.038	0.032	0.036	0.036	0.009	0.006	0.000		0.009	0.008	0.008	0.008	0.009	0.008	0.008
ro247mcFG_{RostratusB}	0.232	0.170	0.222	0.178	0.171	0.170	0.170	0.153	0.153	0.172	NC	NC	0.164	0.115	0.112	0.115	0.112	0.113	0.239	0.224	0.227	0.123	0.070	0.068	0.060	0.060	0.065	0.049	0.039	0.040	0.039	0.008	0.008	0.009	0.008	0.008	0.008
roJPH918S48COrdoba_{RostratusA}	0.195	0.133	0.233	0.176	0.135	0.143	0.144	0.126	0.126	0.141	0.034	0.133	0.111	0.103	0.103	0.103	0.096	0.192	0.183	0.186	0.094	0.057	0.057	0.053	0.053	0.057	0.041	0.046	0.039	0.039	0.030		0.003	0.006	0.005	0.005	0.005
roJPH919S48COrdoba_{RostratusA}	0.184	0.131	0.233	0.175	0.127	0.132	0.133	0.122	0.122	0.130	0.026	0.129	0.109	0.097	0.099	0.097	0.091	0.183	0.174	0.176	0.092	0.052	0.052	0.049	0.049	0.052	0.039	0.044	0.036	0.036	0.031	0.004		0.005	0.005	0.005	0.005
roMAV322S63COY_{RostratusA}	0.184	0.137	0.230	0.175	0.121	0.132	0.133	0.125	0.125	0.135	0.034	0.131	0.115	0.103	0.103	0.103	0.096	0.189	0.178	0.180	0.097	0.054	0.054	0.054	0.054	0.058	0.045	0.049	0.042	0.041	0.034	0.019	0.022		0.003	0.003	0.003
roMAV396SR49COY_{RostratusA}	0.202	0.140	0.230	0.182	0.138	0.150	0.150	0.143	0.143	0.152	0.067	0.143	0.115	0.111	0.111	0.111	0.103	0.208	0.195	0.197	0.107	0.061	0.061	0.056	0.056	0.060	0.046	0.045	0.042	0.042	0.032	0.017	0.020	0.004		0.002	0.002
roAJC3422SCOVCh_{RostratusA}	0.184	0.129	0.230	0.169	0.124	0.138	0.139	0.131	0.132	0.140	0.038	0.133	0.113	0.103	0.103	0.103	0.092	0.189	0.178	0.180	0.099	0.057	0.057	0.053	0.053	0.057	0.044	0.047	0.041	0.040	0.034	0.018	0.020	0.006	0.002		0.000
roAJC3506COSVCh_{RostratusA}	0.184	0.129	0.230	0.169	0.124	0.138	0.139	0.131	0.132	0.140	0.038	0.133	0.113	0.103	0.103	0.103	0.092	0.189	0.178	0.180	0.099	0.057	0.057	0.053	0.053	0.057	0.044	0.047	0.041	0.040	0.034	0.018	0.020	0.006	0.002	0.000	

B2.

PedroAra		0.015	0.017	0.015	0.015	0.016	0.014	0.015	0.016	0.016	0.015	0.015	0.015	0.016	0.021	0.016
Acuminatus	0.158		0.017	0.014	0.013	0.013	0.012	0.013	0.013	0.016	0.014	0.014	0.014	0.014	0.019	0.014
Uruguayus	0.180	0.178		0.018	0.016	0.017	0.026	0.016	0.017	0.018	0.018	0.017	0.017	0.017	0.021	0.017
Nebulosus	0.169	0.122	0.195		0.014	0.014	0.011	0.013	0.013	0.016	0.014	0.014	0.014	0.014	0.019	0.014
PedromedinaeB	0.163	0.123	0.183	0.120		0.008	0.010	0.013	0.013	0.016	0.013	0.013	0.013	0.014	0.019	0.014
PedromedinaeA	0.163	0.113	0.178	0.124	0.043		0.009	0.012	0.013	0.016	0.013	0.013	0.013	0.014	0.018	0.014
Boulengeri_sugillatus	0.133	0.106	0.206	0.099	0.089	0.078		0.011	0.010	0.014	0.009	0.011	0.011	0.012	0.017	0.011
GarbeiD	0.171	0.124	0.174	0.118	0.113	0.109	0.088		0.010	0.015	0.010	0.013	0.012	0.013	0.016	0.013
GarbeiA	0.175	0.113	0.173	0.117	0.109	0.112	0.084	0.065		0.014	0.008	0.012	0.011	0.012	0.016	0.012
GarbeiB	0.194	0.190	0.221	0.185	0.180	0.179	0.145	0.167	0.156		0.014	0.016	0.016	0.016	0.020	0.016
GarbeiC	0.168	0.128	0.191	0.127	0.114	0.110	0.085	0.063	0.046	0.152		0.012	0.012	0.012	0.016	0.012
KennedyA	0.181	0.134	0.201	0.138	0.117	0.113	0.096	0.105	0.101	0.187	0.101		0.006	0.008	0.013	0.009
KennedyTopotypic	0.172	0.131	0.201	0.135	0.118	0.119	0.098	0.101	0.090	0.188	0.102	0.020		0.008	0.012	0.009
RostratusTopotypic	0.186	0.135	0.187	0.129	0.125	0.123	0.103	0.097	0.086	0.182	0.092	0.045	0.040		0.009	0.008
RostratusB	0.232	0.170	0.200	0.171	0.170	0.153	0.168	0.114	0.113	0.230	0.123	0.069	0.062	0.042		0.007
RostratusA	0.189	0.133	0.203	0.128	0.139	0.130	0.104	0.106	0.095	0.185	0.098	0.056	0.054	0.042	0.032	

C1.



wAJC_3464COSJAra_{SJArama}																			
wAJC_3461COMiraf_{SJArama}	0.002																		
wAJC_1743COSJAram_{SJArama}	0.000	0.002																	
KP149372.1COSJAram_{SJArama}	0.000	0.002	0.000																
crRPB327S19COCh_{S_cruentomma}	0.179	0.181	0.179	0.179															
crJDL27074B32COLet_{S_cruentomma}	0.086	0.086	0.084	0.084	0.175														
spJPH860ACO_{spCh}	0.098	0.098	0.096	0.096	0.175	0.068													
spJPH863ACO_{spCh}	0.098	0.098	0.096	0.096	0.175	0.068	0.000												
wPalmasS64,_2380_bases,_0_checksum._{SanMartin}	0.106	0.105	0.104	0.104	0.162	0.076	0.036	0.036											
wCmrlSR65Topot_{Topotypic}	0.106	0.105	0.104	0.104	0.162	0.076	0.036	0.036	0.000										
wAFAG486S3COMan_{SanMartin}	0.104	0.104	0.102	0.102	0.162	0.077	0.034	0.034	0.002	0.002									
wAFAG515S5COMan_{SanMartin}	0.104	0.104	0.102	0.102	0.162	0.077	0.034	0.034	0.002	0.002	0.000								
wAFAG536S2COMan_{SanMartin}	0.104	0.104	0.102	0.102	0.162	0.077	0.034	0.034	0.002	0.002	0.000	0.000							
wAFAG962S59COCmrb_{Cumaribo}	0.137	0.137	0.135	0.135	0.191	0.110	0.070	0.070	0.043	0.043	0.044	0.044	0.044						
wAJC_3974COCas_{Casanare}	0.106	0.105	0.104	0.104	0.165	0.077	0.036	0.036	0.011	0.011	0.013	0.013	0.013	0.032					
wAJC_4105COCas_{Casanare}	0.106	0.105	0.104	0.104	0.165	0.077	0.036	0.036	0.011	0.011	0.013	0.013	0.013	0.032	0.000				
wAJC_4120COCas_{Casanare}	0.106	0.105	0.104	0.104	0.165	0.077	0.036	0.036	0.011	0.011	0.013	0.013	0.013	0.032	0.000	0.000			
wAFAG552S61COAr_{Arauca}	0.107	0.107	0.105	0.105	0.164	0.077	0.038	0.038	0.013	0.013	0.014	0.014	0.014	0.035	0.002	0.002	0.002		
wAFAG608S62COAr_{Arauca}	0.106	0.105	0.104	0.104	0.162	0.076	0.036	0.036	0.011	0.011	0.013	0.013	0.013	0.033	0.000	0.000	0.000	0.002	
wAFAG570S60COAr_{Arauca}	0.106	0.105	0.104	0.104	0.162	0.076	0.036	0.036	0.011	0.011	0.013	0.013	0.013	0.033	0.000	0.000	0.000	0.002	0.000

C2.

SJArama		0.012	0.012	0.012	0.012	0.014	0.012	0.012
S_cruentomma	0.132		0.012	0.011	0.011	0.013	0.011	0.011
spCh	0.097	0.122		0.008	0.008	0.011	0.008	0.008
SanMartin	0.103	0.120	0.034		0.001	0.008	0.004	0.004
Topotypic	0.105	0.119	0.036	0.001		0.008	0.004	0.004
Cumaribo	0.136	0.150	0.070	0.044	0.043		0.007	0.007
Casanare	0.105	0.121	0.036	0.012	0.011	0.032		0.001
Arauca	0.105	0.119	0.036	0.013	0.011	0.034	0.001	

D.

sphUWIZM4635TT		0.003	0.003	0.004	0.002	0.003	0.003	0.007	0.003	0.005	0.002	0.003	0.003	0.013	0.011	0.011
sphCFBH15725BRAc	0.006		0.004	0.004	0.003	0.003	0.003	0.007	0.003	0.005	0.003	0.003	0.003	0.014	0.011	0.011
sphAS0516BO	0.006	0.007		0.004	0.002	0.003	0.003	0.007	0.003	0.005	0.002	0.002	0.003	0.013	0.011	0.011
sphA19PELo	0.009	0.011	0.011		0.003	0.004	0.004	0.007	0.004	0.006	0.003	0.004	0.004	0.013	0.011	0.011
sphA20PELo	0.002	0.004	0.004	0.007		0.002	0.002	0.007	0.002	0.004	0.000	0.002	0.002	0.013	0.011	0.011
lacLetS93	0.004	0.006	0.006	0.009	0.002		0.000	0.007	0.000	0.004	0.002	0.003	0.003	0.013	0.011	0.011
sphSR85ASM312COPtoNar	0.004	0.006	0.006	0.009	0.002	0.000		0.007	0.000	0.004	0.002	0.002	0.002	0.013	0.011	0.011
E1MNCN-ADN2406BO	0.028	0.030	0.026	0.033	0.026	0.028	0.028		0.007	0.008	0.007	0.006	0.007	0.014	0.012	0.012
E5MNCN-ADN27047PELo	0.004	0.006	0.006	0.009	0.002	0.000	0.000	0.028		0.004	0.002	0.003	0.003	0.013	0.011	0.011
EF217515.1_Sphaenorhynchus_lacteus_16S_ribosomalGF	0.008	0.010	0.011	0.016	0.005	0.008	0.008	0.029	0.008		0.004	0.004	0.004	0.018	0.015	0.015
16S_lacteus_CI_090Manu	0.002	0.004	0.004	0.007	0.000	0.002	0.002	0.026	0.002	0.005		0.002	0.002	0.013	0.011	0.011
16S_lacteus_MJ1358BO	0.004	0.006	0.002	0.009	0.002	0.004	0.004	0.024	0.004	0.008	0.002		0.003	0.013	0.011	0.011
SphUSNM_268930Tambopata	0.004	0.006	0.006	0.009	0.002	0.004	0.004	0.028	0.004	0.008	0.002	0.004		0.014	0.011	0.011
SR86_16S_ASM	0.121	0.125	0.116	0.122	0.118	0.121	0.120	0.135	0.121	0.162	0.118	0.118	0.123		0.014	0.014
SR87_16S_ASM	0.075	0.079	0.076	0.071	0.073	0.073	0.073	0.096	0.074	0.103	0.073	0.074	0.077	0.145		0.000
SR88_16S_ASM	0.075	0.079	0.076	0.071	0.073	0.073	0.073	0.096	0.074	0.103	0.073	0.074	0.077	0.145	0.000	

4. Capítulo 4: The frogs of war: unraveling the history of some Spix's (1824) types and old South American specimens deposited in the Zoologische Staatssammlung München – ZSM

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Keywords: Phylogeography, historical biogeography, Hylidae, *Scinax*, *Scarthyla*, *Sphaenorhynchus*, cryptic diversity, Llanos.

4.1 Abstract

The Zoologische Staatssammlung München – ZSM custodies almost all the types of the amphibian collections obtained during the expedition of Johann Spix and Karl Martius to Brazil, which were preserved after the devastating effects of World War II. Most of the remaining types and hylid specimens from South America were reviewed, identified, and described, to improve the understanding of the real diversity and to contribute to clarifying the taxonomy of tree frogs in the Northwestern Amazon basin. After the review, some important taxonomic changes should be made.

Keywords: amphibians, tree frogs, taxonomy, biological collections, World War II, Brazil, Northwestern Amazonia, Colombia.

4.2 Resumen

La colección herpetológica de las Colecciones Zoológicas de Baviera (Zoologische Staatssammlung München – ZSM) custodia casi todos los tipos restantes de las colecciones de anfibios obtenidas durante la expedición de Johann Spix y Karl Martius a Brasil, que fueron diezmados, dañados o se perdieron durante la Segunda Guerra Mundial. La mayoría de los tipos que subsistieron, así como los especímenes de hílidos de Sudamérica que se encontraron en dicha colección fueron revisados, identificados y descritos, con el fin de mejorar la comprensión de la diversidad real y contribuir a aclarar la taxonomía de las ranas arborícolas de la cuenca amazónica noroccidental. Después de la revisión, es necesario hacer algunos cambios taxonómicos.

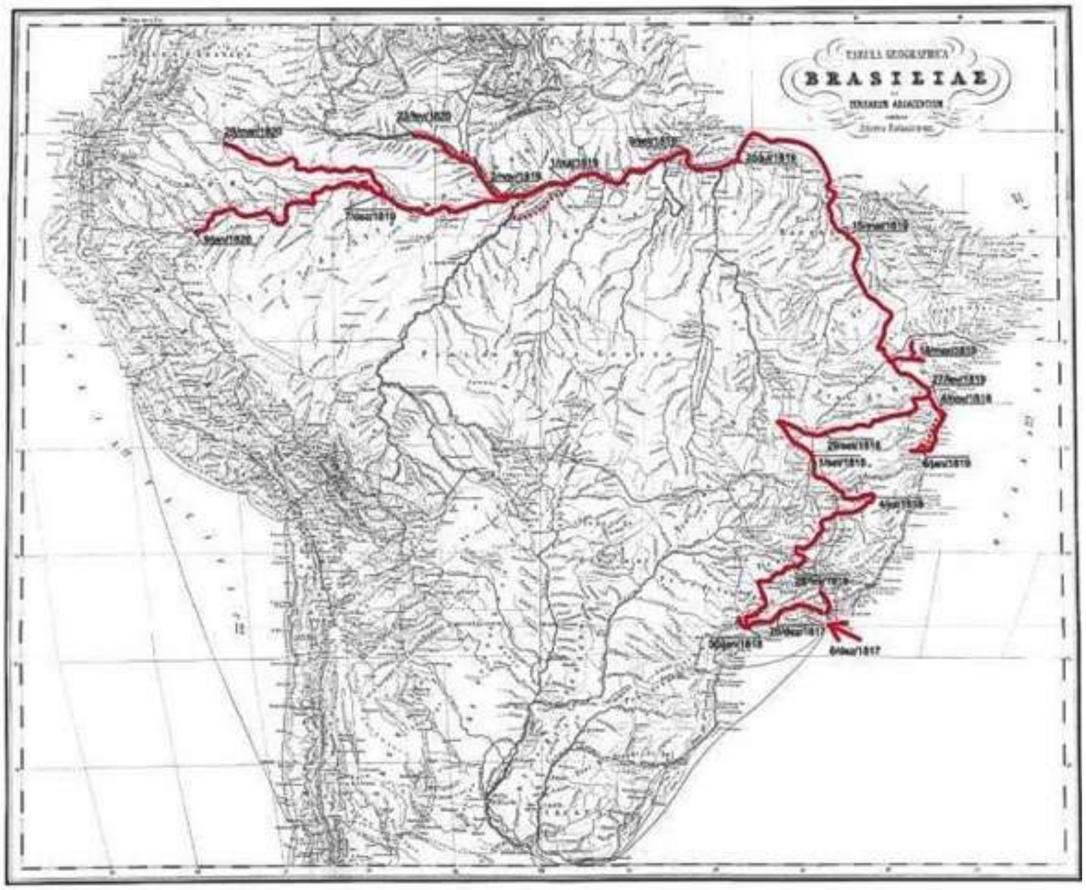
Palabras clave: anfibios, ranas arborícolas, taxonomía, colecciones biológicas, Segunda Guerra Mundial, Brasil, Colombia, Amazonia noroccidental.

4.3 Introduction

In 1824 Johann Baptist Ritter von Spix published a compendium of the species of amphibians and turtles collected during his expedition with K. F. von Martius to Brazil between 1817 and 1820 (Spix, 1824). The expedition was a huge endeavor (Figure 1), comprising more than 10.000 km traveled from the Atlantic coast to the western Amazon basin, which took the scientists as far as the Caquetá river in Colombia (Mori, 2013). It provided dozens of new vertebrates and hundreds of new plants to science, and a superb number of specimens for the ZSM collection, which still contains a very important part of the history of Herpetology in South America. Unfortunately, the geographic focus of the collection changed from America to Madagascar and Asia during the 21rst century, and much of the material remained unseen after Duellman's Das Tierreich (William E. Duellman, 1977).

In 2018, during a doctoral research stay of nine months in Germany at Senckenberg Dresden, the author had the opportunity to embark in her own expedition to visit the most relevant biological collections to the doctoral project, which aimed to disentangle the evolutionary lineages and the distributions hidden under the names *Scinax ruber, S. rostratus* and *S. wandae* (among others) in the study area. The fact that 23 of the species described by Spix (1824) were included in the genus *Hyla* (**Table 4-1**) and 6 of those (*H. affinis, H. coerulea, H. lateristriga, H. nebulosa, H. strigilata* and *H. x-signata*) were associated to *Scinax* in the past, made evident that ZSM was the most important collection to visit. Fortunately, Drs. Michael Franzen and Frank Glaw kindly opened the doors and cabinets of their collection for a short but very productive review of types and, specially, of the nearly forgotten hylid specimens from the North-western South America collected by Spix himself, Wagler, Peters, Hellmich, and several other German expeditioners along the North-western South America. Some remarkable outcomes of that visit are presented herein.

Figure 4-1: Route of the expedition of Spix & Martius to Brazil, as depicted by Martius for the Flora Brasiliensis. Published as a part of the Flora Brasiliensis project, leaded by CRIA and the Missouri Botanical Garden (http://florabrasiliensis.cria.org.br/project).



4.4 Data obtention

Based on Spix's publication (Spix, 1824), his identifications of specimens as *Hyla* were probably related to the presence of expanded disks on fingers and toes; that would explain the inclusion of some craugastorids, a dendrobatid and one hylodid among *Hyla*. On the contrary, the later allocation of some of those *Hyla* in *Scinax* was a responsibility of the reviewers from 1872 to the present, starting with the critical review of Peters (1873) and the outcomes of Duellman's work in Europe (William E. Duellman, 1977), a century later. Unless not a formal review, remarks about the specimens mentioned by Müller (1927) were also considered.

Table 4-8. Species of *Hyla* described by Spix (1824) and their current taxonomic identity. The asterisk means that the synonymy is not trusted by the author based on the specimens reviewed.

Original Name	Actual name	Family	Original synonymy
Hyla abbreviata	*Haddadus binotatus*	Craugastoridae	Peters (1873) in Frost, 2022
Hyla affinis	*Scinax x-signatus**	Hylidae	Hoogmoed & Grüber (1983)
Hyla albomarginata	*Boana albomarginata*	Hylidae	--
Hyla albopunctata	*Boana albopunctata*	Hylidae	--
Hyla bicolor	*Phyllomedusa bicolor*		Peters (1873)
Hyla bipunctata	*Dendropsophus bipunctatus*	Hylidae	--
Hyla bufonia	*Trachycephalus typhonius*	Hylidae	Duméril & Bibron, (1841) in Frost, 2022
Hyla cinerascens	*Boana cinerascens*	Hylidae	--
Hyla coerulea	*Scinax x-signatus**	Hylidae	Peters (1873)
Hyla geographica	*Boana geographica*	Hylidae	--
Hyla geographica var. semilineata	*Boana semilineata*	Hylidae	--
Hyla lateristriga	*Scinax ruber*	Hylidae	Peters (1873)
Hyla nebulosa	*Scinax nebulosus*	Hylidae	--
Hyla nigerrima	*Ameerega trivittata*	Dendrobatidae	Peters (1873) in Frost, 2022
Hyla papillaris	*Boana punctata**	Hylidae	Peters (1873) in Frost, 2022
Hyla pardalis	*Boana pardalis*	Hylidae	--
Hyla ranoides	*Hylodes nasus*	Hylodidae	Peters (1873) in Frost, 2022
Hyla stercoracea	*Hylodes nasus*	Hylodidae	Peters (1873) in Frost, 2022
Hyla strigilata	*Scinax strigilatus*	Hylidae	--
Hyla trivittata	*Amereega trivittata*	Dendrobatidae	--
Hyla variolosa	*Boana punctata*	Hylidae	Duméril & Bibron, (1841) in Frost, 2022
Hyla x-signata	*Scinax x-signatus*	Hylidae	--
Hyla zonata	*Trachycephalus typhonius*	Hylidae	Duméril & Bibron, (1841) in Frost, 2022

To check on the actual taxonomy and the physical and historic condition of those specimens and the species they represent, I searched for the South American hylids inside the drawers of the ZSM collection, and I found and reviewed 12 hylid specimens plus the specimens/types identified as *Hyla vogli* Müller, 1938 and *Hyla trivitatta* Spix, 1824 (**Table**

4-2). Then, I read again their original descriptions and some of the revisions which incorporated their names, seeking to identify the similarities to the original publications.

It is worth to mention that most of the Spix types and ancient specimens in the collection were temporarily or definitively lost during World War II, and the specimens surviving that dark period of History were possibly manipulated in or out their original containers and labels by unknown and untrained people. Hence, the research question became wider: to identify which types of *Scinax* and noteworthy hylids from South America does ZSM still hold and what are their current identities and collection status. Finally, with the aim of solving the identities and evolutionary placements of the types related to *Scinax ruber* and *S. x-signatus*, Dr. Christian Kehlmaier of Senckenberg Dresden attempted to obtain 16S and 12S sequences of the types.

4.5 Results

An overview of the taxonomic work developed in the ZSM collection is presented in **Table 4-2**, under the columns “Current accepted name”, “Collection locality” and “Sex”. However, the most important achievement of this work is that it allows a specimen-based sorting of the taxonomic history of the Scinax described by Spix and of some of the specimens collected later (19th and 20th century) by other German explorers in the North-western Amazon basin. As it could be anticipated, several taxonomic annotations and changes should be made to comply to these findings:

1) The only Spix’s (1824) type whose morphology corresponds to *Hyla rubra* Daudin, 1802 (**Figure 4-2**) is *H. lateristriga* (specimen in a very poor condition, ZSM 048/2005). No DNA sequences were obtained from the sample donated to the doctoral project. Curiously, no indication of a collection locality is mentioned in Spix’s text either, adding to the uncertainty on the original locality of Laurenti (1768), which was restricted by W. E. Duellman & Wiens (1993).

Table 4-2. Hylid and *Hyla* type specimens reviewed in the ZSM collection, as of February, 2018.

ZSM No.	Name in jar	Type status	Current accepted name	Actual collection locality	Sex
67/1937	*Hyla vogli* Müller, 1938	Holotype	*Gastrotheca ovifera* (Lichtenstein and Weinland, 1854)	Venezuela, Caracas	male
102/1937	*Hyla wilsoniana krausi* Hellmich, 1940	Holotype	*Dendropsophus molitor* (Schmidt, 1857)	Colombia, PNN Sumapaz	male
1182/0/1	*Hyla variabilis* Boulenger, 1896	Syntype	*Dendropsophus columbianus* (Boettger, 1892)	Colombia, Cali	female
1182/0/2	*Hyla variabilis* Boulenger, 1896	Syntype	*Dendropsophus columbianus* (Boettger, 1892)	Colombia, Cali	male
1182/0/3	*Hyla variabilis* Boulenger, 1896	Syntype	*Dendropsophus columbianus* (Boettger, 1892)	Colombia, Cali	female
43/0	*Hyla trivitatta* Spix, 1824	Paralectotype	*Ameerega trivittata* (Spix, 1824)	Brazil, Amazonas	male
2710/0/1	*Hyla coerulea* Spix, 1824	Lectotype	*Scinax x-signatus* (Spix, 1824)	Brazil, Amazonas	male
2710/0/2	*Hyla coerulea* Spix, 1824	Paralectotype	*Scinax x-signatus* (Spix, 1824)	Brazil, Amazonas	female
80/1921	*Hyla ehrhardti* Müller, 1924	Holotype	*Aplastodiscus ehrhardti* (Müller, 1924)	Brazil, Santa Catharina	male
1175/0	*Hyla auraria* Peters, 1873	Holotype	Not stated. *nomen inquirendum* according to (Hoogmoed, 2018)	Sudamérica	juv.
2495/0	*Hyla affinis* Spix, 1824	Holotype	*Scinax x-signatus*	Brazil, Amazonas	male
sn 214	Hellmich - Unidentified	none	None. It is a *Scinax*.	Colombia, Meta	Not stated
sn 181	Hellmich – Unidentified	none	None. It is a *Scinax wandae*	Colombia, Meta	Not stated
sn	Hellmich - Unidentified	none	None. It is a *Scinax*.	Colombia, Meta	Not stated

Figure 4-2: Daudin's (1802) illustration of *Hyla rubra*, clearly showing the reduced digital membrane between toes I and II and the characteristic light/dark mottling of the hidden thighs in Guianan populations.

2) *Hyla coerulea* (**Figure 4-3)** is undoubtedly a *Scinax*, but its morphology and color suggest that it belongs to the green-boned group, aff. *S. funereus* (Cope, 1874). The pattern of spots on the concealed thighs of both specimens (ZSM 2710/0/1 and 2710/0/2) is also reminiscent of *S. sateremawe* (Sturaro & Peloso, 2014). The specimens were said to be collected near the “pagum Ecgá” (Brazil, near Tefé, 03°21'S, 64°42'W according to Vanzolini, 1981) and Solimões, so there could be several today known species of Scinax possibly referred to it. Based on the morphology and crypticity of the group, this taxon should be considered a valid species instead of a synonym of *Scinax x-signatus*.

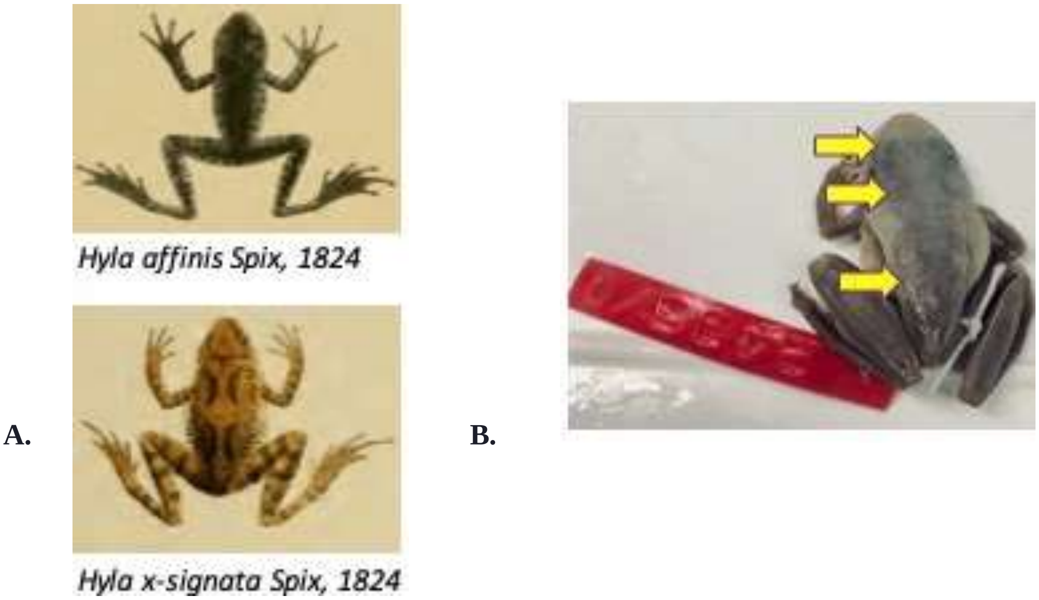
3) *Hyla affinis* (**Figure 4-4**) was mentioned to be “similar to” *H. nebulosa* by Spix (1824) in the original description (the syntypes of which were also lost during the World War II according to Glaw & Franzen, 2006), so that was reflected in the etymology of the specific epithet, and it is also evident in the original illustration (Tab. VII, Fig. 3). However, the specimen reviewed in ZSM does not comply neither with the original illustration of *H. affinis* nor with any *Scinax* of the *rostratus* group, but it does comply with a very “ruber-like” pattern of finger size and hidden thighs color pattern. Besides, according to Peters (1873), the holotype of *H. affinis* (ZSM 2495/0) was a female, but the specimen stored in ZSM is a male with open vocal

slits (**Table 4-2**). It has a beautiful, but partially fainted, X-mark on the dorsum, in the form of two inverted parentheses over the scapulae, a dark interorbital bar (nearly an inverted triangulum) and a pair of thick inverted parentheses on the sacral area.

Figure 4-3: Reproduction of Spix's (1824) illustration of *Hyla coerulea* (left) and picture of the collection type specimen of the same species and the supposed original label included in the jar, as it was found in the ZSM collection. Picture: ASM.

4) The nominal taxon to which almost every other Spix's *Hyla* has been related, *Hyla x-signata* –because of its distinctive dorsal X-markings– was delimited by Araujo-Vieira *et al*. (2020) on the basis of distribution and morphology of the X-marked *Scinax* of the Northeastern Brazil (**Figure 4-4A**). Those authors erected also a neotype, the specimen CFBH 44688 of the Universidade Estadual de Santa Cruz – UESC collection, supported on the long-lasting uncertainty about the holotype, which was considered lost for about 40 years. However, considering that the 19th century "Bahia" should include localities in the "Provincia Bahiae", which was extended further to the Northwestern Amazon basin, and the absence of data about the collecting event in the original description –which Spix was normally careful in providing when he has collected the specimens by himself–, a level of uncertainty on the taxon locality and its external look must be maintained. Besides, due to the extensive convergence of that X pattern among members of *Scinax, Pristimantis* and *Eleutherodactylus*, and several others, it is not surprising that the recorded distribution of the species could be mistaken.

Figure 4.4 A. *Hyla affinis* (top) and *H. x-signata* (bottom) as illustrated in Spix (1824) **B**. Picture of the type specimen ZSM 2495/0 contained in the jar marked as *Hyla affinis*, as it was found in the ZSM collection. Yellow arrows signal the still visible dark markings, totally compliant to the *H. x-signata* illustration in A - bottom. Picture: ASM.



5) *Hyla wilsoniana krausi* Hellmich, 1940 is an undisputed synonym of *Hyla labialis* Peters, 1863, but neither the former nor the later are synonyms of *Hyla molitor* Schmidt, 1857. Moreover, the specimens stated as the holotype and the lectotype of *Hyla labialis* are not conspecific despite Peters' and Savage's indications. This statement is based on the morphology of both taxa (rounded or nearly truncated snout, expanded digital disks, thickened arm, and enlarged tympanum in Hyla molitor), the localities in which *Hyla labialis* was collected and actually lives (as *Dendropsophus:* the high Andean forests and paramos of the Cordillera Oriental of Colombia, in the same places that are still occupied by another taxon described by Peters, 1863, *Bolitoglossa adspersa*) and the expert taxonomic opinion that, based on morphology and distribution, *Hyla molitor* could be a member of *Dendropsophus* or *Smilisca* but never conspecific to this very particular high Andean *Dendropsophus*.

6) The specimens collected by Hellmich in Colombia (**Table 4-2**, sn 181, sn 214 and sn) are members of *Scinax*, and probably they represent the oldest *Scinax* of the ruber-like and wandae clades already known from the country. Specimens sn214 and sn are members of the Scinax6 clade, at present being formally described to science (Á. M. Suárez-Mayorga, 2021). Specimen sn 181 is the first *Scinax wandae* known from Colombia, existing in the vicinities of Villavicencio, Meta for at least 30 years before its formal scientific description.

4.6 Conclusion

Based on the above, some taxonomic precisions must be remarked. The first one is that there was no need of a neotype for *Hyla x-signata* as described by Araujo-Vieira et al., (2020), simply because the long-thought-to-be-lost holotype of *H. x-signata* exists in the ZMS collection, hidden under the label "Hyla affinis". That automatically implies that the lost Spix's (1824) type is that of *Hyla affinis*. No ancient specimens in the ZSM collection were morphologically similar to S. nebulosus nor to any other member of the *Scinax rostratu*s group. Moreover, after our identification of morphological color patterns characteristic of forest/non forest clades, we can only conclude that the pattern described by Spix (1824), and L. Müller (1927) for *Hyla x-signata* is a forest one.

The second one is that *Hyla caerulea* and *H. affinis* are not synonyms of *H. x-signata*, but different and well-defined species which should be considered valid, and the populations to which their names apply should be found in the Northwestern Amazonia. The same applies to all the *Scinax ruber*-like taxa which seemed like our collective idea of *Scinax ruber* (or *Hyla rubra* Laurenti, 1768) with orangish dorsi and dark/orange patterned hidden limbs. An attempt to disentangle that diversity is being made by the author through the publication of her doctoral thesis manuscripts.

And the last consequence is that the frog fauna of Colombia and the northern South America is much larger and complex that normally anticipated, and biological collections with digitized catalogues associated to pictures or illustrations, as well as permanent communication among curators and taxonomists, is essential to describe, understand and use the knowledge accumulated over the years inside them.

4.7 Acknowledgements

The curators of the Zoologische Staatsamlung München – ZSM, doctors Frank Glaw and Michael Franzen allowed the review of the specimens, provided tissues for molecular analyses, and gave advise on the literature during the doctoral stay in Germany of the author. Dr. Walter Winhard was the best travel guide and hosted the author and her family during their trip to Bayern. The Colombian-German Fundation for Science and Technology - Funcytca, the Universidad Nacional de Colombia, the Senckenberg Collections at Dresden and the Ministry of Science of Colombia provided financial support for the development of the doctoral thesis. Dr. Javier Fuertes, from the Real Jardín Botánico de Madrid, helped in identifying the location of the original locality of specimen sn 181. Dr. Christian Kehlmeier tried to obtain sequences of the Spix types, with a very limited success. Dr. Raffael Ernst provided some space in his collection to review München specimens. Drs. Uwe Fritz and Mario Vargas Ramirez were scientific advisors, professors, and friends during the doctoral dissertation development.

Part of this work was presented as a poster in the Congreso Argentino de Herpetologìa, 2021.

5. Conclusión

La integración de los contenidos presentados a lo largo de los cuatro capítulos que componen este manuscrito de Tesis Doctoral permitió el cumplimiento completo del Objetivo General propuesto: describir los patrones evolutivos y proporcionar hipótesis sustentadas sobre los procesos, a nivel específico y poblacional, que han influido en la distribución y diversificación actual de tres clados de ranas arborícolas de amplia distribución en las tierras bajas del norte de Sudamérica (*Scarthyla*, el clado *Scinax ruber* y *Sphaenorhynchus lacteus*). Además, el análisis comparativo de la información molecular, morfológica y distribucional que aquí se proporciona, apoyó la intuición inicial de que la historia sobre las ranas de tierras bajas que habitan en el área de estudio fue (aún es) escasa e inapropiada.

En cuanto al **Objetivo 1**. "Describir la variación histórica y actual de las poblaciones y los hábitats de las especies estudiadas", en los capítulos 1 a 3 se ofrecen los patrones de diversificación y las distribuciones de 24 linajes evolutivamente independientes recientemente identificados (o especies candidatas confirmadas), además de un número incierto de unidades taxonómicas aún mal definidas (especies candidatas no confirmadas), que habían quedado al descubierto por una persistente confusión entre taxonomía, nomenclatura y morfología.

Como se planteó en primer lugar, las áreas de distribución actuales de la mayoría de las especies incluidas en el clado terrestre (*Scinax*) son coherentes con las historias de diversificación en macrosimpatría de sus poblaciones: todos los linajes recién identificados de *Scinax* del grupo *ruber* son filopátricos, ya sea a bosques o a zonas no forestales, y parecen depender de la presencia de sus hábitats preferidos para sobrevivir. Fue posible, al menos para las especies que viven en el valle del Magdalena Medio, estudiar ejemplares simpátricos pertenecientes a linajes diferentes y no relacionados, pero morfológicamente similares -al menos externamente. Aunque esa filopatría no fue evaluada para los grupos *rostratus* y *wandae* de *Scinax,* cada uno de ellos contiene linajes únicos y no identificados previamente en cuatro áreas diferentes de los Llanos Orientales de Colombia: el piedemonte, los Llanos del norte de Arauca, la región de Manacacías en los alrededores de San Martín, Meta, y la región de Cumaribo en Vichada, a lo largo de la frontera entre Colombia y Venezuela. Esto apoya la idea de una diversificación macrosimpátrica, pero también recupera la historia de sus hábitats.

Para la mayoría de especies de *Scinax* se encontraron distribuciones pequeñas y especializadas como se había predicho (por variaciones ecofisiológicas y ambientales), y eso fue revelado a través de los modelos de distribución y los reducidos datos de ecología térmica que existen para algunas de las especies bajo estudio, incluyendo lo producido durante el transcurso de la tesis por la estudiante doctoral (Nowakowski et al., 2018). Sin embargo, las distribuciones restringidas definidas aquí pueden considerarse todavía amplias (y especialmente comparadas con las distribuciones andinas), si bien son mucho más pequeñas de lo que se pensaba anteriormente.

Las dos especies más acuáticas dentro del presente muestreo, *Scarthyla goinorum* y *Sphaenorhynchus lacteus* mostraron patrones de diversificación diferentes: la primera se estructuró en una clina de suroeste a noreste, con poblaciones del piedemonte peruano más distantes genéticamente de las brasileñas topotípicas que del grupo amazónico colombiano, hasta el punto de que una de las muestras fue considerada en el análisis bayesiano del capítulo 3 como más relacionada con *Scarthyla vigilans* que con el taxón amazónico. Esto debe revisarse con más material de la zona y evaluar aislamiento por distancia.

Por otro lado, la diversificación (o la ausencia de ella) en *Sphaenorhynchus* parece haberse producido por la migración a lo largo de los cursos de agua, de forma coherente con su hábito de vida de remar en sobre embarcaciones de vegetación flotante a lo largo de los ríos Amazonas y, supuestamente, Orinoco. Las secuencias mitocondriales de

Sphaenorhynchus muestran una muy ligera variación entre dos puntos extremos de distribución dentro del presente muestreo (Bolivia y Perú-Colombia), pero no entre Bolivia y el Caribe, por lo que las distancias *p* no corregidas dentro del clado son en todo caso inferiores al 1%. Esto significa que, o bien existe un flujo genético permanente SW-NE a lo largo de toda la distribución súper extensa de *Sphaenorhynchus lacteus* (que vive desde el norte de Argentina hasta Trinidad y Tobago), lo que es consistente con el drenaje real del Amazonas, o bien que *Sphaenorhynchus* está más cerca de *Pseudis* no sólo en el hábito de vida sino en sus historias evolutivas. *Pseudis*, el otro hílido acuático considerado en los análisis filogenéticos de los capítulos 1 y 3, se considera de origen caribeño, pero ese taxón no era el objetivo de este trabajo.

Scarthyla goinorum, por su parte, presenta estructura filogeográfica y probablemente específica, pero la información disponible a lo largo de esta disertación no es suficiente para distinguir entre un patrón de diversificación original o particular y una muestra identificada erróneamente. Esto es sorprendente pues mientras que la mayoría de las especies cisandinas al oriente de los Andes parece haberse diversificado desde el sureste hacia el noroeste del continente, este clado semiacuático o acuático amazónico evolucionó de forma diferente (de los Andes al mar, o del oeste al este), siguiendo el curso del río Amazonas.

La última hipótesis para este objetivo, que implica los tiempos de divergencia de los clados terrestres frente a la divergencia de los acuáticos, debe ser rechazada. De acuerdo con la filogenia datada presentada en el Capítulo 3, la divergencia entre los diferentes clados terrestres considerados no ocurrió antes de la primera y gran divergencia de los clados acuáticos (Pseudinae y *Sphaenothynchus*), que es, de hecho, el primer evento de divergencia recuperado en la filogenia. Esto implica necesariamente que la divergencia de esos clados no se produjo debido a las barreras paleobiogeográficas conocidas, sino de una manera más consistente con el aislamiento por distancia, si es que se puede rastrear algún aislamiento. Algo a destacar a este respecto es que la localidad registrada para la muestra atípica KU 215423, de posición conflictiva en la filogenia, está bien situada al oeste, pero también en un área más seca que las restantes localidades amazónicas.

En cuanto al último resultado esperado de este objetivo, "Los intervalos de confianza de los tiempos de divergencia de los clados considerados incluirán las fechas estimadas de formación de barreras", hay que hacer dos precisiones: 1) en el análisis bayesiano que se utiliza para la estimación de las dataciones no se obtienen intervalos de confianza, sino intervalos de credibilidad. Y a menos que los intervalos obtenidos aquí puedan incluir realmente fechas relacionadas con las posibles barreras, no pueden considerarse precisos -son tan amplios como la edad media del clado, o más. Esto se explica porque la información molecular nuclear disponible para la estimación era escasa para una filogenia en la que no hay parientes fósiles cercanos del grupo propio, pero también porque en muchos casos de diversificación terrestre las barreras, siempre que hubieran existido, no tenían nada que ver con la historia de la diversificación, dado que aparentemente aparece en macro-simpatría y mediada por preferencias ecológicas y de historia de vida.

Con respecto al **Objetivo 2.** "Evaluar el impacto de la presencia de bosques y sus variables ambientales relacionadas (precipitación, humedad y temperatura) en la formación de los límites de las distribuciones de los anuros de tierras bajas cisandinas y transandinas de Colombia", los tres capítulos presentan diferentes evidencias que apoyan la importancia de las coberturas boscosas/no boscosas en la diversificación simpátrica de las especies de *Scinax*. Sin embargo, parece que se presentan dos patrones diferentes de diversificación entre el muestreo de especies:

1) El patrón *bosque-bosque* de las especies similares a *Scinax ruber*, que es evidente en la diversificación de los clados de bosque cis-andino vs. trans-andino y recupera la historia geológica de la formación del río Magdalena y el levantamiento de la Cordillera Oriental.
2) El patrón de *bosque interior*, que se da a lo largo de la OTU UR en la cuenca amazónica noroccidental. La diversificación puede relacionarse en este caso con la combinación de dos fragmentos nucleares que reflejan la capacidad de una población para adaptarse visualmente a las condiciones del bosque (por ejemplo, las secuencias nucleares Rho + Tyr que evolucionan con patrones similares), así como con la dimensión de altura, equivalente a la de una columna de agua, inherente a los bosques maduros de esa sección del noroeste amazónico.

El patrón 1) es consistente con la primera predicción de este objetivo, según la cual "Las áreas de distribución actuales de las especies consideradas estarán limitadas por barreras ambientales que pueden estar separando diferentes linajes genéticos dentro de cada área". Eso ocurre especialmente en las especies ligadas al bosque. A su vez, la segunda predicción: "Las áreas de distribución de las especies consideradas en la propuesta estarán fragmentadas en (o comprenderán) los diferentes rangos de distribución de los linajes evolutivos revelados por el análisis" resulta evidente, pero no sólo para las especies ligadas al bosque sino para todas las especies terrestres. Las historias de vida de las especies nominales seleccionadas, y sus adaptaciones a sus ambientes preferidos tienen un rastro genético, y las áreas que habitan han sufrido de muchos cambios estructurantes durante los últimos 20 My.

Por último, el **Objetivo 3** de "Resolver los interrogantes taxonómicos que se han planteado sobre la identidad y distribución de *Scinax rostratus*, *S. ruber*, *S. "x-signatus"* y *S. wandae* para Colombia" se ha desarrollado ampliamente a lo largo de esta tesis, con muchos hallazgos esperados pero también inesperados. En ese sentido, la Hipótesis 3.1. "Scinax rostratus, S. ruber y S. wandae se componen cada uno de más de un linaje evolutivo (especie) en Colombia, de la misma manera que se ha visto para Brasil y Guyana" debe ser aceptada, y la cuenta total de los linajes crípticos dentro de las especies nominales está aún lejos de ser terminada. Los capítulos 1 y 3 proporcionan, sin embargo, avances muy contables en este sentido: un gran total de 25 linajes recién identificados dentro de las especies nominales *Scarthyla goinorum*, *Scinax garbei*, *S. cruentomma*, *S. kennedy, S. rostratus*, *S. ruber* y *S. wandae*, más las numerosas especies candidatas no confirmadas que seguirán necesitando mejor evidencia y una visión ntegradora, son una estimación conservadora de la diversidad inexplorada de los países andino-amazónicos. La filogeografía es ciertamente útil para desentrañar ese desorden, pero la necesidad de una taxonomía responsable es innegable, lo cual se ejemplifica con el redescubrimiento del holòtipo de *Hyla x-signata* en la colección ZSM de München, Baviera, Alemania. Nuevas revisiones deberían hacerse, a partir de lo aquí expuesto, para verificar las identidades de los *Scinax* de toda la región.

Varias revisiones recientes que identifican linajes crípticos (todos los trabajos de Antoine Fouquet citados a lo largo de los capítulos, por ejemplo) son coherentes al utilizar los genomas mitocondriales y los modelamientos de distribución para ayudar en la identificación de poblaciones efectivamente aisladas al interior de amplias distribuciones. Sin embargo, la falta de detalles taxonómicos y de cuidado hacen difícil relacionar sus hallazgos con las poblaciones reales en el campo, y en las colecciones biológicas. Como se dijo en la 3ra predicción de este objetivo, la diversidad de anuros de la familia Hylidae para Colombia y para otros países de la región andino-amazónica se incrementará significativamente con especies crípticas pertenecientes a *Scinax* --una vez que esas especies sean descritas formalmente para la ciencia--, lo cual le tomará a la autora y a sus colegas un buen tiempo. Lamentablemente, la situación actual de la pandemia de COVID-19 ha retrasado y seguirá retrasando esa enorme tarea, principalmente porque las colecciones biológicas no son de interés y ni se consideran una necesidad primaria, por lo que han sido cerradas temporalmente para la investigación y para la identificación de holotipos de cada linaje, y porque el trabajo de campo necesario para precisar los datos de diagnóstico no ha sido posible por un tiempo. Esa tarea seguramente tendrá que ver con la identificación de sinónimos actuales de especies previamente descritas que podrían revalidarse con mejores datos en la mano, e influirá en la revisión crítica de la literatura taxonómica antigua y novedosa sobre el tema. En el contexto de la diversidad neotropical, todo parece posible, pero el acceso a las colecciones, al trabajo de campo, a la literatura, a las publicaciones y a la financiación es de suma importancia para obtener un resultado. En ese sentido, el acceso a las colecciones de anfibios del siglo XX, y los esfuerzos por preservarlas adecuadamente, son la base del redescubrimiento de las grandes expediciones del pasado, pero también de las respuestas integradoras que se necesitan en el presente para empezar a resolver los retos del futuro, como se muestra en el capítulo 4.

Aunque en este objetivo sólo se plantearon inicialmente dos predicciones específicas, ambas relacionadas con miembros del grupo *Scinax rostratus*, de los presentes análisis se pueden extraer otras varias conclusiones taxonómicas que involucran a *Scinax wandae* y *S. garbei*, la especie de bosque. La primera y obvia es que nuestra ignorancia colectiva supera nuestro interés por describir y proteger nuestra biodiversidad regional. Todos los demás clados considerados dentro del presente muestreo contienen linajes crípticos, y muchos de ellos están ya amenazados de alguna manera. Pero esa ignorancia sobre las especies y sus patrones de distribución no debería ser la base de las decisiones sobre su supervivencia.

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