Lizards of Paraguay: an integrative approach to solve taxonomic problems in central South America

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To the ones who led my early way:
Frederick Bauer and Norman Scott

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List of abbreviations and acronyms

ABGD: Automatic barcode gap discovery.

AE Buffer: TE Buffer provided by Qiagen.

AICc: corrected Akaike Information Criterion.

AL Buffer: Lysis buffer provided by Qiagen.

Am: Equatorial monsoonal climate.

ATL Buffer: Tissue lysis buffer provided by Qiagen.

Aw: Equatorial winter dry climate.

AW1: First washing buffer provided by Qiagen.

AW2: Second washing buffer provided by Qiagen.

BI: Bayesian inference.

BIC: Bayesian Information Criterion.

BSh: Arid steppe, hot, arid climate.

Cfa: Warm temperate, fully humid, hot summer climate.

Cwa: Warm temperate, winter dry, hot summer climate.

EDTA: Ethylenediaminetetraacetic acid.

GuSCN: Guanidinium thiocyanate.

masl: Meters above sea level.

ML: Maximum Likelihood.

NaCl: Sodium chloride.

PLT: Para La Tierra (Paraguayan non profit organization).

SDS: Sodium dodecyl sulfate.

TAE Buffer: Tris - Acetic acid - EDTA Buffer.

Taq-DNA: Thermus aquaticus – DNA polymerase.

TE Buffer: Tris - EDTA Buffer.

Tris-HCl: Tris hydrochloride.

Abstract

Paraguay is a country located in the center of South America and divided by the Paraguay River in two regions: the Occidental Region or "Chaco" (60.7% of the total surface of the country), and the Oriental or Eastern Region (39.3% of the national area). Both regions have different environmental characteristics (drier and warmer in the west), and population densities, with more than 95% of the inhabitants concentrated in the Oriental Region where the capital is located. The terrain of Paraguay is relatively flat with five major rocky mountain ranges in the east, where the highest peak (Cerro Tres Kandú) reaches 842 masl, situated in the Cordillera del Ybyturuzú mountain range. Paraguay is located in the Río de la Plata river basin and, apart from the superficial water bodies (mostly found in the eastern part of the country), also has two important underground systems of water reserves (Acuífero Patiño and Acuífero Guaraní), with which the country is an important source of fresh water.

Biogeographically, Paraguay is a key spot in the region where several ecoregions converge such as Chaco, Pantanal, Cerrado, Alto Paraná Atlantic Forest, and the Southern Cone Mesopotamina Grasslands. The Chaco is differentiated by many authors in Dry Chaco and Humid Chaco, because even though they have the same origin (based on the amount of shared taxa and proximity), these two environments differ notably in several traits of vegetation, soil, and availability of water. There is another ecoregion, endemic to Paraguay, located in the middle of the country (east of the Paraguay River) and that represents a transition or intergradation area between Humid Chaco and Alto Paraná Atlantic Forest. According to some authors this area has a relict Pleistocene flora. Two ecoregions in Paraguay (Alto Paraná Atlantic Forest and Cerrado) are recognized as global hotspots of biodiversity. Finally, in the last years, two ecoregions strongly associated with the Dry Chaco were described from the northern part of the country: Médanos del Chaco, and Cerrados del Chaco. These areas are poorly studied and sampled due to the difficult access.

In my study, I sampled most of the ecoregions of Paraguay (with the exception of Médanos del Chaco, and Cerrados del Chaco), collecting samples of Squamata for genetic and morphological analyses. The main objective of my work is to solve taxonomic problems, identified through genetic barcoding analyses, in the central region of South America. To achieve this objective, I use selected taxa of the Paraguayan Squamata as models taking into consideration the crucial geographic position of the country, plus the scarce available genetic data of Paraguayan reptiles.

The collecting activities were performed in the framework of a barcoding inventory project of the Paraguayan herpetofauna and carried out mostly in rural areas searching for animals in different types of habitats. The methods used in the field were traditional techniques for herpetological sam-

pling: active searching at different times of the day and night examining potential shelters (e.g.: barks, logs, caves, leaf litter, etc.). A total of 147 days of field work were accounted for this project, and about 1246 specimens collected. The greatest portion of the specimens were amphibians (including their larvae). Among reptiles, only one tissue sample of a Crocodilian (*Caiman yacare*) was collected and the animal released, whereas entire specimens were preserved of one turtle (*Kinosternon scorpioides*) and several Squamata that were used in this work.

The extraction of DNA was performed with two different methods. For sets containing few samples (usually eight or fewer) I used the DNeasy® Blood & Tissue Kit of Qiagen®, whereas for sets of 96 samples I used the fiber glass plate protocol. The first procedure was always to sequence the mtDNA genes 16S and COI, which were used as a first reference to examine potential taxonomic issues. Thus, these sequences were used as barcodes for taxonomic comparisons. When a taxonomic conflict was identified, in some cases other genes were sequenced to generate more data and stronger taxonomic evidence. In total, I tried to amplify four mitochondrial (16S, COI, Cytb, ND4) and five nuclear (PRLR, c-mos, c-myc, pomc, Rag1) genes. Some amplifications did not work at all, and some (mostly of mitochondrial genes) produced good results. I assessed the quality of sequences after amplification in agarose gel electrophoresis.

A DNA barcode is a genetic identifier for a species, and two of the most commonly used markers are partial sequences of Cytochrome oxidase, subunit 1 (COI) and 16S rRNA. Both markers seem to work rather equally well for species identification. Nevertheless, given the low representation of COI sequences available in GenBank for comparison of Squamata from Paraguay, I used barcodes of 16S to evaluate conspecificity. For the genera *Homonota* and *Teius* I also sequenced gene fragments from tissues provided by colleagues from Argentina and Uruguay.

All the analyses to test phylogenetic hypotheses (based on single genes or concatenated datasets) were performed under Maximum Likelihood and Bayesian approaches. To root the phylogenetic trees, I chose the available taxon (or taxa) most closely related to the respective studied group as outgroups. For the general tree of Paraguayan Squamata, based on barcodes of 16S, I chose *Sphenodon punctatus*.

To complement molecular evidence generated with the ML grouping of 16S barcodes, I took a morphological approach based on voucher specimens collected during fieldwork (usually the same specimens that I used for genetic analysis), supplemented by the revision of museum collections. The morphological characters that I used depended on the taxonomic group. Usually I worked with characters traditionally used for the specific group, but also I explored additional characters that could help to diagnose the different taxa.

Given that nowadays taxonomy is a rather integrative discipline, the species concept that a scientist can apply often depends on the tools and methods that were used for the respective analyses. Thus, I follow the Unified Species Concept because it is grounded on the basis that lineages evolve separately from other lineages and independent from the tool that a scientist uses do measure the degree of divergence. To assess conspecificity I first inspected the monophyly of the samples in the 16S gene tree of Squamata from Paraguay. Once I got evidence that the taxonomy of a given cluster was in need of a revision, I looked for stronger signals such as: acquisition of more GenBank sequences from related species to calculate intra- and interspecific genetic distances, sequencing of more genes, and use of species delimitation analyses (usually ABGD). Then, the stronger line of evidence is a genetic comparison which is interpreted as reproductive isolation.

I generated a total of 142 sequences of 64 species of Squamata from Paraguay, including one exotic species: *Hemidactylus mabouia* (Appendix I). The final alignment of 615 bp comprised 249 samples. The best substitution model for the Barcoding dataset based on the gene 16S was GTR+G, according to the BIC. The sample of *Sphenodon punctatus* was recovered as the sister clade to the Squamata. Deep nodes have low bootstrap values, meaning that these phylogenetic relationships are weakly resolved. Nevertheless, the shallowest divergences have higher support values supporting the monophyly of most genera included in the analysis, with the exception of *Manciola* (Scincidae) and the tribe Xenodontini (Colubridae).

In the tree, the samples of *Vanzosaura rubricauda* from the Cerrado (field number "ALA") show a high branch distance compared with *Vanzosaura rubricauda* from the Chaco (GK 3801), which is even larger than the distance with *V. multiscutata*. According to the results, the samples of *Colobosaura* also exhibit large genetic distances, and accordingly I revalidated *Colobosaura kraepelini* (Appendix II -Published-).

The *Tropidurus* samples are monophyletic in the species of the *torquatus* group (*T. catalanensis* and *T. etheridgei*), but several issues were detected within the *spinulosus* group (*T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara*, and *T. teyumirim*). Thus, a deep genetic and morphological analysis of Paraguayan samples of this genus was performed, including additional markers (COI and PRLR) resulting in the recognition of *T. tarara* and *T. teyumirim* as synonyms of *T. lagunablanca* and *T. guarani* as a synonym of *T. spinulosus* (Appendix III -Published-).

Within the family Phyllodactylidae some taxonomic uncertainties were solved. First, the holotype of *Homonota fasciata* was recognized as a different taxon from those specimens known by that name in South America (Appendix IV -Published-). Second, we provided evidence that the samples of *H. horrida* from the type locality were different from populations of "*H. horrida*" from Paraguay

(Appendix IV -Published-). Third, we provided evidence for the recognition of two different taxa previously assigned to *H. horrida* from Paraguay (Appendix V -Accepted-).

In the same family, the barcoding tree shows a large branch length between samples of *Phyllopezus przewalskii* and samples from an isolated population in southern from Paraguay associated with rocky hills. With the inclusion of additional genes, we recognized this isolated population as a different species (Appendix VI -In review-).

In this work I present the most comprehensive analysis of genetic samples of Squamata from Paraguay. The results obtained here will be useful to help to clarify further taxonomic issues regarding the squamate fauna from the central region of South America. Moreover, the data generated for this study will have a positive impact in a larger geographic context, beyond Paraguayan borders.

The taxonomic implications of this project were the revalidation of *Colobosaura kraepelini*, the recognition of *Homonota fasciata* as a nomen nudum, the description of two new species of *Homonota* (*H. septentrionalis* and *H. marthae*) and the description (still under review) of a *Phyllopezus* from the south-east of Paraguay. Additionally, some other taxonomic conflicts were identified. For example, I found the samples of *Xenodon merremii* to form the sister group to *X. pulcher* + *Erythrolamprus* spp. It is desirable to perform phylogenies in this group using more nuclear data to achieve more robust relationships in the deep nodes. Also, the two analyzed species of *Phimophis* (*P. guerini* and *P. vittatus*) are clustered separately, and a deeper integrative (morphological and molecular) analysis is needed to understand their relationships. With respect to the venomous snakes, species of the genus *Micrurus* from the southern cone are particularly poorly represented in GenBank. In our analysis I found a sample of *M. pyrrhocryptus* in a polytomy with samples of *Micrurus* with a different coloration pattern. Evidently, a more detailed analysis is needed but precluded by the lack of samples at this moment. Finally, the genus *Bothrops* hasexperienced several taxonomic changes in the last decade, and there are many genetic samples for comparison. Nevertheless, it still needs a macro analysis at a regional scale including morphological characters to assess the concordance between molecular genetics and morphological diagnostic characters, that are not reliable to distinguish Paraguayan specimens of the *neuwiedi* group so far.

Not all goals aimed at in this project were achieved eventually. A detailed and integrative taxonomic analysis is a time-consuming project since the person performing it needs access to genetic, geographic, and morphological data. For this, it is necessary to deal with several legal issues involving national bureaucratic matters, as well as with budget restrictions, implementation of new methodologies, and even climatic conditions and logistics when performing field work. Particularly I had troubles working with the genus *Teius*. In collaboration with GK and colleagues from Argentina, I generated a large dataset of geographic information and morphologic data for lizards of this genus.

We published a detailed and extensive geographic account for the genus *Teius* (Appendix VII -Published-), with a perspective of further integrative morphological and molecular analyses to assess the parthenogenetic origin of *Teius suquiensis*, and to clarify the taxonomic status of a putatively undescribed taxon in this genus. I generated a large dataset based on about 300 specimens of *Teius* (including type specimens) with 38 morphological variables for each specimen from Argentina, Bolivia, Paraguay, and Brazil; but the molecular analyses (based on ~100 samples from Argentina, Paraguay, and Uruguay) did not yield conclusive information yet, due to problems in the amplification of most of the genes used. Thus, this is still ongoing research.

Regarding the conservation of the Paraguayan reptiles, and considering the taxonomic changes accomplished here, it is important to note that many species lack legal protection. In Paraguay, the major problem for conservation is habitat loss due to extensive crop farming. Thus, currently, the protected areas are the best strategy for conservation of biodiversity in the country. However, many such areas face legal problems (e.g., lack of official measurements, management plans, forest guard"s, infrastructure, etc.) so that the maintenance of their biodiversity over time is not guaranteed. According to the taxonomic modifications presented here, *Colobosaura kraepelini* is not known to occur in any protected area. Although it seems possible that the species occurs within “Monumento Natural Cerro Chororí” and “Monumento Natural Cerro Kõi”, its presence in a conservation unit remains to be confirmed.

In conclusion, in this study I present contributions on the taxonomy of mostly lizards from Paraguay, most of them already published (Appendices II-VI). Due to lack of samples, I was not able to deal with a deep taxonomic revision of the country's snakes. Based on my results, I can argue that analyses of Xenodontini and Pseudoboini are currently a pressing research issue. This barcoding project may continue since some colleagues in Paraguay are interested in collaboration. Given that the sequenced specimens are yet a small portion of the actual diversity of Paraguay, it will be of utmost importance to continue and expand these studies that will further improve our taxonomic knowledge. Furthermore, it is desirable to have Paraguayan scientists not only involved, but to see them taking the lead of high quality taxonomic research.

Resumen

Paraguay es un país ubicado en el centro de Sudamérica, y se encuentra dividido por el río Paraguay en dos regiones: Región Occidental o "Chaco" (60,7% de la superficie total del país), y Región Oriental (39.3% del área nacional). Ambas regiones tienen diferentes características ambientales (más secas y cálidas en el oeste) y densidad poblacional con más del 95% de los habitantes concentrados en la Región Oriental donde se encuentra la capital. El terreno de Paraguay es relativamente plano con cinco cordilleras rocosas principales en el este, donde el pico más alto (Cerro Tres Kandú) alcanza los 842 msnm, situado en la Cordillera del Ybyturuzú. Paraguay está situado en la cuenca del Río de la Plata, y además de los cuerpos de agua superficiales, cuenta también con dos importantes sistemas subterráneos de reservas de agua (Acuífero Patiño y Acuífero Guaraní), por lo cual el país es una importante fuente de agua dulce.

Biogeográficamente, Paraguay es un lugar clave en la región, donde convergen varias ecorregiones como el Chaco, el Pantanal, el Cerrado, el Bosque Atlántico del Alto Paraná y los Pastizales de la Mesopotamina de Sudamérica. El Chaco es diferenciado por muchos autores en Chaco Seco y Chaco Húmedo, porque incluso cuando tienen el mismo origen (basado en la cantidad de taxones compartidos y proximidad), estos dos ambientes difieren notablemente en varios rasgos de la vegetación, el suelo y la disponibilidad de agua. Existe otra ecorregión, endémica de Paraguay, ubicada en el centro del país (al este del río Paraguay) y que representa un área de transición o integración entre el Chaco Húmedo y el Bosque Atlántico del Alto Paraná. De acuerdo con algunos autores, esta área tiene una flora relictual pleistocénica. Dos ecorregiones en Paraguay (Bosque Atlántico del Alto Paraná y Cerrado) son reconocidas como puntos importantes de la biodiversidad global. Finalmente, en los últimos años, se describieron dos ecorregiones del norte del país, fuertemente asociadas al Chaco Seco: Médanos del Chaco y Cerrados del Chaco. Estas áreas son poco estudiadas y muestreadas debido al difícil acceso.

En mi estudio, muestreé la mayoría de las ecorregiones de Paraguay (a excepción de Médanos del Chaco y Cerrados del Chaco), colectando muestras de Squamata para análisis genéticos y morfológicos. El objetivo principal de mi trabajo es resolver problemas taxonómicos, identificados a través de análisis de códigos de barras genéticos, en la región central de América del Sur. Por lo tanto, tomo taxa seleccionados de Squamata de Paraguay como modelos para lograr este objetivo, tomando en consideración la crucial posición geográfica del país, además de los escasos datos genéticos disponibles de los reptiles de Paraguay.

Las actividades de colecta se llevaron a cabo principalmente en las zonas rurales en busca de animales en diferentes tipos de hábitats. Los métodos utilizados en el trabajo de campo fueron téc-

nicas tradicionales para el muestreo herpetológico: búsqueda activa en diferentes momentos del día y de la noche examinando refugios potenciales (por ejemplo, cortezas, troncos, cuevas, hojarasca, etc.). Se contabilizaron un total de 147 días de trabajo de campo para este proyecto, y se colectaron alrededor de 1246 especímenes. La mayor parte de los especímenes fueron anfibios (incluidas sus larvas). Entre los reptiles, solo se colectó una muestra de tejido de un cocodriliano (*Caiman yacare*) tras lo cual fue liberado, una tortuga acuática (*Kinosternon scorpioides*) y varios Squamata que se utilizaron en este trabajo.

La extracción de ADN se realizó mediante dos métodos diferentes. Para los conjuntos que contienen pocas muestras (generalmente ocho o menos) utilicé el kit DNeasy® Blood & Tissue de Qiagen®, mientras que para los conjuntos de 96 muestras utilicé el protocolo de placa de fibra de vidrio. El primer procedimiento fue siempre secuenciar los genes de ADN mitocondrial 16S y COI, que se usaron como primera referencia para examinar posibles confusiones taxonómicas. Por lo tanto, estas secuencias se utilizaron como códigos de barras para comparaciones taxonómicas. Cuando se identificó un conflicto taxonómico, en algunos casos se secuenciaron otros genes para generar más datos y conclusiones taxonómicas más sólidas. En total, traté de amplificar cuatro genes mitocondriales (16S, COI, Cytb, ND4) y cinco genes nucleares (PRLR, c-mos, c-myc, pomc, Rag1) pero algunos no funcionaron en absoluto, y algunos (la mayoría de genes mitocondriales) produjeron buenos resultados. Evalué la calidad de las secuencias después de la amplificación en electroforesis en gel de agarosa.

Un código de barras de ADN es un identificador genético para una especie, y dos de los marcadores más comúnmente usados fueron secuencias parciales de citocromo oxidasa, subunidad 1 (COI) y rRNA 16S, y ambos marcadores parecen funcionar bastante bien para la identificación de especies según estudios anteriores. Sin embargo, dada la baja representación de las secuencias de COI disponibles en GenBank para la comparación con Squamata de Paraguay, utilicé códigos de barras de 16S para evaluar la conespecificidad. En algunos casos (para *Homonota* y *Teius*) también secuencié fragmentos de genes extraídos de tejidos proporcionados por colegas de Argentina y Uruguay.

Todos los análisis para probar hipótesis filogenéticas (en base a genes individuales o conjuntos de datos concatenados) se realizaron bajo máxima verosimilitud y enfoques bayesianos. Para enraizar los árboles filogenéticos, elegí agrupar el taxón (o taxa) más cercano disponible en relación con el grupo de estudio. Para el árbol general de Squamata de Paraguay, en base a los códigos de barras de 16S, elegí *Sphenodon punctatus*.

Para acompañar las incertidumbres moleculares identificadas con la agrupación de ML de códigos de barras 16S, se tomó un enfoque morfológico basado en ejemplares testigo colectados du-

rante el trabajo de campo (generalmente especímenes utilizados para el análisis genético), complementados con revisión de colecciones de museos. Los caracteres morfológicos que utilicé dependían del grupo taxonómico. Usualmente trabajé con caracteres tradicionalmente utilizados para el grupo específico, pero también exploré caracteres adicionales que podrían ayudar a diagnosticar los diferentes taxones.

Dado que hoy en día la taxonomía es una disciplina bastante integrativa, muchas veces el concepto de especie que un científico debe seguir depende de las herramientas y los métodos que se utilizaron para los análisis respectivos. Por lo tanto, yo sigo el Concepto de Especies Unificadas forjado en la base de que los linajes evolucionan por separado de otros linajes independientemente de la herramienta que utiliza un científico para medir el grado de divergencia. Para evaluar la conspecificidad, primero inspeccioné la monofilia de las muestras en el árbol del gen 16S en base a muestras de Squamata de Paraguay. Una vez que obtuve evidencia de que la taxonomía de un grupo dado no era correcta, busqué señales más fuertes tales como: adquisición de más secuencias de GenBank, de especies relacionadas para calcular distancias genéticas intra e interespecíficas, secuenciación de más genes y uso de análisis de delimitación de species (generalmente ABGD). Entonces, la línea de evidencia más sólida es una comparación genética que se interpreta como aislamiento reproductivo.

Genere un total de 142 secuencias de 64 especies de Squamata de Paraguay (Apéndice I), incluida una especie exótica: *Hemidactylus mabouia*. La alineación final consistió en un conjunto de datos de 249 muestras de 615 pares de bases de longitud. El mejor modelo de sustitución para el juego de códigos de barras de 16S fue GTR+G, según el BIC. La muestra de *Sphenodon punctatus* se recuperó como el clado hermano de los Squamata. Los nodos profundos tienen valores de bootstrap bajos, lo que significa que las relaciones filogenéticas son débiles. Sin embargo, las divergencias más superficiales tienen valores de soporte más altos, lo que produce monofila en la mayoría de los géneros incluidos en el análisis, con excepción de *Manciola* (Scincidae) y la tribu Xenodontini (Colubridae).

En el árbol, las muestras de *Vanzosaura rubricauda* del Cerrado (número de campo "ALA") muestran una gran distancia de ramificación en comparación con *Vanzosaura rubricauda* del Chaco (GK 3801), que es incluso mayor que la distancia con *V. multiscutata*. De acuerdo con los resultados, las muestras de *Colobosaura* también muestran grandes distancias genéticas, por lo que se revalidó *Colobosaura kraepelini* (Appendix II -Published-).

Las muestras de *Tropidurus* son monofiléticas en las especies del grupo *torquatus* (*T. catalanensis* y *T. etheridgei*), pero varias incertidumbres fueron encontradas dentro del grupo *spinulosus* (*T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara* y *T . teyumirim*). Por lo tanto, se realizó un

profundo análisis genético y morfológico de muestras paraguayas de este género, incluyendo marcadores adicionales (COI y PRLR) que dieron como resultado el reconocimiento de *T. tarara* y *T. teyumirim* como sinonimos de *T. lagunablanca* y *T. guaraní* como sinónimo de *T. spinulosus* (Appendix III -Published-).

Dentro de la familia Phyllodactylidae se resolvieron algunas incertidumbres taxonómicas. Primero, el holotipo de *Homonota fasciata* fue reconocido como un taxón diferente de aquellos especímenes conocidos con ese nombre en América del Sur (Appendix IV -Published-); segundo, proporcionamos evidencia de que los ejemplares de *H. horrida* de la localidad tipo eran diferentes de las poblaciones de "*H. horrida*" de Paraguay (Appendix IV -Published-); y tercero, proporcionamos evidencia para el reconocimiento de dos taxones diferentes previamente asignados a *H. horrida* de Paraguay (Appendix V -Published-).

En la misma familia, el árbol de códigos de barras muestra una gran longitud de rama entre muestras de *Phyllopezus przewalskii* y muestras de una población aislada al sur de Paraguay asociada con colinas rocosas. Con la inclusión de genes adicionales, reconocimos esta población aislada como una unidad taxonómica diferente (Appendix VI -Published-).

En este trabajo presento el análisis más completo de muestras genéticas de Squamata de Paraguay. Los resultados obtenidos aquí serán útiles para ayudar a aclarar algunas cuestiones taxonómicas de la fauna de Squamata de la región central de América del Sur. Además, los datos generados aquí tendrán un impacto positivo en un contexto geográfico más amplio, más allá de las fronteras de Paraguay.

Las implicancias taxonómicas de este proyecto fueron la revalidación de *Colobosaura kraepelini*, el reconocimiento de *Homonota fasciata* como nomen nudum, la descripción de dos nuevas especies de *Homonota* (*H. septentrionalis* y *H. marthae*) y la descripción (aún en revisión) de un *Phyllopezus* del sudeste de Paraguay. Además, se identificaron algunos otros conflictos taxonómicos. Por ejemplo, encontré las muestras de *Xenodon merremii* como hermanas de *X. pulcher* + *Erythrolamprus* spp. Es aconsejable realizar filogenias en este grupo utilizando más datos nucleares para obtener relaciones más sólidas en los nodos profundos. Además, las dos especies analizadas de *Phimophis* (*P. guerini* y *P. vittatus*) se agrupan por separado, y se necesita un análisis integrativo (morfológico y molecular) más profundo para comprender sus relaciones. Con respecto a las serpientes venenosas, las especies del género *Micrurus* del cono sur están particularmente poco representadas en el GenBank. En nuestro análisis, encontré una muestra de *M. pyrrhocryptus* en una politomía con muestras de *Micururus* con un patrón de coloración diferente. Evidentemente, se necesita un análisis más detallado, pero la falta de muestras lo dificulta en este momento. Finalmente, el género *Bothrops* tuvo varios cambios taxonómicos en la última década, y hay muchas mues-

tras genéticas para comparar. Sin embargo, es necesario un macroanálisis a escala regional que incluya caracteres morfológicos para ver la concordancia entre la genética molecular y los caracteres de diagnóstico morfológicos, que no son confiables para distinguir los especímenes del grupo de neuwiedi de Paraguay.

Hubieron algunas debilidades en este proyecto. Un análisis taxonómico detallado e integrador es un proyecto que requiere mucho tiempo ya que se necesita acceso a datos genéticos, geográficos y morfológicos, para lo cual es necesario tratar varios asuntos legales que involucran asuntos burocráticos nacionales, restricciones presupuestarias, implementación de nuevas metodologías, e incluso condiciones climáticas y logística al realizar el trabajo de campo. Particularmente tuve problemas trabajando con el género *Teius*. En colaboración con GK y colegas de Argentina, generé un gran conjunto de datos de información geográfica y datos morfológicos para las lagartijas de este género. Publicamos una extensiva reseña geográfica detallada para el género *Teius* (Appendix VII -Published-), con una perspectiva de un análisis integrador morfológico y molecular para evaluar el origen partenogenético de *Teius suquiensis* y para aclarar el estado taxonómico de un taxón putativamente no descrito en este género. Generé un gran conjunto de datos basado en aproximadamente 300 especímenes de *Teius* (incluidos materiales tipo) con 38 variables morfológicas para cada espécimen de Argentina, Bolivia, Paraguay y Brasil; pero los análisis moleculares (basados en ~100 muestras de Argentina, Paraguay y Uruguay) no dieron información concluyente aún, debido a problemas en la amplificación de la mayoría de los genes utilizados. Por lo tanto, esto sigue siendo una investigación en curso.

Con respecto a la conservación de los reptiles de Paraguay, y considerando los cambios taxonómicos realizados aquí, es importante señalar que muchas especies carecen de protección legal. En Paraguay, el principal problema para la conservación es la pérdida de hábitat debido a cultivos extensivos. Por lo tanto, actualmente, las áreas protegidas son la mejor estrategia para la conservación de la biodiversidad en el país, aunque muchas unidades de conservación enfrentan problemas legales (por ejemplo, falta de: mediciones oficiales, planes de manejo, guardias forestales, infraestructura, etc.) y además el mantenimiento en el tiempo de su biodiversidad no está garantizado. De acuerdo con las modificaciones taxonómicas presentadas aquí, *Colobosaura kraepelini* se distribuye en zonas donde no se encuentran áreas protegidas. Sin embargo, es posible que la especie se encuentre en el “Monumento Natural Cerro Chororí” y “Monmento Natural Cerro Kõi”, aunque su presencia en una unidad de conservación debe ser confirmada.

En conclusión, en este estudio presento contribuciones sobre taxonomía principalmente en lagartos de Paraguay, muchas de las cuales ya fueron publicadas (Appendices II-VI). Debido a la falta de muestras, no fui capaz de lidiar con una profunda revisión taxonómica de las serpientes. Con

base en los resultados, puedo argumentar que los análisis de Xenodontini y Pseudoboini son actualmente un tema de investigación acuciante. Este proyecto de códigos de barras puede continuar dado que algunos colegas en Paraguay están interesados en colaboración. Dado que los especímenes secuenciados son aún una pequeña porción de la diversidad real de Paraguay, será de la mayor importancia continuar y ampliar estos estudios que mejorarán aún más nuestro conocimiento taxonómico. Y es deseable no solo contar con la participación sustancial de científicos paraguayos, sino también verlos liderando investigaciones taxonómicas de alta calidad.

Zusammenfassung

Paraguay ist ein Land im Zentrum Südamerikas, das durch den gleichnamigen Fluss in zwei Regionen geteilt wird: die westliche Region oder „Chaco“ (60,7% der Gesamtfläche des Landes) und die östliche Region (39,3% der Gesamtfläche). Beide Regionen haben unterschiedliche Umwelteigenschaften (trockener und wärmer im Westen) und Bevölkerungsdichten, wobei sich mehr als 95% der Einwohner auf die östliche Region konzentrieren, in der sich auch die Hauptstadt befindet. Das Terrain von Paraguay ist relativ flach mit fünf großen felsigen Bergketten im Osten, wo der höchste Gipfel (Cerro Tres Kandú im Ybyturuzú-Gebirge) 842 m hoch ist. Paraguay befindet sich im Rio de la Plata-Becken und hat neben seinen Oberflächengewässern (hauptsächlich im östlichen Teil des Landes) auch zwei wichtige unterirdische Systeme von Wasserreserven (Aquifer Patiño und Aquifer Guaraní), wodurch das Land eine wichtige Quelle für Süßwasser ist.

Biogeographisch ist Paraguay von großer regionaler Bedeutung, da hier mehrere Ökoregionen konvergieren: der Chaco, das Pantanal, der Cerrado, der Atlantische Regenwald von Alto Paraná und die Mesopotamina-Grasländer von Südamerika. Der Chaco wird von vielen Autoren weiter in Trockenen und Feuchten Chaco differenziert, denn auch wenn sie den gleichen Ursprung haben (bezogen auf die Menge der gemeinsamen Taxa und ihre Nähe zueinander), unterscheiden sich diese beiden Naturräume in mehreren Merkmalen ihrer Vegetation, Böden und der Verfügbarkeit von Wasser deutlich voneinander. Es gibt eine weitere Ökoregion, endemisch für Paraguay, die sich im Zentrum des Landes (östlich des Paraguay-Flusses) befindet und einen Übergangsbereich zwischen dem Feuchten Chaco und dem Atlantischen Regenwald von Alto Paraná darstellt. Einigen Autoren zufolge hat dieses Gebiet eine pleistozäne Reliktflora. Zwei Ökoregionen in Paraguay (Atlantischer Regenwald von Alto Paraná und Cerrado) gelten als globale Hotspots der Artenvielfalt. Schließlich sind in den letzten Jahren zwei Ökoregionen im Norden beschrieben worden, die stark mit dem Trockenen Chaco assoziiert sind: Médanos del Chaco und Cerrados del Chaco. Diese Bereiche sind wegen des schwierigen Zugangs bisher nur wenig erforscht und beprobt.

In meiner Studie besuchte ich die meisten Ökoregionen Paraguays (mit Ausnahme von Médanos del Chaco und Cerrados del Chaco) und sammelte Exemplare von Squamata für genetische und morphologische Analysen. Das Hauptziel meiner Arbeit ist die Lösung taxonomischer Probleme, die durch die Analyse von DNA-Barcodes in der zentralen Region Südamerikas identifiziert wurden. Um dieses Ziel zu erreichen verwende ich ausgewählte squamate Taxa aus Paraguay als Modelle, , unter Berücksichtigung der entscheidenden geografischen Position des Landes und den begrenzten genetischen Daten von Reptilien aus Paraguay.

Die Sammelaktivitäten fanden im Zuge eines umfassenden Barcoding Inventars Project der Herpetofauna Paraguays statt und wurden hauptsächlich in ländlichen Gebieten durchgeführt und bestanden aus der Suche nach Tieren in verschiedenen Arten von Lebensräumen. Die Methoden, die in der Feldarbeit verwendet wurden, waren traditionelle Techniken für die herpetologische Inventarisierung: aktive Suche zu verschiedenen Tages- und Nachtzeiten einschließlich der Untersuchung potenzieller Zufluchtsorte (z. B. Rinde, Totholz, Höhlen, Abfall usw.). Insgesamt wurden 147 Tage Feldarbeit für dieses Projekt absolviert, und ungefähr 1246 Exemplare gesammelt. Die meisten dieser Exemplare waren Amphibien (einschließlich ihrer Larven). Innerhalb der Reptilien wurde von den Krokodilen nur eine Gewebeprobe von einem später freigelassenen Individuum (*Caiman yacare*) gesammelt, wohingegen ganze Exemplare von einer Wasserschildkröte (*Kinosternon scorpioides*) und mehreren Squamata, die in dieser Arbeit verwendet wurden, konserviert wurden.

Die DNA-Extraktion wurde mit zwei verschiedenen Methoden durchgeführt. Für Sätze von wenigen Proben (in der Regel acht oder weniger) benutzte ich das DNeasy® Blood & Tissue Kit von Qiagen®, während ich für größere Mengen von 96 Proben das Glasfaserplatten-Protokoll verwendete. Als erstes sequenzierte ich immer die mitochondrialen Gene 16S und COI, die als Referenz verwendet wurden um erste Hinweise mögliche taxonomische Problemfälle zu erhalten. Dementsprechend wurden diese Sequenzen als Barcodes für taxonomische Vergleiche verwendet. Wenn ein taxonomischer Konflikt festgestellt wurde, wurden in einigen Fällen andere Gene sequenziert, um mehr Daten für solidere taxonomische Schlussfolgerungen zu erhalten. Insgesamt habe ich versucht, vier mitochondriale Gene (16S, COI, CytB, ND4) und fünf Gene des Kerngenoms (PRLR, c-mos, c-myc, pomc, Rag1) zu amplifizieren, wobei einige Amplifikationen überhaupt nicht funktionierten und andere (die meisten mitochondrialen Gene) zu guten Ergebnissen führten. Ich bewertete die Qualität der Sequenzen nach der Amplifikation mittels Agarosegelelektrophorese.

Ein DNA-Barcode ist ein genetischer Identifikator für eine Spezies, und zwei der sind häufigsten verwendeten Marker waren Teilsequenzen der Cytochromoxidase-Untereinheit 1 (COI) und 16S rRNA, die früheren Studien zufolge beide recht gut für die Identifizierung von Spezies zu funktionieren scheinen. Aufgrund der geringen Repräsentation von COI-Sequenzen die in GenBank zum Vergleich mit Squamata aus Paraguay zur Verfügung stehen, verwendete ich 16S-Barcodes, um die Artzugehörigkeiten zu bewerten. Für die Gattungen *Homonota* und *Teius* sequenzierte ich auch Genfragmente aus Gewebeproben, die mir von Kollegen aus Argentinien und Uruguay zur Verfügung gestellt wurden.

Alle Analysen zum Testen phylogenetischer Hypothesen (basierend auf einzelnen Genen oder verknüpften Datensätzen) wurden unter Maximum-Likelihood- und Bayes‘schen Ansätzen durchgeführt. Um phylogenetische Stammbäume zu wurzeln, verwendete ich jeweils das der untersuchten Gruppe nächstverwandte verfügbare Taxon (oder Taxa) als Außengruppe. Für den allgemeinen Baum der Squamata von Paraguay, basierend auf 16S Barcodes, wählte ich *Sphenodon punctatus*.

Ergänzend zu der mit der ML-Gruppierung von 16S-Barcodes generierten molekularen Beweislinie verfolgte ich auch einen morphologischen Ansatz, basierend auf während der Feldarbeit gesammelten Exemplaren (normalerweise die gleichen, die auch für die genetischen Analysen verwendet wurden), und ergänzt durch die Untersuchung bestehender Museumssammlungen. Die morphologischen Merkmale, die ich verwendete, hingen von der taxonomischen Gruppe ab. Ich habe normalerweise mit solchen Merkmalen gearbeitet, die traditionell für die jeweilige Gruppe verwendet wurden, aber auch zusätzliche Merkmale überprüft, die bei der Diagnose der verschiedenen Taxa hilfreich sein könnten.

Angesichts der Tatsache, dass Taxonomie heute eine ziemlich integrative Disziplin ist, hängt das Artkonzept, dem ein Wissenschaftler folgen kann, oft von den Werkzeugen und Methoden ab, die für die jeweiligen Analysen verwendet wurden. Ich folge hierin dem Unified Species Concept, das auf der Grundlage entwickelt wurde, dass sich die genealogischen Linien unabhängig von anderen Linien entwickeln, unabhängig davon, unter welchen Gesichtspunkten ein Wissenschaftler den Grad der Divergenz misst. Um Artzugehörigkeiten zu bewerten, untersuchte ich zunächst die Monophylie der Proben im 16S-Genbaum der Squamata aus Paraguay. Sobald sich Hinweise ergaben, dass die Taxonomie einer bestimmten Gruppe revisionsbedürftig war, suchte ich nach stärkeren Signalen, beispielsweise durch: Verwendung von mehr GenBank-Sequenzen verwandter Arten zur Berechnung intra- und interspezifischer genetischer Distanzen, Sequenzierung weiterer Gene und die Verwendung von Artabgrenzungs-Analysen (in der Regel ABGD). Die stärkste Beweislinie ist also ein genetischer Vergleich, der als reproduktive Isolation interpretiert wird.

Ich generierte insgesamt 142 Sequenzen von 64 Arten von Squamata aus Paraguay, einschließlich einer exotischen Spezies, *Hemidactylus mabouia* (Anhang I). Das endgültige 16S gene Alignment mit einer Länge von 615 Basenpaaren umfasste 249 Individuen. Das beste Substitutionsmodell für den Barcode-Datensatz war GTR+G, laut BIC. Die Sequenz von *Sphenodon punctatus* wurde als Schwesterngruppe der Squamata gefunden. Basale Knoten haben niedrige Bootstrap-Werte, was bedeutet, dass diese phylogenetische Beziehungen schwach sind. Die terminaleren Divergenzen haben jedoch höhere Bootstrap-Werte, und die Monophylie der

meisten in der Analyse enthaltenen Gattungen wurde bestätigt, mit Ausnahme von *Manciola* (Scincidae) und dem Stamm Xenodontini (Colubridae).

Im Baum zeigen die Proben von *Vanzosaura rubricauda* aus dem Cerrado (Feldnummer „ALA“) eine große Distanz zu *Vanzosaura rubricauda* aus dem Chaco (GK 3801), die sogar größer ist als die Distanz zu *V. multiscutata*. Den Ergebnissen zufolge zeigen die *Colobosaura*-Proben auch große genetische Distanzen, so dass *Colobosaura kraepelini* revalidiert wurde (Appendix II -Published-).

Die *Tropidurus*-Proben sind monophyletisch in den Arten der *torquatus*-Gruppe (*T. catalanensis* und *T. etheridgei*), aber einige Unsicherheiten wurden innerhalb der *spinulosus*-Gruppe gefunden (*T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara* und *T. teyumirim*). Daher wurde eine tiefgreifende genetische und morphologische Analyse von paraguayischen Individuen dieser Gattung durchgeführt, einschließlich zusätzlicher Marker (COI und PRLR), die zur Erkennung von *T. tarara* und *T. teyumirim* als Synonyme von *T. lagunablanca* und *T. guarani* als Synonym von *T. spinulosus* führten (Appendix III -Published-).

Innerhalb der Familie Phyllodactylidae wurden ebenfalls einige taxonomische Unsicherheiten gelöst. Erstens wurde der Holotyp von *Homonota fasciata* als ein von den in Südamerika unter diesem Namen bekannten Exemplaren verschiedenes Taxon erkannt (Appendix IV -Published-). Zweitens konnten wir belegen, dass sich die *H. horrida*-Exemplare der Typlokalität von den Populationen von „*H. horrida*“ in Paraguay unterscheiden (Appendix IV -Published-), und drittens lieferten wir Beweise für die Anerkennung von zwei verschiedenen Taxa, die in Paraguay zuvor als *H. horrida* angesprochen wurden (Appendix V -Published-).

In der gleichen Familie zeigt der Barcoding-Baum eine große Astlänge zwischen Proben von *Phyllopezus przewalskii* und Proben von einer mit felsigen Hügeln assoziierten, isolierten Population aus dem südlichen Paraguay. Unter Einbeziehung zusätzlicher Gene entlarvten wir diese isolierte Population als eine neue Art (Appendix VI -Published-).

In der vorliegenden Studie präsentiere ich die vollständigste Analyse von genetischen Proben paraguayischer Squamata. Die hier gewonnenen Ergebnisse werden dazu beitragen, weitere taxonomische Probleme der Squamata-Fauna der zentralen Region Südamerikas zu klären. Darüber hinaus werden sich die hier generierten Daten in einem breiteren geografischen Kontext, über die Grenzen Paraguays hinaus, positiv auswirken.

Taxonomische Konsequenzen dieses Projektes waren die Revalidierung von *Colobosaura kraepelini*, die Anerkennung von *Homonota fasciata* als nomen nudum, die Beschreibung von zwei neuen Arten der Gattung *Homonota* (*H. septentrionalis* und *H. marthae*) und die Beschreibung (derzeit noch im Review-Prozess) eines neuen *Phyllopezus* aus dem Südosten Paraguays. Darüber

hinaus wurden noch einige weitere taxonomische Konflikte identifiziert. Zum Beispiel stellten sich die Proben von *Xenodon merremii* als Schwesterngruppe von *X. pulcher* + *Erythrolamprus* spp. heraus. Es ist angeraten, Phylogenien für diese Gruppe zu erstellen, in denen mehr nukleäre Daten verwendet werden, um bessere Unterstützungen in den basalen Knoten zu erhalten. Darüber hinaus clustern die beiden paraguayischen analysierten Arten von *Phimophis* (*P. guerini* und *P. vittatus*) getrennt voneinander, und eine integrative Analyse (morphologischer und molekularer Merkmale) ist für ein tieferes Verständnis ihrer Verwandtschaftsbeziehungen nötig. In Bezug auf Giftschlangen muss bemerkt werden, dass Arten der Gattung *Micrurus* besonders schlecht in GenBank repräsentiert sind. Unserer Analyse positionierte ein Individuum von *M. pyrrhocryptus* inmitten einer Polytomie von *Micururus*-Exemplaren mit einem vollkommen unterschiedlichen Farbmuster. Offensichtlich ist eine detailliertere Analyse erforderlich, aber durch das Fehlen von entsprechenden Sequenzen derzeit unmöglich. Schließlich hat die Gattung *Bothrops* im letzten Jahrzehnt mehrere taxonomische Veränderungen erfahren, und es gibt viele genetische Proben, die verglichen werden könnten. Allerdings müssen wir eine Makroanalyse auf regionaler Ebene durchführen, um die Korrelation zwischen der molekularen Genetik und diagnostischen morphologischen Merkmalen zu überprüfen, denn letztere sind zumindest für die Unterscheidung paraguayischer Vertreter der*neuwiedi*-Gruppe immer noch sehr unzuverlässig.

Nicht alle in diesem Projekt gesetzten Ziele konnten auch erreicht werden. Eine detaillierte und umfassende taxonomische Analyse ist ein sehr zeitaufwändiges Projekt, das den Zugang zu genetischen, geografischen und morphologischen Daten erfordert. Die durchführende Person muss sich mit diversen rechtlichen Fragen und nationalen bürokratischen Problemen auseinanderzusetzen. Hinzu kommen Schwierigkeiten durch Budgetzwänge, bei der Umsetzung neuer Methoden sowie durch klimatische Bedingungen und Logistik bei der Feldarbeit. Ich hatte besonders Probleme mit der Gattung *Teius* zu arbeiten. In Zusammenarbeit mit GK und Kollegen aus Argentinien erzeugte ich eine große Datenmenge von geographischen Informationen und morphologischen Daten für Echsen dieser Gattung. Wir publizierten eine umfassende und detaillierte geografische Abhandlung über die Gattung *Teius* (Appendix VII -Published-), mit der Perspektive einer weiterführenden morphologische und molekulare Analysen vereinigenden integrativen Studie zum möglichen parthenogenetischen Ursprung von *Teius suquiensis* und zur Klärung des taxonomischen Status eines mutmaßlich unbeschriebenen Taxons innerhalb der Gattung. Ich erzeugte einen große Datensatz für etwa 300 *Teius*-Exemplaren (einschließlich Typusmaterial) mit 38 morphologischen Variablen für jedes Individuum aus Argentinien, Bolivien, Paraguay und Brasilien, aber die molekulare Analyse (basierend auf ~100 Exemplaren aus Argentinien, Paraguay und Uruguay) lieferten aufgrund von Problemen bei der Amplifikation der

meisten Gene bisher noch keine schlüssigen Informationen. Daher ist dies noch eine laufende Untersuchung.

In Bezug auf den Schutz der Reptilien in Paraguay und unter Berücksichtigung der hier vorgenommenen taxonomischen Veränderungen ist zu beachten, dass viele Arten keinen rechtlichen Schutzsstatus genießen. In Paraguay besteht das Hauptproblem des Naturschutzes in dem Verlust von Lebensräumen aufgrund extensiven Nutzpflanzenanbaus. Daher sind Schutzgebiete derzeit die beste Strategie für den Erhalt der Biodiversität in diesem Land, obwohl viele Schutzgebiete mit rechtlichen Problemen konfrontiert sind (zum Beispiel das Fehlen von offiziellen Vermessungen, Bewirtschaftungsplänen, Waldschutz, Infrastruktur, etc.), so dass die Erhaltung der Biodiversität mittelfristig nicht gewährleistet ist. Nach den hier vorgestellten taxonomischen Änderungen ist *Colobosaura kraepelini* aus keinem einzigen geschützten Gebiet bekannt. Es scheint zwar möglich, dass die Art im „Monumento Natural Cerro Chororí“ und im „Monumento Natural Cerro Kõi“ vorkommt, allerdings muss ihre Anwesenheit in einem geschützten Gebieten erst noch bestätigt werden.

Zusammenfassend präsentiere ich in dieser Studie Beiträge zur Taxonomie vor allem der Echsen Paraguays (Anhänge II-VI). Aufgrund des Fehlens ausreichender Probenmengen war ich nicht in der Lage, eine tiefgreifende taxonomische Überarbeitung der Schlangen des Landes vorzunehmen. Aufgrund meiner Ergebnisse kann ich jedoch argumentieren, dass Analysen von Xenodontini und Pseudoboini derzeit ein drängendes Forschungsthema sind. Dieses Barcoding-Projekt kann fortgesetzt werden, da einige Kollegen in Paraguay an einer Zusammenarbeit interessiert sind. Da die sequenzierten Exemplare immer noch lediglich einen kleinen Teil der wahren Vielfalt Paraguays ausmachen, wird es von größter Wichtigkeit sein, diese Studien fortzusetzen und auszuweiten, um unser taxonomisches Wissen weiter zu verbessern. Dabei ist es ist höchst wünschenswert, dass paraguayische Wissenschaftler sich nicht nur beteiligen, sondern auch selbst die treibenden Kräfte qualitativ hochwertiger taxonomischer Forschung darstellen.

1. INTRODUCTION

1.1. Preface

Unbelievably, this work started nearly at the end of 2011, and it seems normal that the persons involved never notice how fast the time is left behind. I met Dr. Köhler on the very last day of his first trip to Paraguay. A few months before I was supposed to start my PhD in Uruguay… which finally did not happen. At the very beginning, my plan was to work on the phylogeography of the lizard genus *Teius*, but due to difficulties (that are detailed in the Discussion section) I had to change the topic of my research. People say that things happen for a reason, and I witnessed that during my PhD in Germany. My current research project came to fill an enormous gap in the knowledge of the central region of South America.

Is well known that Paraguay was considered for many years as a black hole of information, not only in biological sciences but also in many other fields of basic and applied sciences. Things are changing now, and research in Paraguay is making important steps forward. I consider my project as a brick in that process.

For biological works that cover the central portion of South America, Paraguay is critical since the country is located in a confluence of different ecoregions such as Cerrado, Pantanal, Atlantic Forest, Chaco (Humid and Dry), and Southern Cone Mesopotamian Grasslands, each of them having its own distinct origin and evolutionary history. Then, Paraguay is key for works in northern Argentina, Uruguay, southwestern Brazil, and Bolivia. In spite of this biogeographical importance, Paraguay was poorly explored, and in the current era of molecular genetics, the investigations that include genetic samples from Paraguay are extremely rare in herpetology. For instance, the natural history museum of Paraguay (Museo Nacional de Historia Natural del Paraguay) started its tissue collection for genetic analyses in this decade, whereas other neighbor countries started already some decades ago.

The herpetofauna from Paraguay, and specifically the squamate diversity is still poorly known, evidenced by the fact that still in the last decade, and without the help of molecular tools, several new records for the country were made (*Ophiodes fragilis* by Cacciali & Scott 2012, *Epictia vellardi* by Cabral & Netto 2016, *Chironius exoletus* by Cacciali & Ca-

bral 2015, *Lygophis paucidens* by Cacciali *et al*. 2013, *Philodryas livida* by Smith *et al*. 2014, *Rachidelus brazili* by Smith *et al*. 2013, *Micrurus silviae* by Cacciali *et al*. 2011) and some species new to science were described (*Tropidurus lagunablanca* Carvalho, 2016; *Tropidurus tarara* Carvalho, 2016; *Tropidurus teyumirim* Carvalho, 2016; *Ophiodes luciae* Cacciali & Scott, 2015; *Phalotris normanscotti* Cabral & Cacciali, 2015). The incorporation of molecular genetics opened a new panorama that was previously hidden in the country. Some herpetologists in the region included genetic samples of Squamata from Paraguay (Gamble *et al*. 2012, Werneck *et al*. 2012, Morando *et al*. 2014, Recoder *et al*. 2014) although only very occasionally.

This is the reason why my current project represents an important advance generating data that were never provided before. Specifically, the aim of my project is to perform a barcoding analysis of the Squamata from Paraguay, generating information available for comparison with data from countries in the region.

It is important to note that, although Dr. Köhler and I broadly collected samples of amphibians and reptiles, I focused on Squamata given my previous experience with that group.

1.2. Paraguay: An overview

1.2.1. Geography and demographics

Paraguay is located in the center of South America (Fig. 1) between parallels 18º18' and 27º30'S, and 54º19' and 62º38'W; and the country was named after the river that divides the land in two regions: Occidental Region or "Chaco", and Oriental or Eastern Region (Fig. 2). The total surface of Paraguay is 406,752 km^2, of which 39.9% (159,827 km^2) belong to the Eastern Region, and 60.7% (246,925 km^2) correspond to the Occidental Region. The longest distance in the country is about 1,051 km (airline) from Cerro Coronel F. Cabrera (limit with Bolivia) in the northwestern corner to Cambyretá in the southeast, near the limit with Argentina.

Paraguay has currently a population of 6,818,180 inhabitants (DGEEC 2015), with an uneven density being far higher in the capital and surroundings, while the huge and remote departments of Boquerón and Alto Paraguay have less than one habitant per square kilometer (Fig. 3, Candia Franco & Varela Cano 2015). Nevertheless, the highest rate of native indigenous people (total in Paraguay 108,308 persons) is located in the department of Boquerón (DGEEC 2004), even when the access to fresh water is extremely difficult in

some areas. Although Guaraní and Spanish are the only official languages, many ethnic populations speak their own distinct languages, such as Guaraní, Nivaklé, Maká, Ishir, Tomaraho, Angaité, Toba, among others. Nowadays, most of the native people seek a place to live in the capital city, trying to improve their economic perspectives, but a good portion of these become beggars asking for charity in the streets. Even if indigenous people are allowed access to natural reserves for hunting (only with traditional tools), the main problem is that they lost their traditional and sustainable nomad way of life since most of the country is covered with crops, or pasture for cattle, fragmented by deforestation, and fenced off. Thus, the natural resources cannot sustain them anymore.

Figure 1. Location of Paraguay (in gray) in the geographical context of South America.

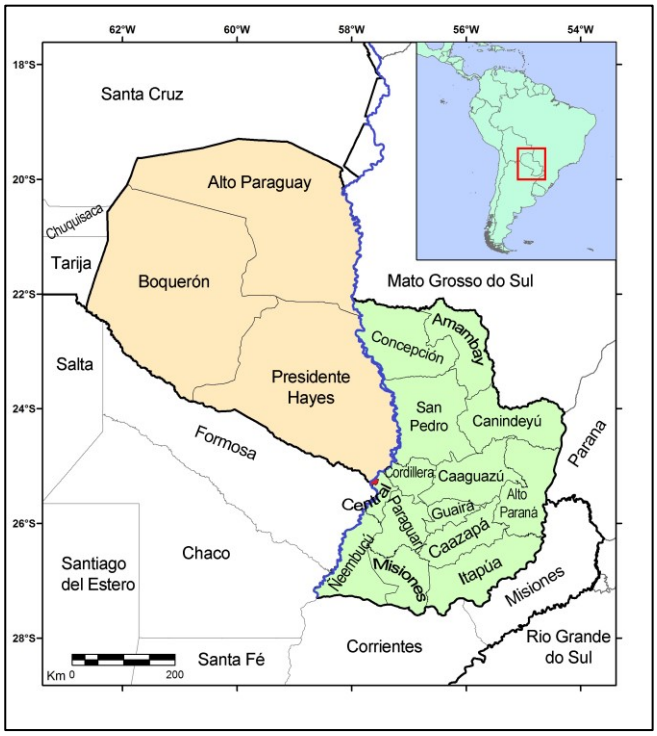

Figure 2. Political divisions of Paraguay and areas of neighbor countries. Thick black lines are countries divisions, and thin gray lines are divisions inside the countries. Paraguay River is highlighted in blue, and it divides Paraguay in the Occidental Region (yellow area) and Oriental Region (green area).

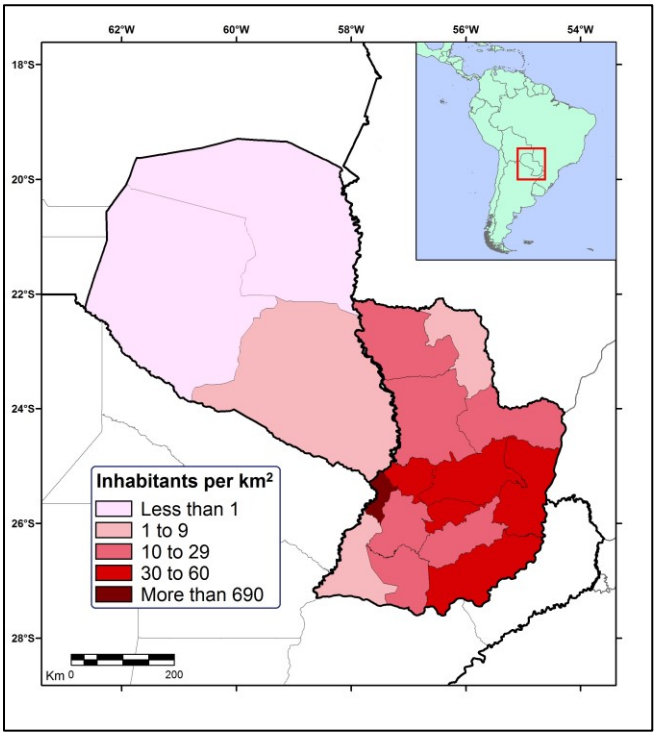

Figure 3. Human population density in Paraguay, by departments. The unevenly highest density is in the capital and surroundings.

1.2.2. Topography

The terrain of Paraguay is relatively flat. The Occidental Region is settled on a very ancient and stable craton platform with low tectonic activity (Fúlfaro 1996), giving place to a flat area with only some scattered hills (Fig. 4), (in Spanish "Cerros"). The erroneously named "Cerro Chovoreca" is actually a gradual a slope located in the northernmost of Paraguay near the border with Bolivia. Some important hills in this region are Cerro Coronel F. Cabrera that is a true hill of 630 masl, with vertical walls at the west and south (Fig. 5). Also, it is important to highlight the presence of a large elevation called Cerro León located in the Defensores del Chaco National Park that has a system of grooves (Fig. 6) on a rocky substrate with at least four geological fractures (Báez Presser *et al*. 2004).

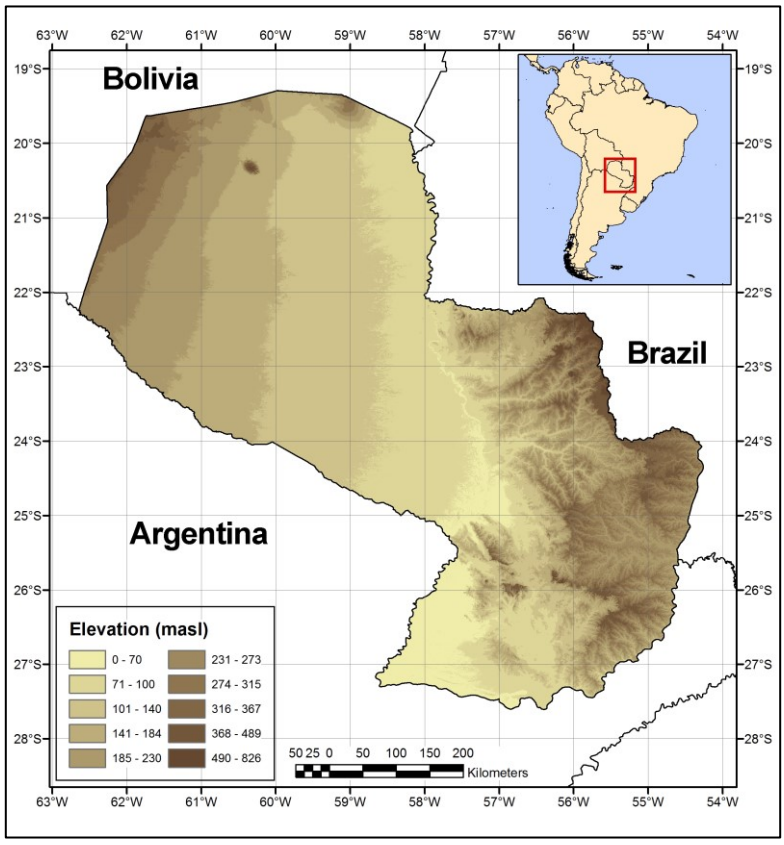

Figure 4. Elevation map showing the difference in the topography between Occidental and Oriental regions of Paraguay.

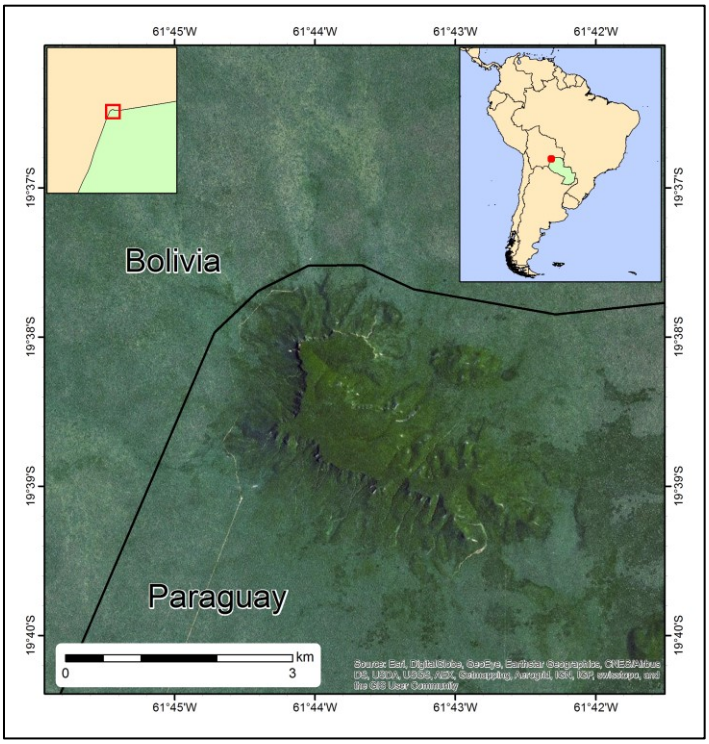

Figure 5. Cerro Coronel F. Cabrera is one of the few hills present in the Occidental Region of Paraguay, and is a boundary post between Bolivia and Paraguay.

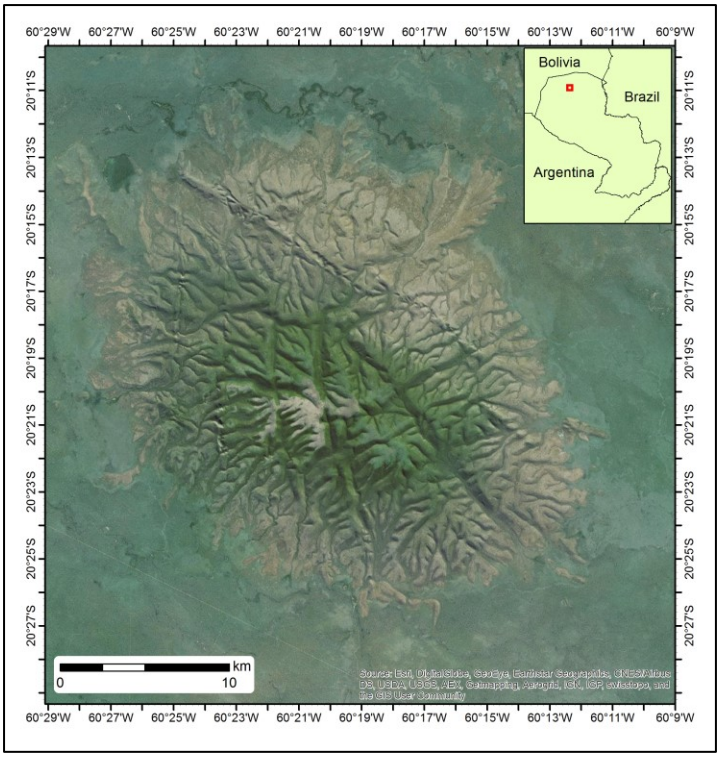

Figure 6. Cerro León is a system of rocky hills and grooves located in the northern portion of the Paraguayan "Chaco" flat plain.

The Oriental Region is more complex, based on orographic systems as a result of tectonic activity in the Paleozoic and Mesozoic (Fúlfaro 1996). The hilly surface of the Oriental Region is shaped by five major main mountain chains, two of them at the north (Cordillera del Amambay and Cordillera de Mbaracayú), and two at the south (Cordillera de San Rafael and Cordillera del Ybyturuzú), plus the Cordillera de los Altos that is the westernmost ridge near the capital of Paraguay. The highest peak of Paraguay (Cerro Tres Kandú) reaches 842 masl, and is located in the Cordillera del Ybyturuzú.

Paraguay is rich in fresh water. Four Paraguayan rivers run along the borders between Paraguay and Brazil (upper portion of Ríos Paraguay and Apa) and Argentina (lower portion of Ríos Paraguay, Paraná, and Pilcomayo). In addition to the profuse superficial riparian systems present mostly in the Oriental Region (Fig. 7), there are two main important underground systems of fresh water: a small aquifer called Acuífero Patiño, and a big underground water system called Acuífero Guaraní (UNESCO 2009).

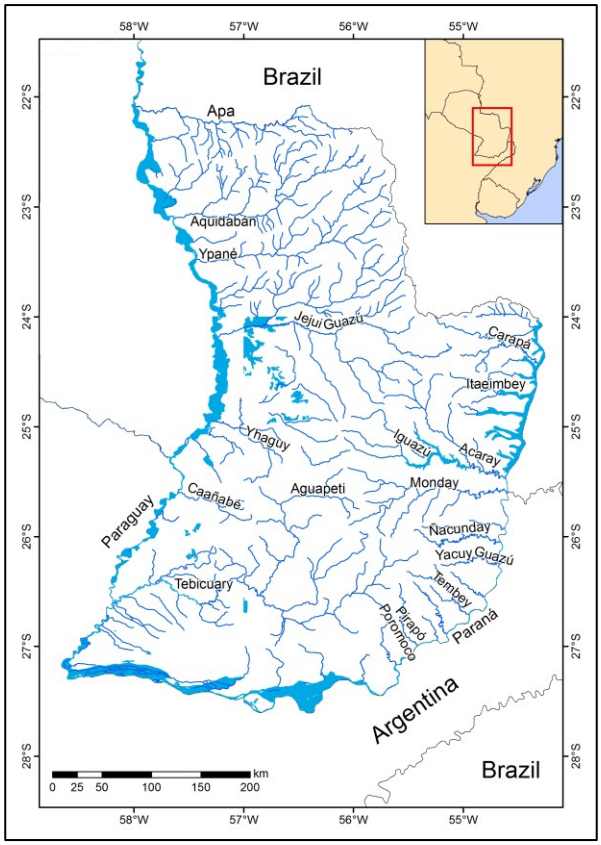

Figure 7. Hydrographic system of the Oriental Region of Paraguay.

Superficial water in the Occidental Region is scarce (Fig. 8), and some rivers are non-perennial, having water only in the rain season. An extreme case is Río Timane that starts near Cerro Coronel F. Cabrera and becomes a big and strong river running from northwest to the east draining into Paraguay River. Nevertheless, most of the year, its course is only a dry basin.

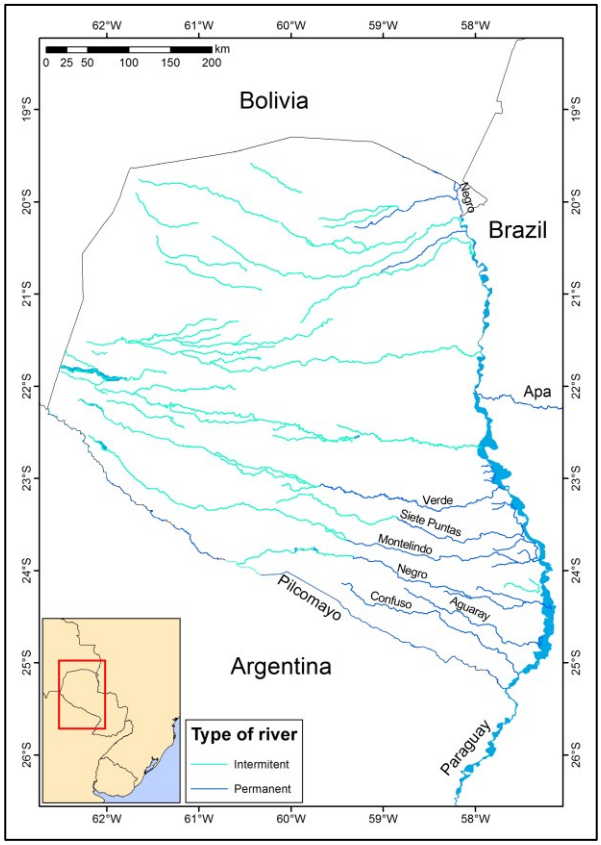

Figure 8. Hydrographic system of the Occidental Region of Paraguay. Light blue lines indicate non-perennial rivers.

The riparian systems of Paraguay belong to the Plata Basin, and two of the most important tributaries of this hydrological basin are the Ríos Paraguay and Paraná, the latter being the fastest flowing river of that basin. This is the reason why many hydroelectric dams are located along this river or some of its tributaries. The most important and notorious cases are the Itaipú and Yacyretá dams. The first was created in 1978, and is the biggest dam in the world according to WWF (2003), and it had flooded 700 km^2 of Atlantic Forest (Fig. 9, Arsenault *et al*. 2007). The second (Yacyretá dam) flooded an important system of

islands in southern Paraguay (Fig. 10). The remaining part of one of these islands has a unique ecosystem with sandy dunes associated to a riparian environment (Fig. 11).

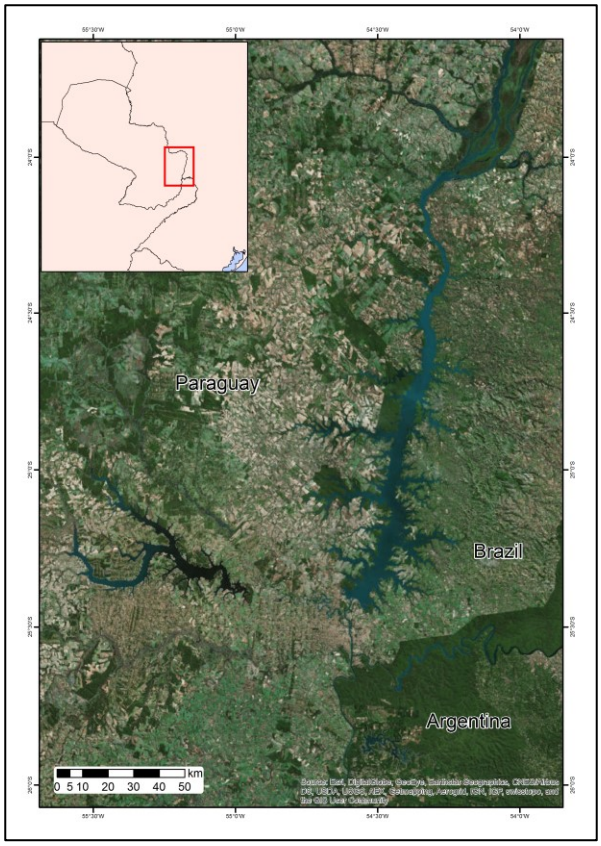

Figure 9. Paraná River, showing the artificial lake formed after the construction of the Itaipú dam. At the left, it is also possible to see a reservoir of a smaller dam: Iguazú dam, situated on the Iguazú River, tributary of the Paraná.

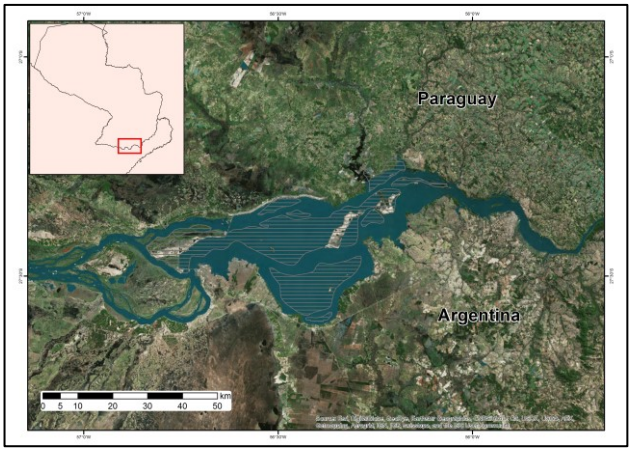

Figure 10. The flooded area of the Yacyretá dam made a system of small islands in south Paraguay (area marked with horizontal lines) disappear. Only two portions of the bigger island, Isla Yacyretá, remain out of the water.

Figure 11. Remainings of the sand dunes of Isla Yacyretá. 90% of the island was flooded by the construction of the Yacyretá dam.

Paraguay is not very rich in lentic systems, and probably the most popular is the erroneously named "Lago Ypacarai", being a lagoon that used to be a touristic place, but was abandoned due to the high pollution of its water, mainly because many factories pouring their effluents into the lagoon, as well two large cities, San Bernardino and Areguá, located around the lagoon (Ritterbusch 1988, JICA 1989). In the Chaco, there are also some salinized lagoons that are important for the maintenance of the biological systems (and therefore declared Ramsar sites), where many components of the fauna got adapted to live in an environment with a high salt concentration (Short 1975, Lesterhuis *et al*. 2008). The only true lake in Paraguay is Laguna Blanca that constitutes a unique ecosystem in Paraguay, being a lake of clear water positioned on white sands, and surrounded by Cerrado (Smith *et al*. 2011, 2012). This place is the first "Important Amphibian and Reptile Area" for conservation in Paraguay supporting an astonish number of species (Smith *et al*. 2016). Finally, another kind of hydrological system in Paraguay, that remains unexplored from a herpetological point of view are the dolines in the north of the Oriental Region (Filippi & Molinas 2014).

1.2.3. Climate

The climatic conditions vary in a northwestern – southeastern gradient, being more humid (Fig. 12) and cooler (Fig. 13) in the southeast. The mean annual temperature in the whole country is about 23º C, being 24.5º C in the western region and 22.5º C in the eastern region. There is a big difference with respect to the variation in temperature, given that the mean maximum is 25º C, but the absolute maximum temperature could reach around 50º C, especially in the northwestern portion of the country in January or February. The coldest month is July, and the absolute minimum temperature could be -6º C in the south. Nevertheless, in Paraguay the "true" winter usually does not last longer than 16 days each year. Thus Paraguay has a warm/hot climate during most parts of the year.

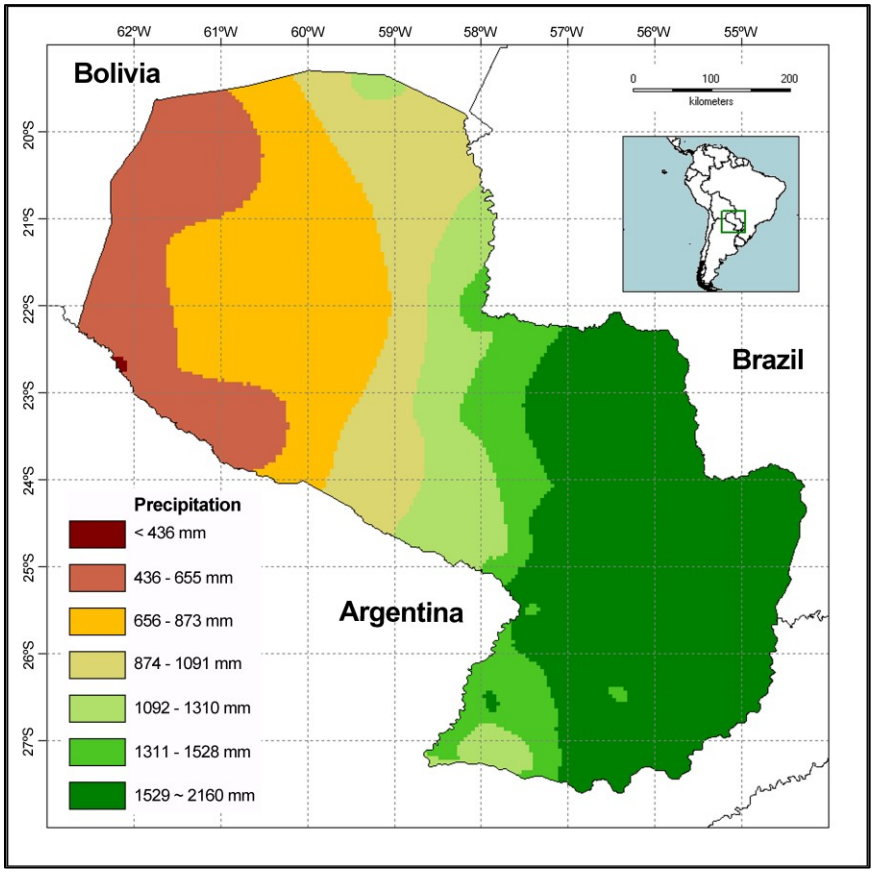

Figure 12. Annual average precipitation. Note how the precipitation increase from north-weast to south-east.

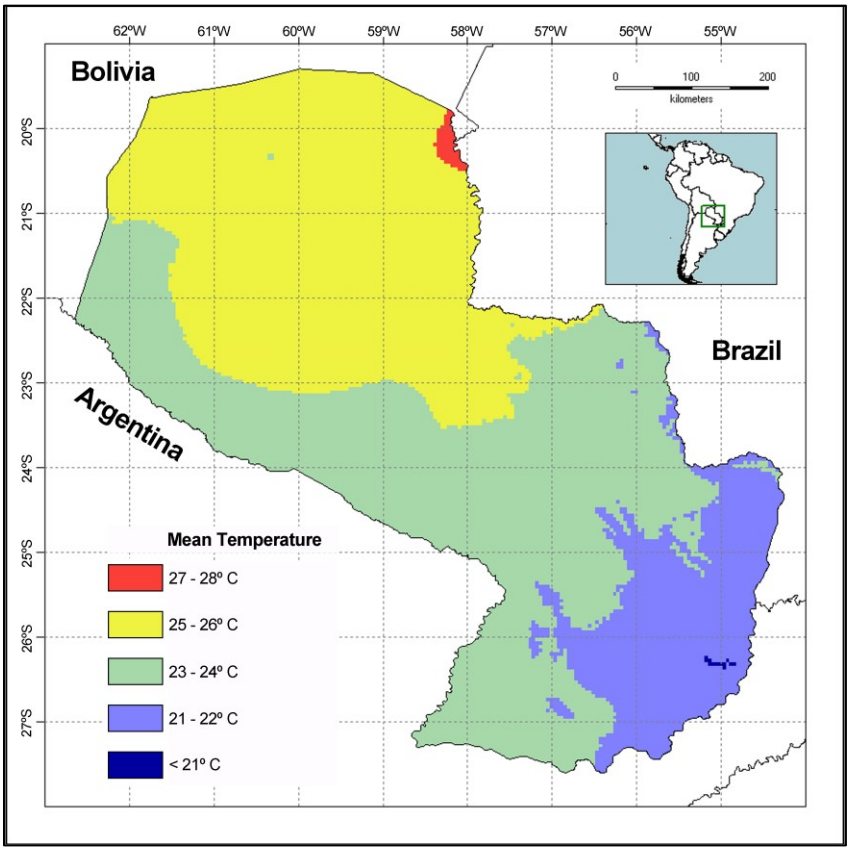

Figure 13. Annual mean temperature. The highest temperatures are recorded in the northernmost part of the country.

According to the Köppen-Geiger classification system, Paraguay has mostly a "Aw" weather in the Occidental Region and upper region of Oriental Region, being "BSh" and "Cwa" in the westernmost area; and a narrow "Am" divides the "Aw" from a big "Cfa" area that covers a large portion of Occidental and Oriental regions at the south (Kottek *et al*. 2006) (Fig. 14).

With respect to the rains, the driest area of Paraguay is in the westernmost side of the country, increasing gradually to the east, the Oriental Region being more humid with precipitation rates above 2000 mm per year (Fig. 12). There are years with precipitation rates out of the normal range, due to El Niño Southern Oscillation (ENSO), when serious flooding can affect several cities in the country (WMO 2014). The three worst flooding periods, due to ENSO, were 1982-1983, 1997-1998, and 2015-2016 (DMH 2016). On the other hand, La Niña Southern Oscillation events also affect the country, causing severe droughts recorded in 1967, 1968, 1978, 1979, 1999, 2000, and 2008 (Báez Benitez & Monte Domeq 2014).

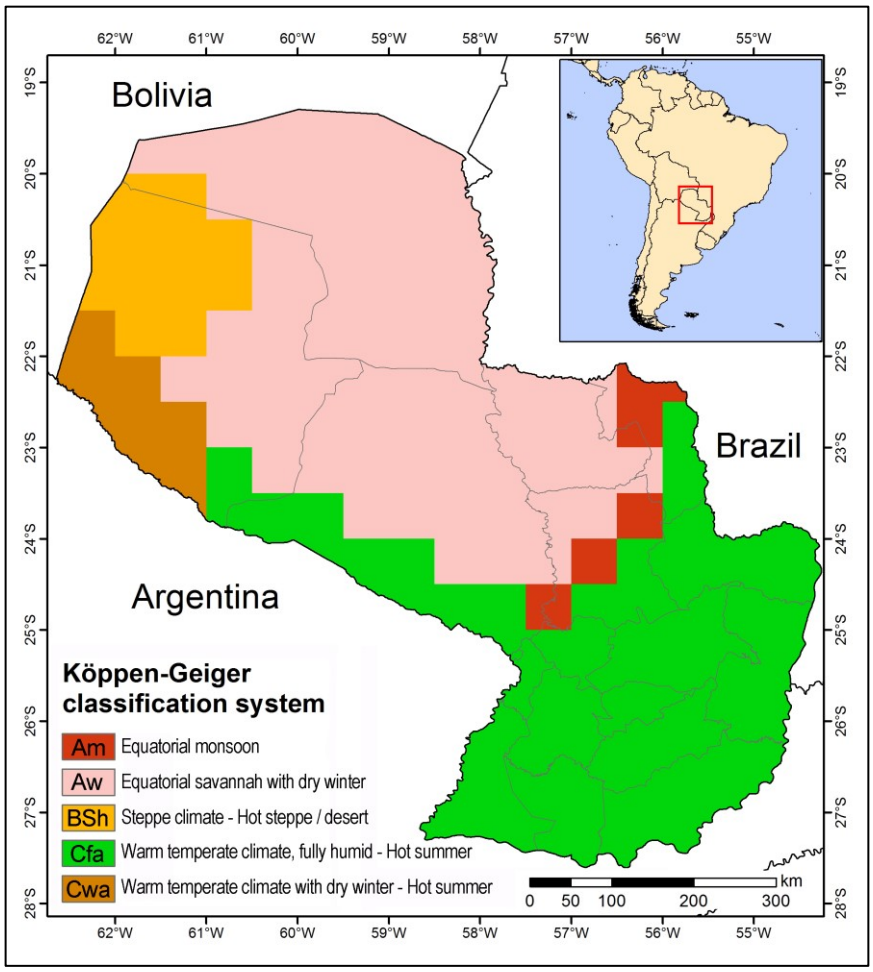

Figure 14. Climate classification of Köppen-Geiger for Paraguay. Based on Kottek et al. (2006), and downloaded from Institute for Veterinary Public Health (http://koeppen-geiger.vu-wien.ac.at/).

1.3. Geological history and biogeography of Paraguay

The tectonic history of Paraguay is not very clear given the lack of fossil records. Nevertheless, placing the country in the regional context, it is believed that a big sea (Mar de Itapucumí) covered most of the Chaco and a boreal portion of the Oriental Region (Fig. 15) around Late Precambrian and Lower Cambrian during existence of Pannotia and later Pangea (Fúlfaro 1996). Currently, even with a relatively small size, and temperate and subtropical climes, the Paraguayan biodiversity is quite high, mainly because is the convergence point of several ecological regions with different evolutionary histories (Spichiger *et al*. 1995), as a result of its geologic history.

The first attempts to differentiate biogeographical zones in South America were made based on animal distributions (Sclater 1858, Sclater & Sclater 1899, Shannon 1927, Mello-

Leitaõ 1938, 1939, 1943, Cabrera & Yepes 1960), and later Cabrera & Willink (1973) made an integrative (botanical and zoological) analysis of zoning, being one of the most important contributions for a long time. In this contribution these authors recognized three biogeographic regions for Paraguay: Provincia Chaqueña, Provincia Paranaense, and Provincia del Cerrado. Already in the 21st Century, the biogeographic regions of South America were reassessed with techniques of historical biogeography (Morrone 2001). The results are rather congruent in the zonification adding only the presence of Pantanal, but are different respect to the origin of the biotas as it will be explained.

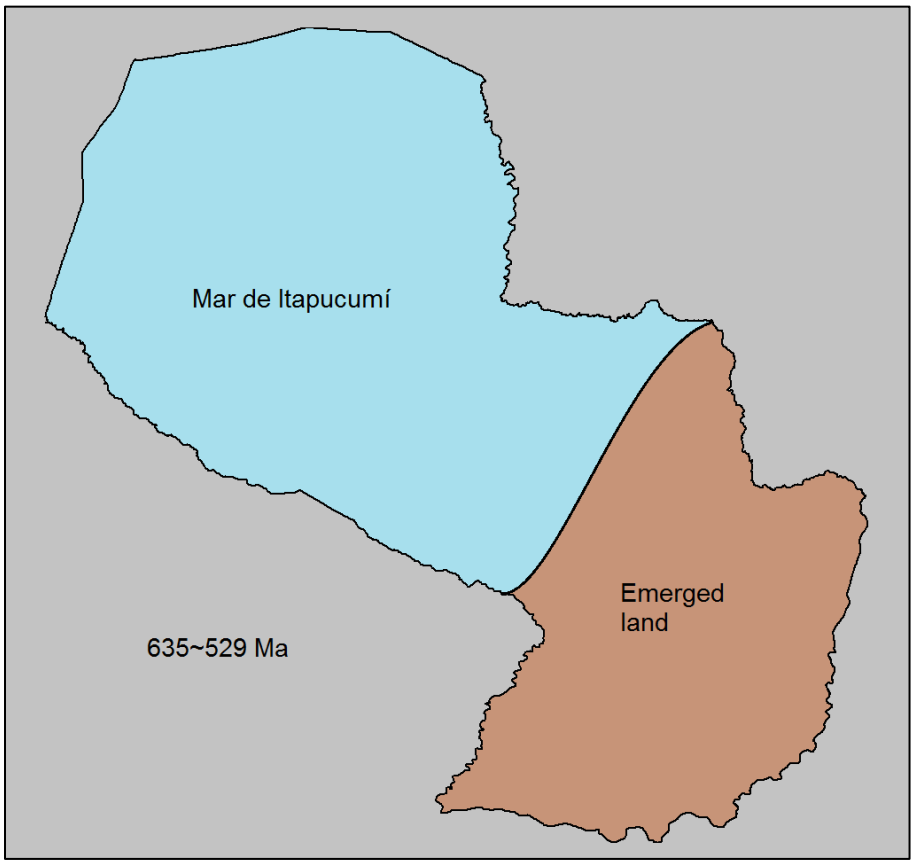

Figure 15. Approximated extention (on Paraguayan territory only) of the "Mar de Itapucumí" in the Late Precambrian and Lower Cambrian according to Fúlfaro (1996).

According to the relations of the ecoregions present in Paraguay, the Pantanal could have double origin being related to biogeographic provinces of Madeira (Central Amazonia), and Pará and Tapajós-Xindú (eastern Amazonia) (Silva & Oren 1996). In Paraguay, only the southernmost portion of Pantanal reaches the north-east of the country. For the

Chaco, Müller (1973) proposed a dual origin Cerrado-Caatinga, with a stronger relation to this last; whereas other authors proposed different origins: Pampas (Cabrera 1976), Yungas (Ayarde 1995), and Monte (Morrone 1993). This may suggest that this is a complex ecoregion formed by elements of different origins. In fact, Prado (1993a, b) recognized a gradual transition in the Chaco biota, considering the "true" Chaco or Dry Chaco as Chaco *sensu stricto* and Humid Chaco as a confluence different flora. This last hypothesis was followed by Dinerstein *et al*. (1995) in a complete assessment of biogeographic regions of Latin America, who differentiated Dry Chaco and Humid Chaco.

With respect to the Cerrado, different relationships were proposed. Cabrera & Willink (1973) linked this ecoregion to Amazonia. Nevertheless, Müller (1973) and Prado & Gibbs (1993) proposed a closer relationship with Chaco ecoregion. This last proposition is based on the common origin of the "Dry diagonal" ecosystems where Caatinga-Cerrado-Chaco form a diagonal of dry environments from the northeast to the southwest (Prado & Gibbs 1993). However, Aguiar & Melo (2007) stated that "Cerrado" is rather a composite area or "biotic crossroad" given that for some elements (mainly Hymenopterans) this ecoregion is in the middle of distribution ranges that fit with the Dry diagonal, but in other cases it is in the middle of ranges that would refer to a "Humid diagonal" formed by Amazonia-Cerrado-Atlantic Forest.

Finally, Cabrera & Willink (1973) suggest a close relation of the Atlantic Forest with Amazonia, but on the other hand Morrone (2001) stated that the former has a Parana origin, totally different from Amazonia elements. But as it was shown, a biogeographic region can display proper chorologic relations for some taxa, but different for others. In spite of this, there is good evidence of a preterit link between the Amazonas and the Atlantic Forest (Nores 1992, Costa 2003) indicating a common evolutionary history, although it is not clear when exactly both ecoregions were divided due to climatic changes that resulted in a breaking of this Neotropical forest bulk.

There is another ecoregion, endemic to Paraguay, recognized by Keel *et al*. (1993), located in the middle of the country, east of the Paraguay River and that represents a transition or intergradation area between Humid Chaco and Alto Paraná Atlantic Forest. This area, according to Oakley & Prado (2011) is dominated by forest species typical for the

Seasonal Forests of the "Pleistocene Arch" described by Prado & Gibbs (1993) and Prado (2000).

In the southern portion of Paraguay, where the Humid Chaco meets the Atlantic Forest (or Alto Paraná Atlantic Forest more precisely), it was identified (although not officially described for Paraguay) as a different ecosystem composed by grasslands with higher moisture and adapted to lower temperatures than the Cerrado grasslands, named Southern Cone Mesopotamian Grasslands, by some authors (Del Castillo & Clay 2005, Cacciali 2010, Cabral & Weiler 2014, Cabral & Bueno-Villafañe 2015).

In an attempt to characterize the reptile fauna of Paraguay, Cacciali & Ubilla (2016) assessed distribution patterns using Parsimony Analysis of Endemisms, but given the scarcity of data records and an evident bias due to collection in urban areas the results were not conclusive. Nevertheless, there are some amphibians and reptiles endemic or exclusive to some biogeographic areas in Paraguay as shown by Brusquetti & Lavilla (2006) and Cacciali *et al*. (2016a) and here summarized in Table 1.

More recently Mereles *et al*. (2013) published a detailed ecoregional analysis of the Occidental Region of Paraguay. The main conclusions are the extension of the Pantanal that was reduced in previous assessments. Also, these authors highlighted the presence of the Cerrado in the northernmost part of Paraguay, in the Chaco, and finally, extremely dry areas of sand dunes at the westernmost point of the country, named "Médanos". These areas were not included by Brusquetti & Lavilla (2006) or by Cacciali *et al*. (2016a) in their examination of ecoregional affinities of the Paraguayan herpetofauna.

1.4. Anthropogenic changes in Paraguay

Habitat loss is the main problem in Paraguay, and given that the taller forests are in the Oriental Region, the demography and the economic development were far more significant in this region since the colonization of the country. But the higher rates of deforestation started in the early 1970s in the Alto Paraná Atlantic Forest, when 73.4% of the original cover still remained and since this time the original cover dropped to 40.7% by 1989, and only to 24.9% by 2000 (Di Bitetti *et al*. 2003, Cartes 2003). Currently less than 13.4% of original vegetation remains in an extremely fragmented landscape with two protected areas (i.e., San Rafael Reserve and Mbaracayú Natural Reserve) containing the two biggest patches of original Atlantic Forest in Paraguay (Huang *et al*. 2007, Cacciali *et al*. 2015).

But unfortunately, even these protected areas are not safe from deforestation (Huang *et al*. 2007, Cartes 2013, Cacciali *et al*. 2015). As it is shown in Table 1, Alto Paraná Atlantic Forest has a high diversity of endemic species, and this not only at a national but also at regional level (Cardoso da Silva *et al*. 2004, Mittermeier *et al*. 2004), and this, added to the high rate of deforestation suffered by this ecoregion, make it a global hotspot for conservation (Myers *et al*. 2000). A similar case occurred with the Cerrado, which was severely modified for anthropogenic use (cattle farms or soybean crops) leading to an extreme fragmentation and population isolation of at least some reptile's species (Smith *et al*. 2014). Given that this ecosystem holds also a significant number of endemic species, not only in Paraguay but in the region (Cardoso da Silva 1997, Ratter *et al*. 1997, de Mello *et al*. 2015) and added to the high deforestation rates in the area, it is also considered a global hotspot for conservation (Myers *et al*. 2000).

Nevertheless, Paraguay is a focus of deforestation in South America (Hansen *et al*. 2013), and the alarming rates of habitat loss have begun to threat the Chaco. The more xeric conditions of the Chaco (especially in the Dry Chaco) resulted a lower productivity scenario, although in the last five years the production has increased substantially (Yanosky 2013, Caballero *et al*. 2014).

The less altered ecoregions in Paraguay are Médanos and the Cerrado Chaqueño. These areas are far from towns and the access is very difficult, being isolated places inhabited only by a few families (sometimes native tribes) who survive thanks to small-scale local hunting and a few cattle.

1.5. History of research on the Paraguayan herpetofauna

To understand the herpetological history in Paraguay, it is worth to know a bit about Paraguayan history, given that the shape of the country was modified since the dawn of natural history's Paraguayan surveys by naturalists of the 18th Century.

The first anecdotic narration of the Paraguayan (and regional) fauna was probably written by Schmidl (1599), who in addition to references to large mammals, he made a brief description of yellow and black big snakes (undoubtedly Anacondas), providing some sensational explanations of attacks by these snakes to men or other big vertebrates. All these observations were made based on a travel between 1534 and 1554 when Paraguay still had no borders, being part of the "Gobernación del Río de la Plata y del Paraguay".

Table 1. Summary of the herpetofauna endemic to the ecoregions present in Paraguay, based on Brusquetti & Lavilla (2006) for amphibians (with modifications based on Brusquetti et al. 2007 and Brusquetti & Lavilla 2008) and Cacciali et al. (2016a) for reptiles. Note that Brusquetti & Lavilla (2006) did not include Central Forest and Mesopotamian Grasslands in the analysis.

	Dry Chaco	Humid Chaco	Pantanal	Cerrado	Central Forest	Southern Grasslands	Atlantic Forest
AMPHINIA	*Pseudis occidentalis* *Lepidobatrachus llanensis*	*Chthonerpeton indistinctum* *Scinax smilis* *Lepidobatrachus asper* *Physalaemus riograndensis* *Rhinella fernandezae* *Melanophryniscus atroluteus* *Melanophryniscus krauczuki*	*Dendropsophus melanargyrea*	*Dendropsophus elianae* *Dendropsophus jimi* *Rhinella scitula*			*Luetkenotyphlus brasiliensis* *Siphonops paulensis* *Hypsiboas curupi* *Itapotihyla langsdorffii* *Phyllomedusa tetraploidea* *Limnomedusa macroglossa* *Proceratophrys avelinoi* *Physalaemus fuscomaculatus* *Rhinella ornata* *Crossodactylus schmidti*
REPTILIA	*Liolaemus chacoensis* *Lygodactylus wetzeli* *Sibynomorphus lavillai* *Phalotris normanscotti* *Phimophis vittatus* *Philodryas baroni* *Erythrolamprus albertguentheri* *Erythrolamprus sagittifer* *Rena unguirostris*	*Phrynops hilarii* *Ophiodes luciae* *Micrurus baliocoryphus* *Tantilla melanocephala* *Psomophis obtusus* *Epictia albipuncta*	*Iguana iguana*	*Paleosuchus palpebrosus* *Bachia bresslaui* *Amphisbaena leeseri* *Amphisbaena roberti* *Amphisbaena steindachneri* *Bothrops pauloensis* *Chironius flavolineatus* *Drymoluber brazili* *Philodryas nattereri* *Erythrolamprus typhlus* *Lygophis paucidens*	*Tropidurus guarani* *Homonota rupicola* *Dipsas cisticeps* *Xenopholis undulatus*	*Liolaemus azarai* *Amphisbaena prunicolor* *Micrurus silviae* *Atractus thalesdelemai* *Mussurana quimi*	*Phrynops williamsi* *Bothrops jararacussu* *Micrurus corallines* *Chironius bicarinatus* *Chironius exoletus* *Atractus reticulatus* *Sibynomorphus mikani* *Apostolepis assimilis* *Clelia plumbea* *Oxyrhopus petolarius* *Liotyphlops beui*

Later, the Jesuit missionary José Sánchez Labrador published a series of manuscripts called "El Paraguay Natural" (unpublished documents dated between 1771 and 1776 in Rávena, Italy) based on his mission in Paraguay and neighbor countries, between 1734 and 1767, when Paraguay was a Governorate of the "Virreinato del Río de la Plata" (Fig. 16). In that work (based on four books, some of them not edited) Sánchez Labrador provides descriptions of all aspects of natural history, including geology, biology and weather (Ottone 2008). Unfortunately, the book of insects and reptiles was not edited (Aguilar 2008, Ottone 2008), but some graphic plates were published by Aguilar (2008).

Figure 16. Political division of South America during the 18th Century. The current territory of Paraguay belonged to the Viceroyalty of Rio de la Plata.

Some years later (1781) the Spanish military engineer Félix Francisco José Pedro de Azara y Perera, commonly known as Félix de Azara, arrived to Paraguay. This marine soldier, impressed by the rich natural diversity that he found, started to describe the vertebrate fauna of Paraguay. Most of his studies were dedicated to the description of birds, most of which he bought from native Indians. The descriptions of amphibians and reptiles were not very academic and sometimes he applied names such as "Lagartija Fea" (ugly lizard). His work was published in French (Azara 1801), Spanish (Azara 1802), and English (Azara 1838).

In the 19th Century, a series of Europeans and North American herpetologists, who provided descriptions of Paraguayan herpetofauna without visiting the country, reporting their findings based on material collected during expeditions of others. In the beginnings of that century, the French zoologist François Daudin provided the first descriptions of reptiles from Paraguay (*Caiman yacare* and *Teius teyou*), following the binomial Linnean classification system (Daudin 1802a,b). Before the next publication related to the Paraguayan herpetofauna, Paraguay became independent from Spain, and the country had boundaries beyond the present country's shape (Fig. 17). The next herpetological contribution for Paraguay was the extensive work of Duméril *et al*. (1854) who described *Bothrops alternatus* using a specimen from Paraguay.

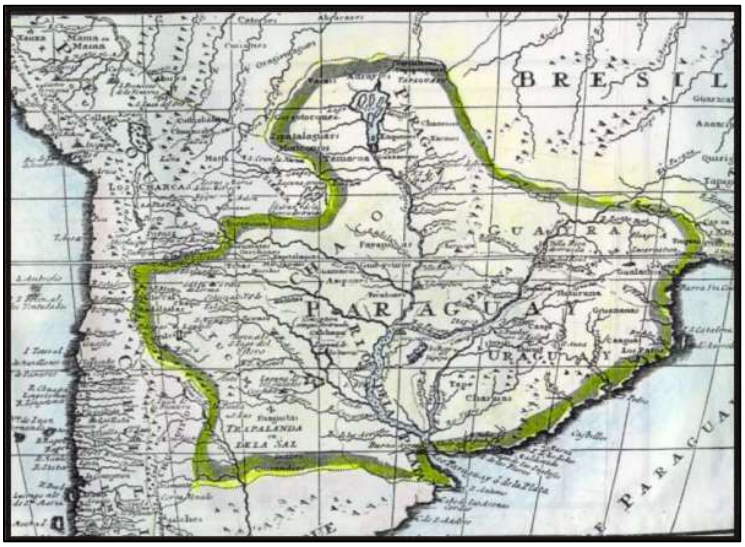

Figure 17. Limits of Paraguay after the independence of Spain, in the 19th Century.

The referred publications using the binomial Linnean classification system were widely dispersed, and Edward Drinker Cope was the first to provide a list of amphibians and reptiles from Paraguay. His most remarkable work for the country was the "Catalogue of the reptiles obtained during the exploration of the Parana, Paraguay, Vermejo and Uruguay rivers, by Capt. Thos. J. Page, U. S. N.; and of those procured by Lieut. N. Michler, U. S. Top. Eng., commander of the expedition conducting the survey of the Atrato River" (Cope 1862) that, as the title indicates, was upon a collection made by a military excursion to some rivers in the central region of South America.

Probably the darkest time in the history of Paraguay was between 1864 and 1870, when Argentina, Brazil, and Uruguay formed an alliance for a war against Paraguay, devastating the country. After that military conflict, Paraguay lost people (only 280,000 inhabitants remained from 900,000) and territory (Fig. 18). Given that the publications mentioned above had no specific localities, it is not possible to know certainly if the specimens came from areas that are now still within the political limits of Paraguay.

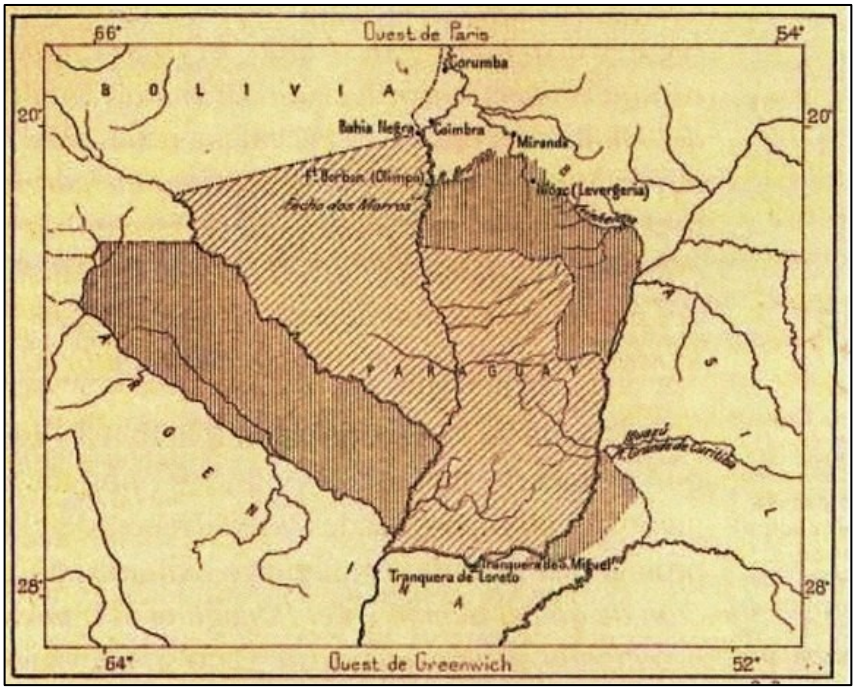

Figure 18. Map of the mid 19th Century, showing the Paraguayan territory lost during the belic conflict with Argentina, Brazil, and Uruguay (dark gray).

The second list of amphibians and reptiles from Paraguay was published by Boettger (1885), upon an animals' collection acquired by the Linnean Institute of Natural History in Berlin from Mr. H. Rohde. In this list he included a total of 63 species (19 amphibians and 14 reptiles) with the original description of five amphibians and 10 reptiles, most of them later synonymized.

Almost ten years later, Boulenger (1894) provided a new list of 20 amphibians and 41 reptiles, describing one amphibian and six reptiles. Nevertheless, a major problem with this collection is that the list is based on specimens from Asunción (Paraguay's capital), but some species are unlikely to be found in Asunción. Thus, probably Asunción was the harbor there the collection left the country, but the actual collecting data of the specimens is unknown. One year later, Peracca (1895) presented another list of the Paraguayan herpetofauna (mainly from the Occidental Region) and northern Argentina. There Peracca (1895) informed about 21 amphibians and 48 reptiles.

Entering the 20th Century, works and publications became more abundant, and collection data were more accurate. The first contribution of that Century was by Schenkel (1901) who provided some data on the herpetofauna from Paraguay, describing some new snakes. Similarly, Peracca (1904) referred to a list of specimens collected in Brazil and Paraguay. That same year, Méhelÿ (1904) published a remarkable work on the Paraguayan batrachofauna, describing some new anurans for Paraguay. One year later, appeared the first publication made by a Paraguayan resident: Arnoldo da Winkelried Bertoni, and for the first time in the herpetological history of Paraguay, data were not only related to taxonomy, but also profusely about natural history. Winkelried Bertoni was son of a family of Swiss immigrants who settled in Alto Paraná Department where almost the whole family was advocated to science. Among his very profuse bibliography, Bertoni published his first document in 1905, about observations of natural history of the Teju lizard *Salvator merianae* (Bertoni 1905); and was the first to publish a catalogue of the vertebrates of Paraguay (Bertoni 1914).

A sporadic and poor contribution was made by Bacqué (1906) who attempted to describe new species of snakes, but with an evident low knowledge about the topic. Years later, Pedro Serié from Argentina published a couple of papers, one about snakes from Paraguay (Serié 1915), very well grounded and referring to voucher specimens too; and anoth-

er about Bacqué's work (Serié 1916), where he discredited the work of Bacqué, saying that the alleged new species were not only already known, but also stating that they were not even vipers, being very common colubrid snakes in Paraguay.

Another herpetologist resident of Paraguay was Guillermo Schouten, who published a contribution to the knowledge on diet and reproduction of Paraguayan lizards (Schouten 1929). The beginnings of the 30's decade were quite prolific. First Parker (1931) authored a small paper based on a zoological expedition where he recorded two amphibians and two reptiles; Schouten (1931) published an extensive list of reptiles from Paraguay, providing data about the biology of some species; and finally Bertoni (1931) recorded for the first time *Helminthophis wilderi* in Paraguay, which probably is actually *Liotyphlops beui*. The next year, Schouten (1937) published a small contribution recording the first caecilian for Paraguay. And then came a gap in the scientific production in Paraguay, probably in a big part due to the last war in which Paraguay was involved, from 1932 to 1935 against Bolivia. In this war both countries were fighting for the domain of Chaco (in fact, the war was called *Guerra del Chaco*), and here, Paraguay gained territory from Bolivia (Fig. 19). The next and last scientific paper of that decade was made by Bertoni (1939) where he provided an update of the catalogue of Paraguayan vertebrates.

Figure 19. Map of Paraguay before the military conflict with Bolivia (light red) and the territory gained from Bolivia (orange area) after the war.

From the 40s to the 70s the herpetological activity decreased considerably, with some brief compilations on the snake fauna provided by Canesse (1966, 1970). Later Scott & Lovett (1975) presented a summary of the amphibians and reptiles collected during an expedition to the Paraguayan Chaco, and at the end of that decade Talbot (1979) published a list (based on collections and literature) of Paraguayan.

In the 1980s a big step was made in biological research in Paraguay: the Museo Nacional de Historia Natural del Paraguay was built in 1983. Since then many herpetologists (especially Aida Luz Aquino) published works which helped to increase the knowledge of the Paraguayan herpetofauna. Since then, the increasingly number of scientific papers on Paraguayan reptiles continued to grow, and Cacciali *et al*. (2016a) presented a graphic showing the constant increase in the documented diversity of Paraguayan reptiles which currently is of 182 recognized by Cacciali *et al*. (2016a) plus three species of *Tropidurus* described by Carvalho (2016) and *Epictia vellardi* recorded by Cabral & Netto (2016) summing up to 186 species.

1.6. Aims of this Study

The main objective of this work is to solve taxonomic problems, identified through genetic barcoding analyses, in the central region of South America. Thereby I am taking selecting taxa of the Paraguayan Squamata as models to achieve this objective taking into consideration the crucial geographic position of the country, plus the scarce available genetic data of Paraguayan reptiles.

2. MATERIALS AND METHODS

2.1. Environmental characteristics of the study sites

Following I present a description of the environmental traits of the ecoregions where the field work was carried out. We were able to collect in nine of the eleven ecoregions of Paraguay (Fig. 20). More information regarding the collecting process is provided in the next section (2.2. Collecting, preservation and legal issues). The descriptions provided are based on Keel *et al*. (1993), Cacciali (2007), Gauto *et al*. (2011), and Mereles *et al*. (2013).

Figure 20. Map of ecoregions from Paraguay, showing sampled sites (brown dots) for this project.

2.1.1. Dry Chaco

The Dry Chaco covers a wide extension in the Occidental Region of Paraguay, west of the Paraguay River (Fig. 21). This is a xeromorphic forest with ephemeral water courses formed during the rainy season. Precipitation range varies from 500 to 900 mm/yr. Maximum absolute temperature is around 48 ºC, and the minimum can reach -5 ºC during dry winter. The vegetation is mainly composed low trees, and thorny vegetation in all the strata. In some parts, the herbaceous stratum is almost absent. The Dry Chaco presents wide parts of soil without vegetation. The soils are hard, mainly of clay origin, what makes the drainage poor. The topography is flat with some scattered hills. There are some lagoons in the center of the Dry Chaco with salty water.

Figure 21. Representative environments of the Dry Chaco in Paraguay.

The tree flora is dominated by *Ceiba insignis*, *Schinopsis quebracho-colorado*, *Aspidosperma quebracho-blanco*, and *Bulnesia sarmientoi*. In lower strata are frequent some species of *Prosopis* and several Cactaceae. In the herbaceous-shrub stratum there is a distinct diversity of bromeliads and cacti. The most emblematic animals in this ecoregion are the living fossil *Catagonus wagneri*, and some smaller species such as *Tolypeutes matacus*, *Nothoprocta cinerascens*, *Eudromia formosa*, *Ortalis canicollis*, and *Chunga burmeisteri*. In Table 1 I present a list of herpetofauna characteristic of this ecoregion.

2.1.2. Humid Chaco

The Humid Chaco is considered an extension of the Pantanal by Mereles *et al*. (2013), and it is placed in the flooding areas of the Pilcomayo and Paraguay rivers (Fig. 22). Given that, the vegetation is adapted to seasonal flooding. This area receives relative high amounts of rain from 1000 to 1200 mm/yr. The temperature ranges from about 40 ºC to 5 ºC. An important vegetation trait here is the presence of floating meadows in the river, being a key transport mechanism for many elements of flora and fauna along the main canal of the river. The Humid Chaco has clayish soils.

Figure 22. Representative environments of the Humid Chaco in Paraguay.

The vegetation is a mosaic of forests-savannas and palm forests-wetlands. In this ecoregion it is possible to find savannas with the palm tree *Copernicia alba* along with a rich herbaceous stratum. The aquatic vegetation is characterized by *Eichhornia azurea*, *Thalia geniculata*, *Canna glauca*, *Alternanthera philoxeroides*, and *Eleocharis montana*. This complex environment holds also some big wetlands often used by many wader birds. Also it is habitat of big mammals like *Hydrochaeris hydrochaeris*, *Lontra longicaudis*, *Chrysocyon brachyurus*, and *Tapirus terrestris*. As evidenced in Table 1 there are several amphibians associated exclusively with the Humid Chaco. One species in particular, the recently described *Ophiodes luciae* is known only from this ecoregion.

2.1.3. Pantanal

The limits of this ecoregion are still under discussion. According to the vegetation, this ecoregion occupies a wide area in the northernmost portion of the Paraguay River, extending from the river to central Chaco where the vegetation is xeromorphic (Fig. 23). Basically, the physiognomy of the vegetation, closely related to the river, is an intergradation of flora from Dry Chaco and Humid Chaco, implanted on sandy soils adapted to seasonal flooding. The precipitations here are between 1300 to 1400 mm/yr, and the temperature fluctuates between 38 ºC and 5 ºC.

Similar to the Humid Chaco, the savannas of the palm tree *Copernicia alba* are abundant in the Pantanal with scarce presence of wooded flora. This is one of the only places in Paraguay where it is possible to find *Blastocerus dichotomus* and *Pteronura brasiliensis*. Some bird species, typical from this ecoregion are *Nyctiprogne leucopyga*, *Furnarius leucopus*, *Synallaxis hypospodia*, and *Ramphocelus carbo*. With respect to reptiles, the distribution of *Iguana iguana* and *Dracaena paraguayensis* are strongly linked to this ecoregion. The fact that *D. paraguayensis* was not contemplated as a typical Pantanal species by Cacciali *et al*. (2016a) because these authors considered the southernmost record of this species to be in the Humid Chaco. Nevertheless, it is in a transition zone; and according to the interpretation of Mereles *et al*. (2013) it would be completely Pantanal.

Figure 23. Representative environments of the Pantanal in Paraguay.

2.1.4. Cerrado (Oriental Region)

The Cerrado in the Oriental Region is better known since it is an introgression of the actual Brazilian Cerrado (Fig. 24). Mostly, this ecoregion is composed of dense grasslands with islands of stunted wooded vegetation, stood on reddish sandy soils. Nevertheless, there are some Cerrado spots with different characteristics, as it can be seen at Laguna Blanca (San Pedro Department, Paraguay) where the sand is light gray with wide portions of bare ground, and vegetation dominated by small shrubs in the lower stratum. In this type of Cerrado the islands of stunted forests are also present. Even when the topography there is mainly flat, in the northeastern portion of the Cerrado there are important hill chains. Pre-

cipitation varies from 1500 to 1700 mm/yr. Maximum absolute temperature is around 50 ºC, and the minimum can be around 5 ºC.

Figure 24. Representative environments of the Cerrado in Paraguay.

The Cerrado is rather diverse in fauna of large-size mammals like *Chrysocyon brachyurus*, *Myrmecophaga trydactyla*, *Ozotoceros bezoarticus*, and threatened birds like *Eleothreptus candicans*, *Anodorhynchus hyacinthinus*, and *Ara ararauna*. As it is shown in Table 1, there are many species endemic to the Cerrado, making it an important ecoregion for the diversity of Gymnophthalmidae, Teiidae, and Dipsadinae.

2.1.5. Central Paraguayan Forest

This is a narrow strip of transitional vegetation between Humid Chaco and Alto Paraná Atlantic Forest (Fig. 25). The soil is red with high concentrations of iron, and at the southernmost part of this region there are several hills of siltstone. The vegetation is varied with mostly tall forested areas, and more stunted forests on the hills. On the top of the hills, where the layer of soil can be reduced, the vegetation does not exceed 3 m, with a predominance of bromeliads and cacti. Precipitation varies from 1400 to 1600 mm/yr. The maximum absolute temperature is around 43 ºC, whereas the minimum is around 3 ºC.

Figure 25. Representative environments of the Central Paraguayan Forest.

The fauna in this area does not show special features, and given the high anthropogenic alterations in this ecoregion, or its reduced size today, there are no big vertebrates endemic to it. Nevertheless, there are two species of lizards that are endemic to the southern rocky hills formations: *Homonota rupicola* and *Tropidurus guarani*.

2.1.6. Alto Paraná Atlantic Forest

This ecoregion is located at the easternmost part of the country (Fig. 26), and has the tallest trees of the country. This is a moist semi-evergreen forest that receives the highest precipitations of the country (1700-2000 mm/yr). The temperature varies from a maximum of 38 ºC to a minimum of around 0 ºC. This ecoregion is implanted on an undulated land with many hills and valleys, forming important chains of hills like Ybyturuzú, San Rafael, and Mbaracayú. Similar to Central Paraguay Forest, the soils are red with high levels of iron, but the layer organic matter in the forest is deeper and rich in nutrients.

Figure 26. Representative environments of the Alto Paraná Atlantic Forest in Paraguay.

The tallest stratum of the forest is composed of different species of the genera *Aspidosperma*, *Tabebuia*, *Astronium*, and *Peltophorum*. The medium stratum has species like *Ocotea* spp., *Myrcianthes pungens*, *Myrciaria rivularis*, and *Guarea kunthiana*. There are

some other species associated to riverine habitats: *Copaifera langsdorfii*, *Luehea divaricata*, *Inga uruguensis*, *Myrciaria rivularis*, and *Bambusa guadua*. The most representative species of the fauna of the Alto Paraná Atlantic Forest are *Procnias nudicollis* (Paraguay's national bird), *Panthera onca*, *Tapirus terrestris*, and in some places it is possible to finde the rare *Speothos venaticus*. There are several endemic herpetofaunal species (Table 1), and it is the ecoregion with the highest diversity of endemic amphibians.

2.1.7. Southern Cone Mesopotamian Grasslands

It is actually not clear if this is a natural environment or the result of anthropogenic modification. One hypothesis is that it was previously Atlantic Forest that was cleared (before European occupation) for production of yerba mate (*Ilex paraguariensis*), and another hypothesis states that it is an original environment and that most of the territory of Uruguay (currently almost completely modified) originally must have been similar to this ecoregion. Regardless of the origin, this habitat is a savanna (with some flood areas), strongly associated to the Alto Paraná Atlantic Forest with which it forms irregular mosaics in some areas (Fig. 27). Precipitation varies from 1500 to 1700 mm/yr. Maximum absolute temperature is around 38 ºC, and the minimum can be below -5 ºC.

The Southern Cone Mesopotamian Grasslands is habitat for many medium to small size mammals and birds, although none endemic. With respect to the herpetofauna, typical of this ecoregion are *Liolaemus azarai*, *Atractus thalesdelemai*, and *Micrurus silviae*.

Figure 27. Representative environments of the Southern Cone Mesopotamian Grasslands.

2.2. Collecting, preservation and legal issues

2.2.1. Sampling sites

Even when there are some blanks in the areas sampled, the coverage of collecting sites in this study is rather vast (Fig. 20). Some areas (especially in the northernmost part of the country) still remain little sampled, and no collecting was done there for this project, given that explorations in those areas should involve advanced logistics and money. Most of the Departments of Paraguay were sampled, and from a biogeographic perspective, all the ecoregions of the Oriental Region of Paraguay were covered. Nevertheless, there are two ecoregions in the Chaco, from where I have no samples. These are the Cerrado Chaqueño and Médanos. These are regions located far from any city, and require expensive logistics as mentioned before.

The collecting activities were carried out mostly in rural areas (cattle farms) searching for animals in degraded habitats and in remaining forests. As mentioned before, only a few areas of Paraguay nowadays still contain "original" habitat, but I was able to sample some tall forests especially in the eastern part of Paraguay.

2.2.2. Sampling methods

The collecting effort was shared with colleagues and friends. The first collecting activities for this project were made by GK with the collaboration of colleagues and students. Then I started to collect with the help of friends and colleagues. Thus, in every collecting spot it was never only one person working.

The methods used in the field were the traditional techniques for herpetology: active searching at different times of the day and night examining potential shelters (e.g.: barks, logs, caves, leaf litter, etc.) (Cacciali 2013). Runner lizards (e.g.: *Ameiva* and *Teius*) were collected using compressed air rifles (Scrocchi & Kretzschmar 1996). Additionally, some habitats such as ant nests and swamps were dug looking for hypogeal organisms (Simmons 2002), and floating vegetation was sampled using a trawl net (Fig. 28).

Special attention deserves Laguna Blanca. This area is located in the northeastern corner of the San Pedro Department, and as it was said before is the first "Important Amphibian and Reptile Area" for conservation in Paraguay (Smith *et al*. 2016). That was a private reserve for a span of 10 years and the reserve status perished in 2015. Currently the place

was sold. In that area there was an organization called Para La Tierra (PLT), which had many active volunteers eager to help. GK and I were there, and we received the help of many volunteers. Additionally, the people from PLT had pitfall, and they also gave us some specimens captured in these traps. Additionally, they gave us tissue samples from their cryo collection, which helped to enlarge the sample size.

Figure 28. Account of different sampling methods employed during field surveys.

A total of 147 days of field work were accounted for this project, and about 1246 specimens collected. The greatest part of the specimens were amphibians (including their larvae). Among reptiles, only one tissue sample of a Crocodilian (*Caiman yacare*) was collected and the animal released, one turtle (*Kinosternon scorpioides*), and several Squamata that were used in this analysis.

2.2.3. Permits and ethics

Reptiles that were captured alive were euthanized with a pericardial injection of a solution of embutramide, mebezonium iodide, and tetracaine hydrochloride (T-61®) (ester) or Sodium Thiopental (Tiopental Sódico®) (barbiturate).

The Secretaría del Ambiente from Paraguay authorized the collecting of specimens though permits SEAM Nº 04/11 and 009/2014). Exportation permits for tissues and specimens were also issued by the same authority through the permits SEAM No 02/14, 016/2016, and 084/2016.

2.2.4. Fixation and preservation

After euthanization I took tissue samples for molecular analyses, either from muscle of thigh, tongue, finger clips, tail (when regenerated), or liver. In any case, I recorded in my field book where the tissue came from. Tissues were preserved in vials containing 98% non-denatured ethanol, and stored at -20ºC as soon as possible.

Hemipenes of Squamata were everted after euthanization, with the injection of 70% ethanol after manually everting the organs. In snakes, and large-sized lizards I also cut the hemipenis retractor muscle to avoid any potential slight retraction. All specimens were fixed with a solution of 36% formalin and 96% ethanol in proportion 5:1000 (e.g., 5 ml formalin in 1 l ethanol), injected in the body cavity, thighs, and thickest part of the tail. Following fixation, the specimens were maintained in ethanol 70%.

2.3. Molecular protocols

2.3.1. Tissue extraction

I used two different methods of DNA extraction. For sets containing few samples (usually eight or fewer) I used the DNeasy® Blood & Tissue Kit of Qiagen®, whereas for sets of 96 samples I used the fiber glass plate according to Ivanova *et al*. (2006). Both methods are detailed below. The DNA was isolated from tissues whenever possible, or taken from preserved specimens that had been stored for considerable time in 70% ethanol at room temperature in some cases.

DNeasy® Blood & Tissue Kit: For this method, I used tissue fragments of ~2 mm^2. When buffers formed precipitates they were warmed up at 56ºC before use. All reagents for this protocol are included in the kit. Tissues were digested adding 180 µl (all values are for individual samples) of ATL Buffer and 20 µl of proteinase K. Samples in that mix were incubated in a rocking platform at 56ºC for 4–12 h until the tissue was completely lysed.

Following digestion, 200 µl of AL Buffer + 200 µl of ethanol (98%) were added. This mix was centrifuged (8000 rpm) in DNeasy® Mini spin columns, discarding all the flow-through. Then, 500 µl of AW1 Buffer was added, and centrifuged (8000 rpm) discarding

the flow-through. Finally, 500 µl of AW2 Buffer was added and centrifuged (14000 rpm) discarding the flow-through. The final elution was made with 200 µl of AE Buffer, after an incubation of one minute, followed by a centrifugation (8000 rpm).

Fiber glass extraction plate for 96 samples: For this method, I used tissue fragments of ~1 mm^2. Specifications of reagents used in this protocol, are detailed in Table 2. Initially, the samples were washed with 50 µl (values per sample) of a solution of 1× TE Buffer to remove the remaining ethanol, for ~15 h. Following, the samples were digested with 50 µl of a solution of Vertebrate lysis Buffer and proteinase K (10:1), and incubated in a rocking platform at 56ºC for 12–24 h.

Table 2. Reagents and buffer ingredients used for molecular protocols. In parentheses volumes of reagenets needed to reach the specified volume of buffer.

Regeants		**Concentration**	**Supplier**
TE Buffer	(100 ml)		
Tris-HCl	(90 ml)	10 mM	Carl Roth
EDTA	(10 ml)	1 mM	Carl Roth
Vertebrate Lysis Buffer	(100 ml)		
NaCl	(4 ml)	100 M	Carl Roth
Tris-HCl	(10 ml)	1 M	Carl Roth
EDTA	(4 ml)	0.5 M	Carl Roth
SDS	(5 ml)	20 %	Carl Roth
Water	(77 ml)		
Proteinase K solution		20 mg/ml	Bioline
Binding Buffer	(14 ml)		
GuSCN	(7 ml)	4 M	Carl Roth
Ethanol	(7 ml)	96 %	Carl Roth
Washing Buffer 1	(20 ml)		
GuSCN	(5.2 ml)	4 M	Carl Roth
Ethanol	(14.8 ml)	96 %	Carl Roth
Washing Buffer 2	(475 ml)		
Ethanol	(300 ml)	60 %	Carl Roth
NaCl	(4.75 ml)	50 mM	Carl Roth
Tris-HCl	(4.75 ml)	10 mM	Carl Roth
EDTA	(0.475 ml)	0.5 mM	Carl Roth
Taq-DNA Polymerase		5 U/µl	Peqlab
Reactionbuffer Y		2.5 mM	Peqlab
$MgCl_2$		25 mM	Peqlab
dNTPs		2.5 mM	Carl Roth
EasyLadder I		100 lanes	Bioline
peqGOLD Universal Agarose			Peqlab
Loading Buffer	(10 ml)		
Bromophenol blue	(0.25 g)		Carl Roth
Sucrose	(4 g)		Carl Roth
Water	(5 ml)		
TE Buffer	(5 ml)	1×	
SYBR™ Green		10.000×	ThermoFisher
TAE Buffer (stock): dilution of 242.3 g Tris and 37.2 g EDTA, in 58 ml Glacial acetic acid. Concentration for 1x TAE is 40 mM Tris and 2 mM EDTA.			

Once the samples were digested, the DNA extraction was made adding 100 µl of Binding Buffer and centrifuging at 2800 rpm. These products were transferred to a Pall® AcroPrep® filter plate, where the plate was vacuumed for 2 min. Then it was added 180 µl of Washing Buffer 1 and vacuumed again for 2 min. Posteriorly, it was added 750 µl of the Washing Buffer 2 and vacuumed for 2. Then TE Buffer was used to elute the DNA, adding 50 µl and incubating it for 20 min at 56ºC.

2.3.2. DNA amplification

In total, I tried to amplify four mitochondrial and five nuclear genes. Genes and primers used are listed in Table 3. Primers were synthetized by Eurofins MWG Operon. Some genes (such as c-mos, pomc, and Rag1) did not work at all, and some (most of mitochondrial genes) produced good results. In all cases, I prepared the Master Mix in the laboratory a few minutes before starting the polymerase chain reactions (PCR). The Master Mix for every gene, are detailed in Table 4. For the amplification, I used an Eppendorf Mastercycler® pro. PCR protocols used are described in Table 5.

The first procedure was always to sequence the mtDNA genes 16S and COI, which were used as a first reference to examine potential taxonomic confusions. Thus, these sequences were used as barcodes for taxonomic comparisons. Whenever a taxonomic challenge or potential conflict arose, other primers could have been sequenced to generate more data and stronger taxonomic conclusions.

I assessed the quality of sequences after amplification in agarose gel electrophoresis, using SYBR™ Green to provide florescence to the DNA fragments. Gels were prepared with a concentration of 1 g of agarose and 8 µl of SYBR™ Green per 100 ml of 1×TAE Buffer. To stain the DNA fragments I used a Loading Buffer. I used EasyLadder I as the molecular-weight size marker.

Table 3. Sets of primers used of gene amplifications. Forward and reverse primers distinguished with "F" and "R" respectively. Primers from Eurofins MWG Operon.

Gene	Abbr.	Primers	Sequence (5' – 3')	Author
16S ribosomal RNA	16S	F: L2510 R: H3056	CGCCTGTTTAACAAAAACAT CGGTCTGAACTCAGATCACGT	Palumbi *et al*. 1991
Cytochrome b	Cyt-b	F: IguaF2 R: IguaR2	CCACCGTTGTTATTCAACTAC GGTTTACAAGACCAATGCTTT	Corl *et al.* 2010
Cytochrome oxidase subunit 1	COI	F: dgLCO-1490 R: dgHCO-2198	GGTCAACAAATCATAAAGAYATYGG TAAACTTCAGGGTGACCAAARAAYCA	Meyer 2003
NADH dehydrogenase subunit 4	ND4	F: ND412931L R: Leu	CTACCAAAAGCTCATGTAGAAGC CATTACTTTTACTTGGATTTGCACCA	Blair *et al*. 2009 Arevalo *et al*. 1994
Oocyte maturation factor Mos	c-mos	F: Fu-F R: Fu-R	TTTGGTTCKGTCTACAAGGCTAC AGGGAACATCCAAAGTCTCCAAT	Gamble *et al*. 2008a
Protooncogene c-Myc	c-myc	F: cmyc1U R: cmyc-ex2R	CAGGACATCTGCAARAARTT TCATTCAATGGGTAAGGGAAGACC	Crawford 2003 Wiencs *et al*. 2005
Pro-opiomelanocortin	pomc	F: POMC-1 R: POMC-2	GAATGTATYAAAGMMTGCAAGATGGWCCT TAYTGRCCCTTYTTGTGGGCRTT	Wiencs *et al*. 2005
Prolactine Receptor	PRLR	F: PRLR_f1 R: PRLR_r3	GACARYGARGACCAGCAACTRATGCC GACYTTGTGRACTTCYACRTAATCCAT	Townsend *et al*. 2008
Recombination Activating 1	Rag-1	F: Rag1BolitoF R: Rag1BolitoR	CTTGAACTAGGGGGCATACTCAGAAC TGCCTGGCATTCATTTTCCGGAAACG	Elmer *et al*. 2013
		F: R182 R: R270	GCCATAACTGCTGGAGCATYAT AGYAGATGTTGCCTGGGTCTTC	Hedges *et al*. 2008

Table 4. Volume of ingredients used in the master mix cocktail for PCR of each genes used.

	Mitochondrial				Nuclear				
	16S	COI	ND4	Cyt-b	c-mos	c-myc	pomc	PRLR	Rag-1
DNA template	1	1	1	1.5	1	1	1	1	1
Y Buffer	2.5	2.5	2.5	3.5	2	2.5	2.5	2	2.5
dNTPs	4	3	4	3.5	2	4	4	1.6	4
TaqPol	0.5	0.5	0.5	0.2	0.5	0.5	0.5	0.4	0.5
$MgCl_2$	1	2	1	1.5	2	1	1	1.4	1
distilled H_2O	14	14	14	11.8	8.5	14	13	10.6	13
Forward primer	1	1	1	1.5	2	1	1.5	1.5	1.5
Revers primer	1	1	1	1.5	2	1	1.5	1.5	1.5

2.3.3. Sequence treatment

I inspected the chromatograms of forward and reverse sequences to generate consensus sequences, using SeqTrace 0.9.0 (Stucky 2012). Since the beginning of my work, I used a different alignment method: Clustal W (Larkin et al. 2007), but currently I'm employing MAFFT2 (Katoh et al. 2002, Katoh & Standley 2013) through the webserver (available at http://mafft.cbrc.jp/alignment/server/), which includes a special search strategy (Q-INS-i) for the secondary structure of the rRNA 16S (Katoh & Toh 2008). Results of MAFFT2 were visualized in MSA Viewer (Yachdav et al. 2016) and exported as fasta files. Every new sequence published was stored in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

To search for the best substitution model, I usually used MEGA 6.06 (Tamura et al. 2013), or IQ-Tree (Nguyen et al. 2015) through its webserver (Trifinopoulos et al. 2016), although currently I use PartitionFinder 2.1.1 (Lanfear et al. 2016), using linked branch lengths (supported by most of phylogenetic programs) using a PhyML 3.0 analysis (Guindon et al 2010). PartitionFinder 2.1.1 allows a high number of substitution models, and at the same time it writes commands to be used in different phylogenetic programs. To estimate the relative quality of the statistical models, I used AICc or BIC because both proved to be good estimators. Nevertheless, when the samples are from different taxonomic groups, showing a large degree of heterogeneity, I select the BIC since it penalizes more the number of parameters in the model (Brewer et al. 2016). Usually the substitution models are associated to specifications of variation among sites, such as +I (significant propor-

tion of invariable sites) or +G (rate of variation among sites follows a gamma distribution), but seldom both together. Nevertheless, according to Yang (2006) it is not proper to use models that include both +G and +I, and then I chose the following model in the best partition schemes when both were suggested by the program.

Table 5. PCR protocols used for each primer. Asterisk (*) indicate a reduction of 0.5º per cycle after the 10th cycle.

Gene	Primers	PCR conditions					Reference
		Den	Den	Ann	Ext	Ext	
16S	L2510 (F) H3056 (R)	94.0º 02:00	94.0º 00:35 ×40	48.5º 00:35	72.0º 01:00	72.0º 10:00	Lotzkat *et al*. 2013
COI	dgLCO-1490 (F) dgHCO-2198 (R)	94.0º 01:30	94.0º 00:40 ×37	45.0º 00:40	72.0º 00:40	72.0º 06:00	Townsend pers. comm.
c-mos	Fu-F Fu-R	94.0º 05:00	94.0º 00:30 ×32	52.0º 00:45	72.0º 01:00	72.0º 05:00	Gamble *et al*. 2008b
ND4	ND412931L (F) ND4LEU (R)	94.0º 03:00	94.0º 00:30 ×35	55.0º 00:30	72.0º 01:00	72.0º 10:00	Morando pers. comm.
PRLR	PRLR_f1 PRLR_r3	95.0º 01:30	95.0º 00:35 ×10 95.0º 00:35 ×10 94.0º 00:35 ×15	63.0º ↓ (*) 00:35 58.0º 00:35 52.0º 00:35	72.0º 02:00 72.0º 01:00 72.0º 01:00	72.0º 10:00	Noonan & Yoder 2009
pomc	POMC-1 POMC-2	96.0º 02:00	95.0º 00:20 ×45	52.0º 00:25	72.0º 02:00	72.0º 07:00	Wiens *et al*. 2005
Rag1	Rag1BolitoF Rag1BolitoR	96.0º 02:00	95.0º 00:20 ×45	52.0º 00:25	72.0º 02:00	72.0º 07:00	Batista *et al*. 2016
Rag1	R182 (F) R270 (R)	96.0º 02:00	95.0º 00:20 ×45	52.0º 00:25	72.0º 02:00	72.0º 07:00	Batista *et al*. 2014
Rag1	R182 (F) R270 (R)	94.0º 05:00	94.0º 00:30 ×40	50.0º 00:30	72.0º 01:00	72.0º 07:00	Hedges *et al*. 2008

When more than one gene was analyzed for a specific taxon, then I concatenated the sequences in MEGA 6.06. I used the online server Alter (Glez-Peña et al. 2010), available at http://sing.ei.uvigo.es/ALTER/, to convert sequences from fasta to nexus format, according to the respective requirements of the different software applications.

2.4. Molecular analyses

2.4.1. Barcoding analysis

The species collected in the field were identified using field guides or descriptive works such as Cei (1993), Norman (1994), Montero & Terol (1999), Giraudo (2001), Achaval & Olmos (2003), Carreira *et al*. (2005), Cacciali (2009), Cabrera (2010), Carreira & Maneyro (2013), Marques *et al*. (2015). Nevertheless, with the current advance of molecular genetics, broadly used as a taxonomic tool nowadays, in addition to external morphology I also used molecular markers to evaluate conspecificity with previously known taxa, and clearly molecular genetics open a path for more detailed taxonomic studies (Hajibabaei *et al*. 2007).

A DNA barcode is a genetic identifier for a species (Lane 2009), and two of the most commonly used markers were partial sequences of Cytochrome oxidase, subunit 1 (COI) (Hebert *et al*. 2003, Murphy *et al*. 2013) and rRNA 16S (Sacchi *et al*. 2002, Jansen & Schultze 2012, Scherz *et al*. 2017), and both markers seems to work rather equally good for species identification (Vences *et al*. 2005, Xia *et al.* 2012, Zheng *et al*. 2014, Wang *et al*. 2017). Nevertheless, given the low representation of COI sequences available in GenBank for comparison of Squamata from Paraguay, I used barcodes of 16S to evaluate conspecificity.

To evaluate whether samples were contaminated with human cells, or if a mixup of specimens had occurred, I performed a first inspection in GenBank using the Blast tool. To evaluate whether the 16S barcodes reveal conspecificity with the species to which they belong, I downloaded sequences from GenBank but did so cautiously because it is known that there are some misidentification problems in that repository (Vilgalys 2003, Zhang 2009, Park *et al*. 2012).

In most cases, I downloaded only sequences from species that are represented in my samples, except for *Bothrops* and *Amphisbaena*. In these two cases, I downloaded samples from all the species present in Paraguay, because of the difficulty of these taxa for morpho-

logical identification. In the case of *Micrurus* there is only one sequence of a species present in Paraguay available in GenBank (JQ627286). Some of the specimens that I have were decapitated (killed by rural farmers) and then without genetic samples for comparison and without head (which contains important diagnostic characters) its specific taxonomic allocation is difficult. A particular case was the only available sample of *Vanzosaura rubricauda* (AF420716) uploaded in the framework of a paper by Pellegrino *et al.* (2001). That specimen (MRT 05059) from Vacaria, Estado de Bahia, Brazil, actually is *V. multiscutata* (Recoder *et al*. 2014). Nevertheless, it was included in the analysis to evaluate the clustering with the genetic sample from Paraguay.

In some cases, and when sequences were not available for comparison in GenBank for some taxa, colleagues from the region (from Argentina, Brazil, and Uruguay) shared aliquots of tissues for this project.

2.4.2. Phylogenetic analyses

All the analyses to test phylogenetic hypotheses (based on single genes or concatenated datasets) were performed under Maximum Likelihood and Bayesian approaches. Usually I used MEGA 6.06 to perform the ML analyses, although currently I use IQ-Tree (Nguyen et al. 2015) through its webserver (Trifinopoulos et al. 2016), typically setting 10,000 non parametric bootstrap replicates plus 10,000 replicates of Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT) (Anisimova et al. 2011) and 10,000 ultrafast bootstrap (UFBoot) approximation replicates (Minh et al. 2013).

For Bayesian inferences I used MrBayes 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), usually running the analyses independent duplicates, each with 1,000,000 generations for MCMC and a sample frequency of 500 generations. Nevertheless, to have stronger support values I run now the analyses using 5 runs and 8 chains and a burn-in period of 25%. I evaluate the convergence of the chains using the standard deviation of split frequencies. If the value is ≥0.02 I continue adding 500,000 generations until the value reaches ~0.018 or lower.

To root the phylogenetic trees, I chose as outgroup the closest available taxon (or taxa) relative to the group of study. For "availability" I mean, if sequences of that taxon are accessible on GenBank. I did not used sequences that were not yet published. For the general

tree of Paraguayan Squamata, based on barcodes of 16S, I chose *Sphenodon punctatus* as suggested by Pyron et al. (2013) as the sister clade to Squamata, under the GenBank accession number DQ267621, published by Miller (2006). Given that the tree of Paraguayan Squamata does not look for a phylogeny but rather for groupings, I performed only ML analysis, and not BI.

For visualization and edition (branch arrangement, colors, font sizes, etc.) of the trees generated through ML and BI analyses, I use FigTree 1.3.1 or the newest version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). In some cases, the trees (and their alignments) were stored in TreeBASE (https://treebase.org/). To do this, I first managed the alignments and trees in nexus format, and combined them in single files (containing one alignment and two -ML and BI- trees) using Mesquite 3.31 (Maddison & Maddison 2017).

2.5. Morphological analyses

Once a taxonomic uncertainty was identified based on the ML grouping of 16S barcodes, a morphological approach was taken based on voucher specimens collected during fieldwork (usually the specimens used for genetic analysis), supplemented by the revision of museum collections.

The morphological characters that I used depended on the taxonomic group. Usually I worked with characters traditionally used for the specific group, but also I explored additional characters that could help to diagnose the different taxa.

For color descriptions I made color descriptions in life written during fieldwork, based on specimens before euthanization, using the color guide of Köhler (2012) as a base to record the data. Measures and counts of pholidosis traits were recorded on preserved specimens.

2.6. Species delimitation and species concept

Given that nowadays taxonomy is a rather integrative discipline (Padial *et al*. 2010), many times the species concept that a scientist has to follow depends on the tools and methods that were used for the respective analyses. Thus, I follow the Unified Species Concept proposed by de Queiroz (2007), grounded on the basis that "They [the lineages or species] only have to be evolving separately from other lineages" (de Queiroz 2007). To

understand which lineages are evolving separately, there are several lines of evidence that an author can explore and interpret.

I first inspected the monophyly of the samples in the 16S gene tree based on samples of Squamata from Paraguay comparing them with available sequences from GenBank and/or sequences provided by colleagues. Then, case by case, I checked for the original paper where the sequences were first published to assess the correct identification and provenance of the samples. Once I got evidence that the taxonomy of a given cluster was not correct, I looked for stronger signals such as: acquisition of more GenBank sequences, from related species to calculate intra- and interspecific genetic distances, sequencing of more genes, and use of species delimitation analyses (usually ABGD). Then, the stronger line of evidence is a genetic comparison which is interpreted as reproductive isolation.

Given that in taxonomy the morphological diagnostic characters for species recognition are a keystone issue, I always analyzed morphological traits traditionally used in the taxonomy of every group, and in each case explored additional characters that could be successfully applied for species identification.

2.7. Taxonomy and checklist

At the end of this work I introduced a species checklist for the Squamata from Paraguay, based on Cacciali *et al*. (2016a), including the taxonomic modifications proposed here. Nevertheless, I follow Pyron *et al.* (2013) and Figueroa *et al*. (2016) for the taxonomy of Family's nomenclature. This means that Dipsadinae is recognized as a subfamily of Colubridae, and not as a family as recognized by Cacciali *et al*. (2016a) based on Grazziotin *et al*. (2012). Nevertheless, I maintain the genus arrangement within Dipsadinae as proposed by Grazziotin *et al*. (2012). Additional changes include the use of Scincidae instead of Mabuyidae (the latter proposed by Hedges & Conn 2012), but maintaining the genus arrangement of Hedges & Conn (2012).

3. RESULTS

3.1. Barcoding analysis

I generated a total of 142 sequences of 64 species of Squamata from Paraguay, including one exotic species: *Hemidactylus maboua*. In Appendix I I present a list of specimens used for genetic analyses based on the field work for this project. I also sequenced 13 samples of non-Paraguayan lizard samples (*Homonota* and *Teius*) for comparison, and downloaded 94 sequences from GenBank, including a sample of *Sphenoton punctatus* used as outgroup. The final alignment constituted of a dataset of 249 samples of 615 bp length. It is important to mention that we received 43 tissues of Squamata from Laguna Blanca, but I was able to amplify only one, since most of the samples were in bad conditions. Also I had some poor quality sequences when I tried to amplify samples from preserved specimens.

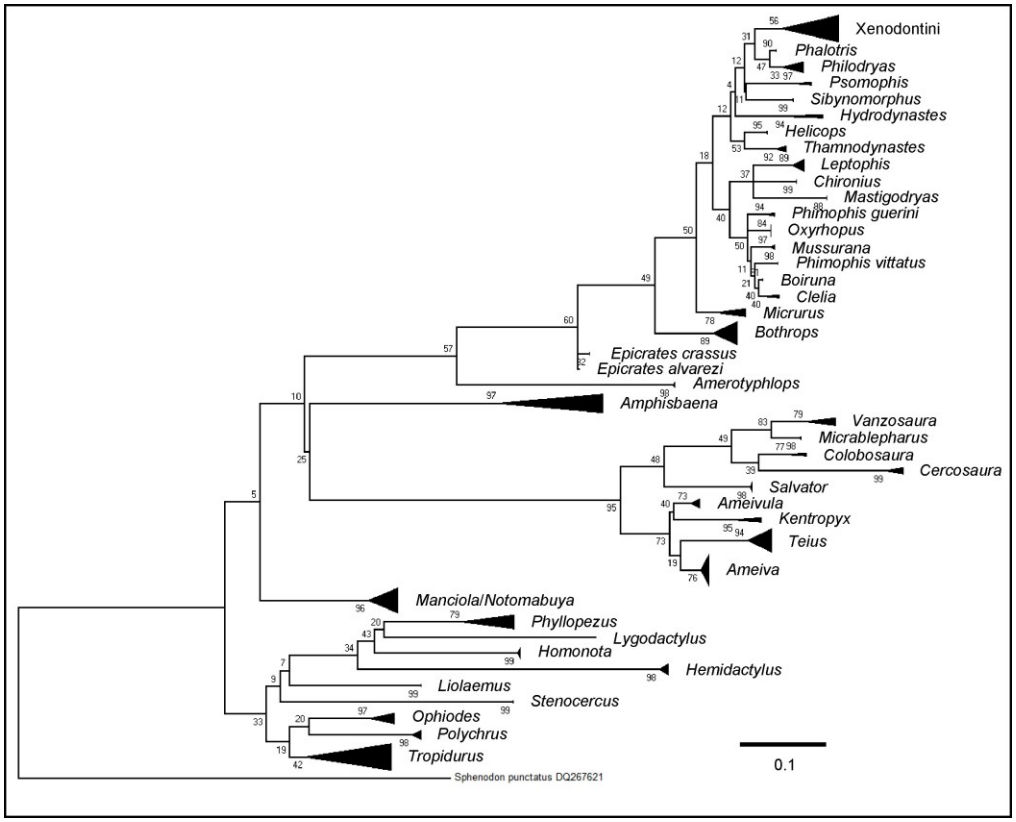

Figure 29. Gene tree based on 16S and a ML approach. Groups collapsed at tribe or genus level. Numbers on nodes denote bootstrap values.

The best substitution model for the Barcoding dataset was GTR+G, according to the BIC. The sample of *Sphenodon punctatus* was recovered as the sister clade to the Squamata (Fig. 29). Deep nodes have low bootstrap values, meaning that the phylogenetic relationships are weak. Nevertheless, the shallowest divergences have higher support values, rendering monophyly in most of the genera included in the analysis, with the exception of *Manciola* (Scincidae) and the tribe Xenodontini (Colubridae).

The tribe Xenodontini (Fig. 30) of the Subfamily Dipsadinae (Colubridae) contains the samples of *Erythrolamprus aesculapii* in a monophyletic clade, whereas *E. poecilogyrus* appears as paraphyletic. *Erythrolamprus reginae* clusters sister to the above mentioned taxa. The genus *Xenodon* seems to be paraphyletic, for two samples of *Xenodon pulcher* is sister to *Erythrolamprus*, whereas *Xenodon merremi* is sister to *Xenodon pulcher* + *Erythrolamprus*. Finally in this clade a sample of *Erythrolamprus sagittifer* is sister to a sample of *Lygophis dilepis*.

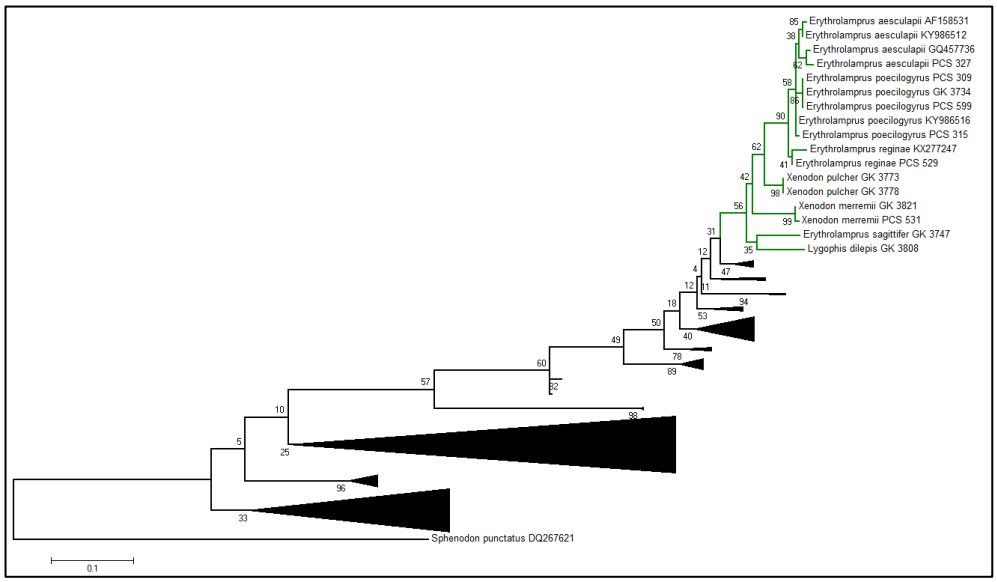

Figure 30. Collapsed tree of Figure 29, with an expanded detail of the Xenodontini tribe.

Sister to Xenodintini is a clade composed by *Phalotris* + *Philodryas* (Fig. 31). Both genera are monophyletic in the tree. The genera *Psomophis* and *Sibynomorphus* are cluster together, and nested as sister to the above mentioned snakes. Other genera of Dipsadinae that are rendered as monophyletic are *Hydrodynastes*, *Helicops*, and *Thamnodynastes* (Fig. 32). A clade grouping members of the Colubrinae subfamily is composed by three genera (*Chironius*, *Leptophis*, and *Mastigodryas*) that also show monophyly (Fig. 33). The Pseu-

doboini (Dipsadinae: Colubridae) is shown in its own clade (Fig. 34) with four of the five genera used in the analysis monophyletic, for the two species of the genus *Phimophis* appear in different positions of the gene tree.

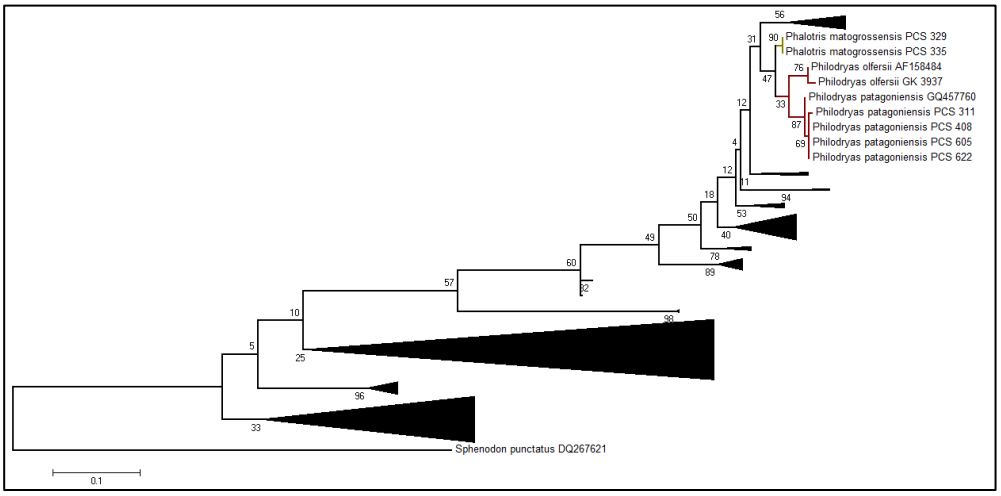

Figure 31. Collapsed tree of Figure 29, with an expanded detail of the genera *Phalotris* and *Philodryas*.

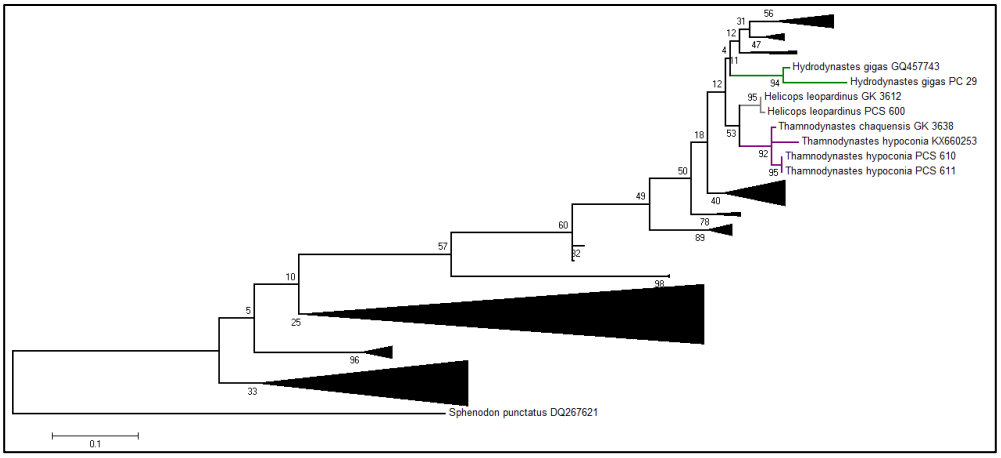

Figure 32. Collapsed tree of Figure 29, with an expanded detail of the genera *Hydrodynastes*, *Helicops*, and *Thamnodynastes*.

The genus *Micrurus* (Elapidae) is the sister clade of the Colubridae, whereas the genus *Bothrops* (Viperidae) seems to be the sister to Elapidae + Colubridae (Fig. 35). In a most basal position among snakes are located the two species of *Epicrates* (Boidae), with *Amerotyphlops* (Typhlopidae) as the sister clade of the remaining snakes (Fig. 36). The genus *Amphisbaena* (Amphisbaenidae) is monophyletic, where *A. alba* and *A. bolivica* are in their own clades, and *A. mertensii* shows also monophyly (Fig. 37). *Amphisbaena angustifrons* is the sister taxon of the other *Amphisbaena*, with a sample of *Amphisbaena* sp. (PCS 314) is the most basal taxon of the clade (Fig. 37). In my analysis, *Amphisbaena* is

sister to Teidae + Gymnophthalmidae. Gymnophthalmidae appears as a monophyletic clade, and the four genera show monophyly as well (Fig. 38).

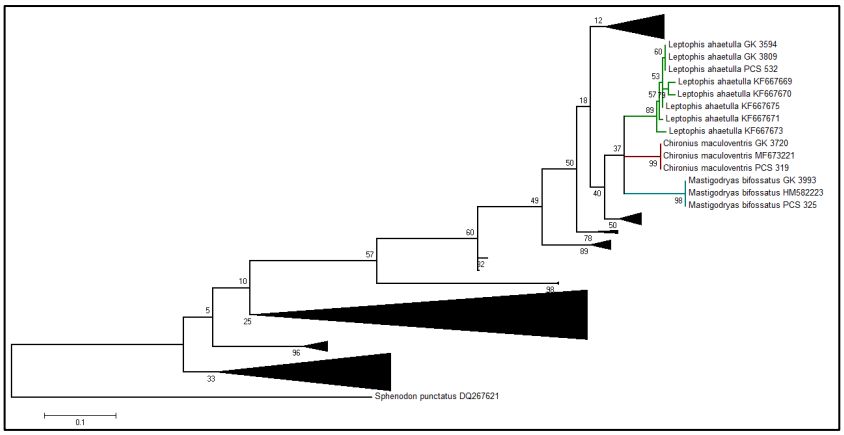

Figure 33. Collapsed tree of Figure 29, with an expanded detail of the subfamily Colubrinae.

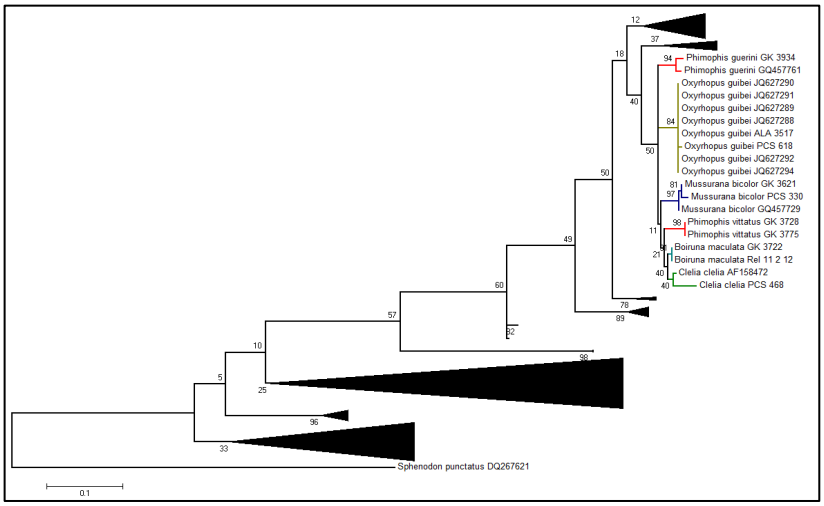

Figure 34. Collapsed tree of Figure 29, with an expanded detail of the tribe Pseudoboini.

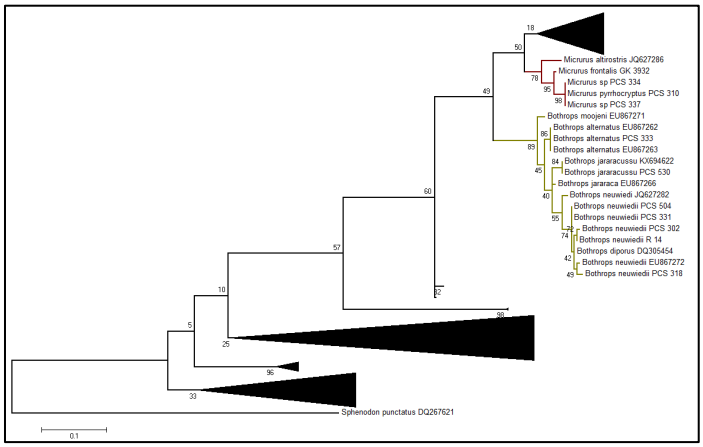

Figure 35. Collapsed tree of Figure 29, with an expanded detail of the genera *Micrurus* and *Bothrops*.

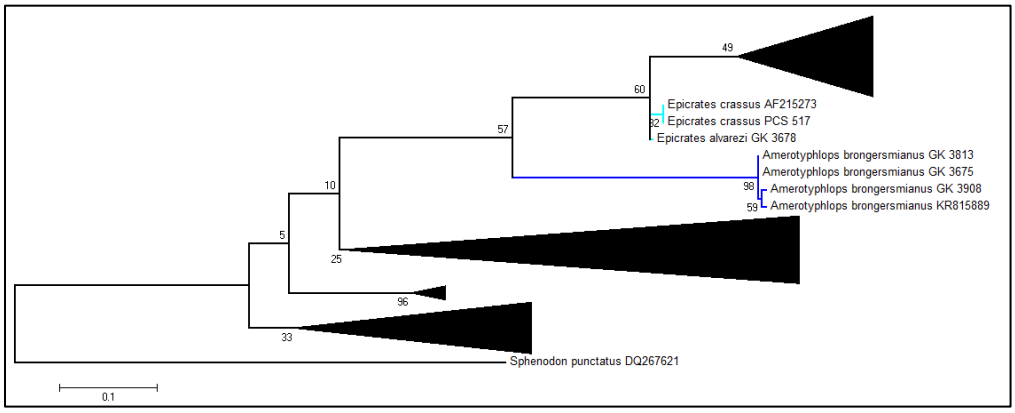

Figure 36. Collapsed tree of Figure 29, with an expanded detail of the families Boidae and Typhlopidae.

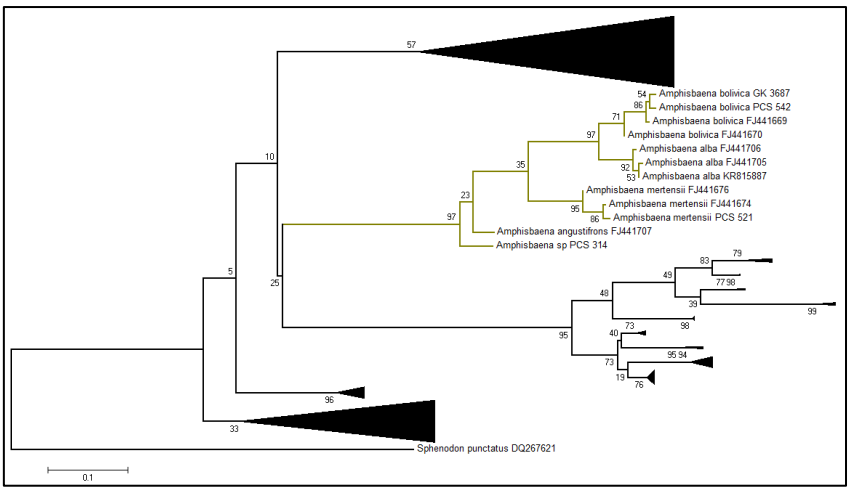

Figure 37. Collapsed tree of Figure 29, with an expanded detail of the family Amphisbaenidae.

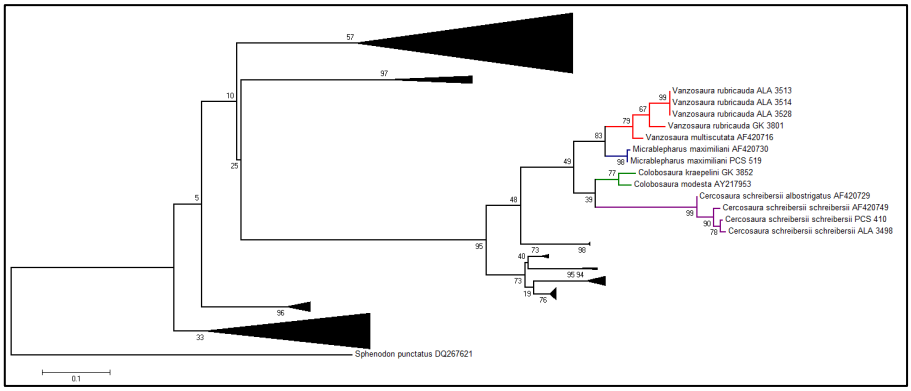

Figure 38. Collapsed tree of Figure 29, with an expanded detail of the family Gymnophthalmidae.

The Family Teiidae is shown as paraphyletic. The only Tupinambinae in my samples was *Salvator*, which is clustered as sister to Gymnophthalmidae (Fig. 39). Samples of Teiinae are clustered together showing monophyly, where *Ameivula* and *Kentropyx* are sister clades (Fig. 39), as are *Teius* and *Ameiva* (Fig. 40). The Family Scincidae is sister to the all

above mentioned clades (Fig. 41). Samples of *Manciola* show paraphyly (Fig. 41). The remaining cluster contains members of the Anguidae, Gekkonidae, Phyllodactylidae, Liolaemidae, Polychrotidae, and Tropiduridae families. The clade composed by geckos shows monophyly in the genera, but not in the families given that *Phyllopezus* and *Homonota* are currently placed in Phyllodactylidae, whereas *Hemidactylus* and *Lygodactylus* are Gekkonidae (Fig. 42). Sister to the Gekkota (Gekkonidae + Phyllodactylidae) is *Liolaemus*, and *Stenocercus* is recovered as sister to the Gekkota + *Liolaemus*.

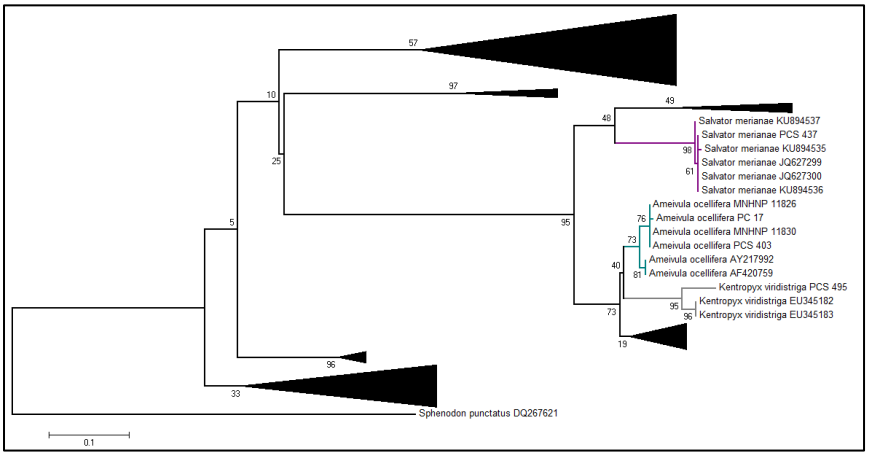

Figure 39. Collapsed tree of Figure 29, with an expanded detail of the genera *Salvator*, *Ameivula*, and *Kentropyx*.

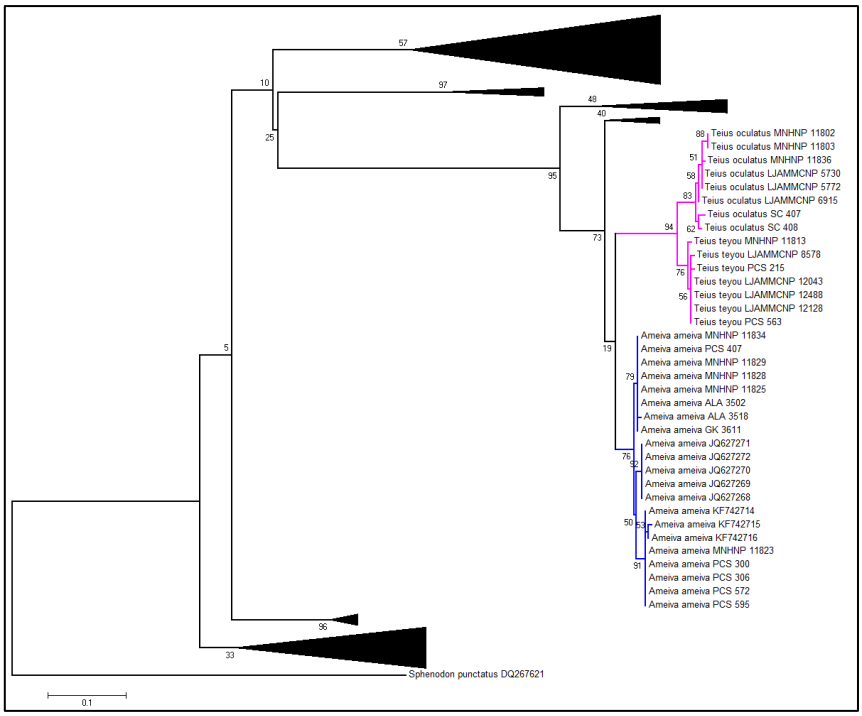

Figure 40. Collapsed tree of Figure 29, with an expanded detail of the genera *Teius* and *Ameiva*.

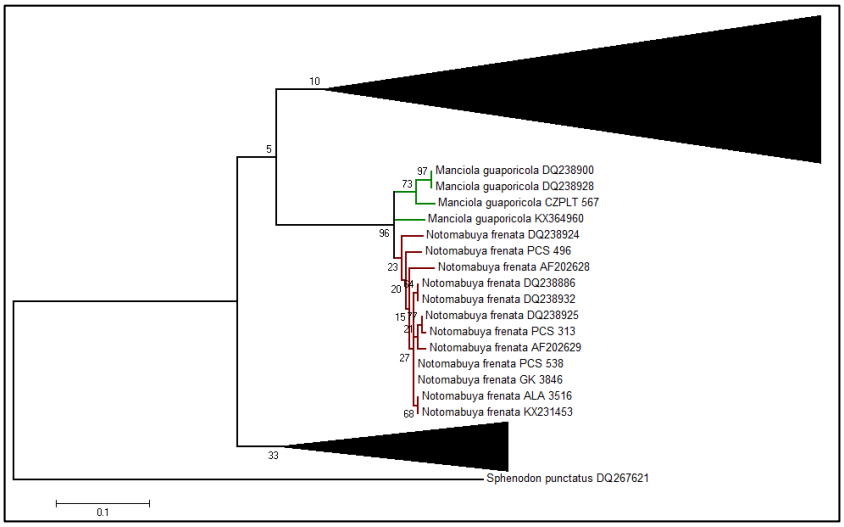

Figure 41. Collapsed tree of Figure 29, with an expanded detail of the family Scincidae.

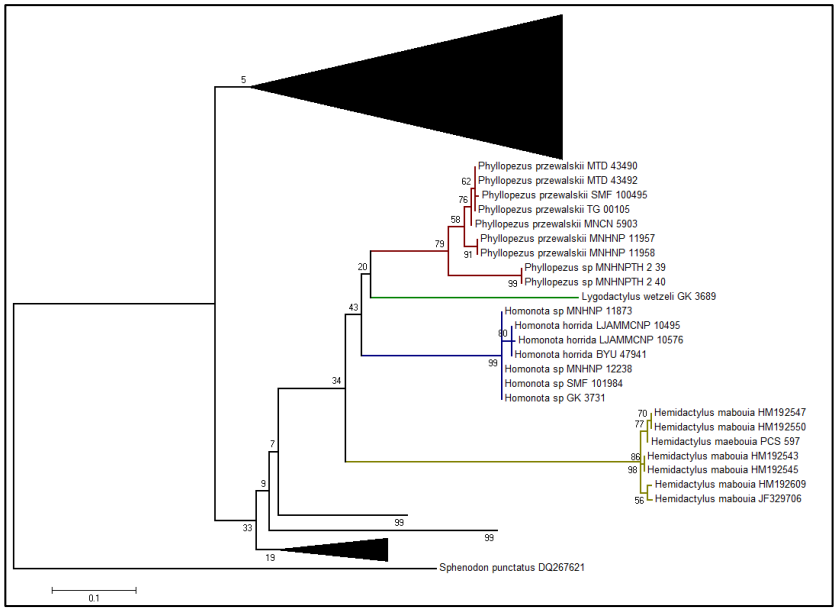

Figure 42. Collapsed tree of Figure 29, with an expanded detail of the Gekkota clade.

The last cluster, sister to Gekkota + *Liolaemus* + *Stenocercus*, is constituted by *Ophiodes* (Anguidae), *Polychrus* (Polychrotidae), and *Tropidurus* (Tropiduridae) (Fig. 43). In this case, the Family Tropiduridae is polyphyletic since the other member of the family (*Stenocercus*) is sister clade to Gekkota + *Liolaemuus*.

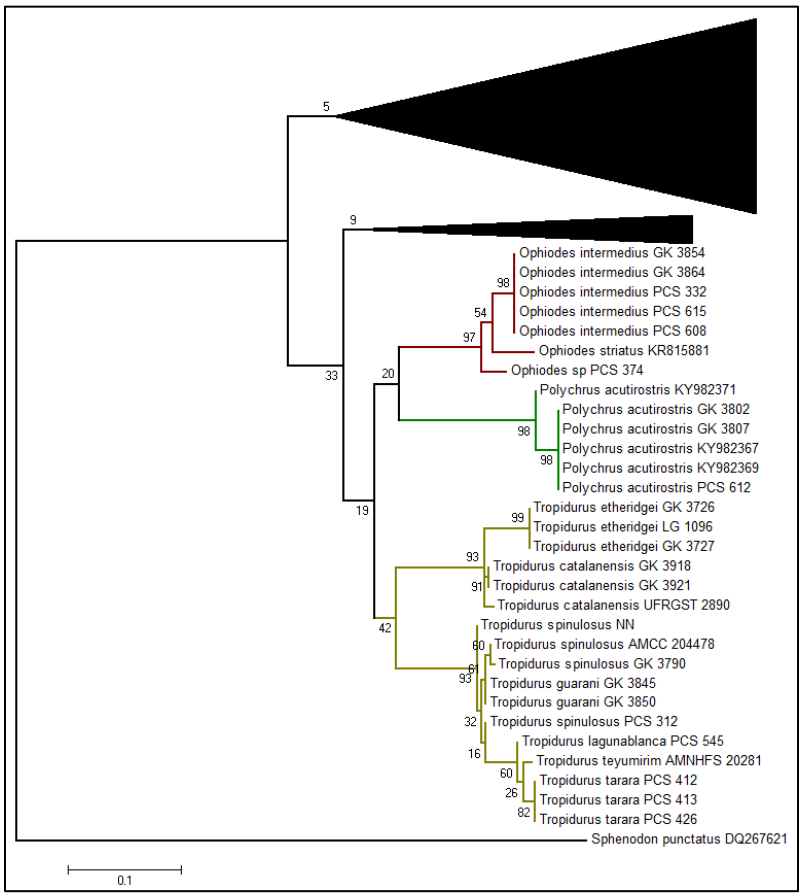

Figure 43. Collapsed tree of Figure 29, with an expanded detail of the genera *Ophiodes*, *Poluchrus*, and *Tropidurus*.

3.2. Taxonomic implications

3.2.1. Taxonomic revisions of selected lizard taxa

Gymnophthalmidae

The samples of *Vanzosaura rubricauda* from Cerrado (field number "ALA") show a high branch distance compared with *Vanzosaura rubricauda* from Chaco (GK 3801), which is even larger than the distance with *V. multiscutata* (Fig. 38).

According to the results, the samples of *Colobosaura* also exhibit large genetic distances, and then *Colobosaura kraepelini* was revalidated (Appendix II). To do this I compared genetic samples of 16S with other species of the Iphisini tribe. I, together with my coauthors, found also morphological differences that support the resurrection of the name *C. kraepelini*.

Cercosaura schreibersii also exhibits a large branch length between the putative subspecies.

Tropiduridae: The *Tropidurus* samples show monophyly in the species of the *torquatus* group (*T. catalanensis* and *T. etheridgei*), but indicate several uncertainties within the *spinulosus* group (*T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara*, and *T. teyumirim*). I, together with GK, performed a deep genetic and morphological analysis of Paraguayan samples of this genus, including an additional mitochondrial marker (COI) and a nuclear gene (PRLR), with the conclusion that the diversity of the *spinulosus* group was overestimated, and we recognize only *T. lagunablanca* and *T. spinulosus* as valid species within the *spinulosus* group (Appendix III)

Phyllodactylidae: This is a family in which I invested some effort, due mostly on some morphological differences that I found a priori. Then I had the collaboration with GK and additional coauthors which brought interesting results: first, we recognized that the holotype of *Homonota fasciata* was significantly different from the taxon known with that name in South America (Appendix IV); second, we provided evidence that the samples of *H. horrida* from the type locality were different from populations of "*H. horrida*" from Paraguay (Appendix IV); and third, we provided evidence for the recognition of two different taxa previously assigned to *H. horrida* from Paraguay (Appendix V).

Finally, the barcoding tree shows a large branch length between samples of *Phyllopezus przewalskii* and samples from an isolated population in southern Paraguay associated with rocky hills (*Phyllopezus* sp. in Fig. 42). Then, we explored additional genes comparing the data with the remaining species of the genus, and recognized the isolated population associated to rocky hills as a different taxonomic unit (Appendix VI).

3.2.2. Additional potential taxonomic revisions

Colubridae: Compared to the diversity of Colubridae, my dataset had only a few samples of this family and then it is not possible to perform a detailed taxonomic analysis. However, the presence of the genus *Xenodon* in two different clusters suggests that more analyses are needed. Also, the revision of the phylogenetic status of *Phimophis* is advisable, since here it appears polyphyletic.

Elapidae: The genus *Micrurus* is scarcely represented in GenBank, and comparison is not possible. The only sample from GenBank is *M. altirostris*, which is differentiated from Paraguayan samples by a rather long branch distance. There is a polytomy with three sam-

ples (PCS 310, 334, and 337), and the only identified specimen is *Micrurus pyrrhocryptus* (PCS 310) from Pantanal (northern part of Paraguay). The other samples are from Concepción at the other side of the river, and with a body color different to the pattern of *M. pyrrhocryptus*.

Viperidae: The monophyly in most of the species of *Bothrops* is well supported in some clades (Fig. 35). I treated the samples of *Bothrops* of the *neuwiedi* group as "*Bothrops neuwiedi*". There are different clusters that indicate that these belong to different species. Nevertheless, it is necessary to identify strong diagnostic characters to differentiate among species within the *neuwiedi* group.

Amphisbaenidae: The most basal sample (*Amphisbaena* sp. PCS 314) seems to be a different species to those that are present in my samples. Further analyses, including more samples and a detailed morphological revision, are necessary to assess the specific status of that sample.

4. DISCUSSION

4.1. Barcoding analysis

Molecular genetics, and in particular barcoding analyses, proved to be a powerful tool to perceive preliminary information about the taxonomic status of problematic taxa (Hajibabaei *et al*. 2007, Chapple & Ritchie 2013). I present here the most comprehensive analysis of genetic samples of Squamata from Paraguay. The results obtained here will be useful to help to clarify some taxonomic issues of the Squamata fauna from the central region of South America, then, the data generated here will have a positive impact in a larger geographic context, beyond Paraguay borders.

Nevertheless, it is well known that genetics alone will not yield a well-founded taxonomy. Researchers have to be cautious because the power of barcoding analysis could lead to misinterpretations if sequences used for comparison belong to misidentified specimens (Shen *et al*. 2013), and it is strongly recommended that molecular genetics tools have to be complemented with morphological, ecological, or even bioacoustic data in those animals that produce vocalizations (Meiri & Mace 2007). In conclusion, molecular genetics open a path for defining operational taxonomic units (OTUs), identifying potential undescribed species and pointing to taxonomic problems, and thus have to be seen as a first informative step and a complementary evidence line in the framework of the modern integrative taxonomic approach (Hajibabaei *et al*. 2007, Padial *et al*. 2010).

4.2. Weaknesses

To perform a detailed and integrative taxonomic analysis is a time-consuming project since the person needs access to genetic, geographic, and morphological data. To do so, it is necessary to deal with various legal issues involving national bureaucratic matters, budget restrictions, implementation of new methodologies, and even climatic conditions and logistics when performing field work.

Field work is rarely completely foreseeable, and I had to face some unexpected problems during my project. First, on a field trip between January and March of 2015, it was an unusually cold and extremely rainy with even the forests grounds becoming flooded. As a result, the activity of lizards and snake was extremely low. The second big problem I en-

countered was when I was working with the genus *Teius*. In collaboration with GK and colleagues from Argentina, I generated a large dataset of geographic information and morphologic data for lizards of this genus. We published a detailed and extensive geographic account for the genus *Teius* (Appendix VII), but then the plan was to make an integrative morphological and molecular analysis to assess the parthenogenetic origin of *Teius suquiensis*, and to clarify the taxonomic status of a putatively undescribed taxon in tis genus. I have measurements of about 300 specimens of *Teius* (with 38 morphological variables for each specimen) from Argentina, Bolivia, Paraguay, and Brazil; but the molecular analyses (based on ~100 samples from Argentina, Paraguay, and Uruguay) did not yield conclusive information yet. I was able to amplify the mitochondrial genes 16S and ND4, and the nuclear gene c-myc, but I failed in the amplification of Cytb, c-mos, pomc, PRLR, and Rag-1. The primers (and PCR conditions) for Cytb and PRLR were appropriate for amplification in other taxa, but did not work in *Teius*. Thus, this is still ongoing research.

4.3. Taxonomic revisions

4.3.1. Selected lizard taxa

Gymnophthalmidae: Integrating molecular and morphological data, Recoder *et al.* (2014) found evidence for the recognition of a new species of *Vanzosaura* (*V. savanicola*) and transferred *Gymnodactylus multiscutatus* to the genus *Vanzosaura*. Nevertheless, the genetic tree showed by Recoder *et al.* (2014) included only a single sample from Paraguay and none from Argentina. In their map, obviously two evident populations of *Vanzosaura rubriauda* are recognized: one west of Paraguay River in the Dry Chaco, and another east of Paraguay River in the Cerrado. Keeping a conservative approach, the authors maintained *V. rubricauda* as a single taxonomic unit, but with our additional samples, it is possible to generate new taxonomic hypotheses. Unfortunately I had no access to most of the material used by Recoder *et al*. (2014).

With respect to *Colobosaura*, the evidence for the recognition of *C. kraepelini* is strong (Appendix II). Now, a further objective is to continue working with gymnophthalmids because they are very scarce in scientific collections due to their secretive life-style.

Tropiduridae: In Appendix III I present the results of a molecular and morphological analysis of Paraguayan samples of *Tropidurus*. These are very common lizards with a wide

distribution in the country. We plan to continue our studies with these group adding samples from different localities, trying to improve the knowledge on the geographic limits of *T. lagunablanca* and *T. spinulosus*.

Phyllodactylidae: Considered as part of the family Gekkonidae for a long time, the family Phyllodactylidae (Gamble *et al*. 2008) includes genera such as *Homonota* and *Phyllopezus* that were object of recent molecular studies where Paraguayan samples were used (Gamble *et al*. 2012, Werneck *et al*. 2012, Morando *et al*. 2014). The inclusion of more genes and more samples in the analyses of *Homonota* demonstrated the presence of more lineages than previously recognized (Appendices IV and V). With respect to *Phyllopezus*, Gamble *et al*. (2012) and Werneck *et al*. (2012) showed a high degree of cryptic diversity within the genus *Phyllopezus*, which coincide with what we found in our analysis of some Paraguayan samples of that genus (Appendix VI).

It is worth to mention that other Gekkota (*Lygodactylus* and *Hemidactylus*) are poorly represented in GenBank. In fact, our sample of *Lygodactylus wetzeli* currently represents the only known genetic data for the species. This is a species endemic to the Dry Chaco (Norman 1994) and seems like it was never included in any molecular analysis.

4.3.2. Perspectives for future taxonomic revisions

Colubridae: Grazziotin *et al*. (2012) presented an influential paper where they propose several taxonomic modifications, including the description of new genera. These authors synonymized the genus *Lystrophis* and *Waglerophis* with *Xenodon* (Grazziotin *et al*. 2012) although in my analyzes, I found the samples of *X. pulcher* (previously *Lystrophis pulcher*) separated from *X. merremii* (previously *Waglerophis merremii*). The analysis of Grazziotin *et al*. (2012) included the genes 12S and 16S for the genus *Lystrophis*, and Cytb and bdnf for one sample of *Waglerophis merremii*. It is desirable to perform phylogenies in this group using more nuclear data to get more robust relationships in the deep nodes.

With respect to *Phimophis*, Grazziotin *et al*. (2012) used two samples of *Phimophis*: *P. guerini* (GQ457761) and *P. iglesiasi* (JQ598891) and due to polyphyly the authors described the genus *Rodriguesophis* to include the latter species. This genus is characterized by the absence of the loreal scale. Both *P. guerini* and *P. vittatus* have a loreal scale, so

cannot be assigned to *Rodriguesophis*. Thus, a deeper integrative (morphological and molecular) analysis is needed to understand their relationships.

Elapidae: Phylogenetic analyses of *Micrurus* are needed, not only from Paraguay but from South America in general, given that the molecular genetic information for this group of snakes is scarce. In my barcoding analysis there is a polytomy showing samples from Concepción (Humid Chaco ecoregion) clustered with *M. pyrrhocryptus* from Alto Paraguay (Pantanal ecorregion). However, *M. pyrhocryptus* was recorded mostly west to Paraguay River with only one record in the north of Concepción (border with Brazil) (Cacciali *et al*. 2016a), whereas the samples PCS 334 and PCS 337 are from ~150 km southwards. Besides, the coloration pattern of *M. pyrrhocryptus* is different from other *Micrurus* from Paraguay, as it is the only species (in the whole *tricolor* group) with the middle black ring more than twice wider than the external rings (da Silva & Sites 1999), which does not coincide with the pattern of the samples from Concepción.

Viperidae: As Cacciali *et al*. (2006) stated, the diagnostic characters suggested by Silva (2004) and Silva & Rodrigues (2008) for the recognition of seven different taxa previously considered subspecies of *Bothrops neuwiedi* are not reliable to distinguish Paraguayan specimens. Carrasco *et al*. (2012) demonstrated that morphological evidence in this group of snakes does not match the patterns generated by phylogenetic analyses based on molecular genetics. As Machado *et al*. (2014) pointed out this is due to the presence of several cryptic lineages that, for the moment, can be diagnosed only based on molecular genetics. Unfortunately, in their comprehensive analysis of Brazilian *Bothrops* Machado *et al*. (2014) did not have access to Paraguayan samples. For a more comprehensive revision, it is necessary to include also samples from also Uruguay, Argentina, Paraguay, and Bolivia. In addition, the exploration of more morphological characters should be attempted.

Amphisbaenidae: One of the major and latest revisions of Amphisbaenidae including molecular analyses was by Mott & Vieites (2009) and included samples of *A. mertensi* from Paraguay, which clusters with the sample PCS 521. Nevertheless, the sample PCS 314 (*Amphisbaena* sp.) is rendered as a sister clade to the remaining species of the group, and Mott & Vieites (2009) found that it was *A. mertensi* and *A. cunhai* (not recorded in Paraguay) the most basal sister group of the genus. There is no doubt that that sample represents

a different taxon, and more deep analyses have to be done, using as a reference the key work for the identification of Paraguayan amphisbaenians (i.e., Montero & Terol 1999).

4.4. Conservation

One of the major problems for conservation in Paraguay since several years was habitat loss due to extensive soybeans crops in the eastern part of Paraguay (Cartes 2003). Nevertheless, habitat fragmentation is currently also affecting the landscapes of the Occidental Region of Paraguay (Huang *et al.* 2009, Yanosky 2013). Thus, currently, the protected areas are the best strategy for conservation of biodiversity in Paraguay, although many conservation units face legal problems (e.g., lack of: official measurements, management plans, forest guards, infrastructure, etc.) and then the maintenance in time of their biodiversity is not guaranteed (Cartes 2013). Cacciali *et al*. (2015) listed species of reptiles that are absent from protected areas, stating that conservation efforts should be intensified on these taxa. Now, with the addition of new species described in the framework of this project, is necessary to update the species list that need further protection.

One species recently revalidated is *Colobosaura kraepelini*. This lizard is known only by the holotype from the locality of Puerto Max (San Pedro Department), the neotype from Altos and an additional specimen from San Bernardino, both localities in Cordillera Department. There are no protected areas in the Cordillera Department, but there are some in the northern portion of Central Department (border with Cordillera), located less than 10 km from the known localities for *C. kraepelini*. The presence of this species in a conservation unit should be confirmed, but is possible that it is protected by "Monumento Natural Cerro Chororí" and "Monumento Natural Cerro Kõi". It is important to note that the conservation unit closer to the distribution of *C. kraepelini* is the "Parque Nacional Lago Ypacaraí", although only the lagoon is protected and not the surroundings. The species *Homonota septentrionalis* was described from the driest part of Paraguay (northwestern Chaco) and is abundant in the "Parque Nacional Teniente Enciso". The four species of *Tropidurus* found in Paraguay (Appendix III) are well represented in several protected areas.

4.5. Perspectives and conclusions

In this study I present contributions on the taxonomy mostly on lizards from Paraguay (Appendices II-VI). Due to lack of samples, I was not able to deal with a deep taxonomic revision of snakes. Based on the results, I can argue that analyses of Xenodontini and Pseudoboini are currently a pressing research issue. With respect to the family Elapidae, the last revision of *Micrurus* from South America was carried out by da Silva & Sites (1999). Currently several new records were added. Nevertheless, the genetic relationships within the genus remain poorly known. A major revision including samples from different areas of South America is necessary for a better understanding of the taxonomy of the group.

As it was shown before, the *neuwiedi* group of the genus *Bothrops* needs more research in a regional context. Currently I am generating data on the morphology of *Bothrops* from Paraguay with some colleagues, and the plan is to include the data into a regional scale along with colleagues from neighbor countries. Finally, it is important to mention the genus *Ophiodes*. Cacciali & Scott (2012) provided an account on the species of the genus *Ophiodes* in Paraguay. The sample included as "*Ophiodes* sp. PCS 374" is none of the species previously recorded for the country. Most probably, it belongs to some of the three species not formally described by Borges-Martins in his thesis work (Borges-Martins 1998).

Finally, this barcoding project may continue since some colleagues in Paraguay are interested in collaboration. Given that the sequenced specimens are yet a small portion of the actual diversity of Paraguay, it will be of the utmost importance to continue and expand these studies that will further improve our taxonomic knowledge. And it is desirable to not only have Paraguayan scientists substantially involved but to see them taking the lead of high quality taxonomic research.

5. CHECKLIST

Species checklist of Squamata from Paraguay based on Cacciali *et al*. (2016a), with the inclusion of the taxonomic modifications accomplished here, and additional taxonomical changes according to Cabral *et al*. (2017), Cacciali & Scott (2015), and Cacciali *et al*. (2016b). The taxonomy for high level taxa follows Pyron *et al*. (2013).

Gekkota

Phyllodactylidae
Homonota borellii (Peracca, 1897)
Homonota horrida (Burmesiter, 1861)
Homonota rupicola Cacciali, Ávila & Bauer, 2007
Homonota septentrionalis Cacciali, Morando, Medina, Köhler, Motte & Avila, 2017
Phyllopezus przewalskii Koslowsky, 1895

Gekkonidae
Hemidactylus mabouia (Moreau de Jonnès, 1818)
Lygodactylus wetzeli (Smith, Martin & Swain, 1977)

Scincoidea

Scincidae
Aspronema dorsivittatum (Cope, 1862)
Copeoglossum nigropunctatum (Spix, 1825)
Manciola guaporicola (Dunn, 1935)
Notomabuya frenata (Cope, 1862)

Lacertoidea

Teiidae
Ameiva ameiva (Linnaeus, 1758)
Ameivula abalosi (Cabrera, 2012)
Dracaena paraguayensis Amaral, 1950
Kentropyx viridistriga (Boulenger, 1894)
Salvator duseni (Lönnberg, 1910)
Salvator merianae Duméril & Bibron, 1839
Salvator rufescens (Günther, 1871)
Teius oculatus (D'Orbigny & Bibron, 1837)
Teius teyou (Daudin, 1802)

Gymnophthalmidae
Bachia bresslaui (Amaral, 1935)
Cercosaura ocellata Wagler, 1830
Cercosaura schreibersii (Wiegmann, 1834)
Colobosaura kraepelini (Werner, 1910)
Colobosaura modesta (Reinhardt & Lütken, 1862)
Micrablepharus maximiliani (Reinhardt & Lütken, 1862)
Vanzosaura rubricauda (Boulenger, 1902)

Amphisbaenidae
Amphisbaena alba Linnaeus, 1758
Amphisbaena albocingulata Boettger, 1885
Amphisbaena angustifrons Cope, 1861
Amphisbaena bolivica Mertens, 1929
Amphisbaena camura Cope, 1862
Amphisbaena darwini Duméril & Bibron (1839)
Amphisbaena leeseri Gans, 1964
Amphisbaena mertensii Strauch, 1881
Amphisbaena microcephalum (Wagler, 1824)
Amphisbaena prunicolor (Cope, 1885)
Amphisbaena roberti (Gans, 1964)
Amphisbaena steindachneri (Strauch, 1881)

Anguimorpha
Anguidae
Ophiodes fragilis (Raddi, 1820)
Ophiodes intermedius Boulenger, 1894
Ophiodes luciae Cacciali & Scott, 2015
Ophiodes striatus (Spix, 1824)
Ophiodes aff. striatus

Iguania
Tropiduridae
Stenocercus caducus (Cope, 1862)
Tropidurus catalanensis Cei, 1982
Tropidurus spinulosus (Cope, 1862)
Tropidurus lagunablanca Carvalho, 2016
Tropidurus torquatus (Wied-Neuwied, 1820)

Iguanidae
Iguana iguana (Linnaeus, 1758)

Polychrotidae
Polychrus acutirostris Spix, 1825

Leiosauridae
Anisolepis longicauda (Boulenger, 1891)

Liolaemidae
Liolaemus azarai Ávila, 2003
Liolaemus chacoensis Shreve, 1948

Dactyloidae
Norops meridionalis Boettger, 1885

Serpentes
Boidae
Boa constrictor Linnaeus 1758
Epicrates alvarezi Abalos, Báez & Nader, 1964
Epicrates crassus Cope, 1862
Eunectes murinus (Linnaeus, 1758)

Eunectes notaeus Cope, 1862

Viperidae
Bothrops alternatus Duméril, Bibron & Duméril, 1854
Bothrops diporus Cope, 1862
Bothrops jararaca (Wied, 1824)
Bothrops jararacussu Lacerda, 1884
Bothrops moojeni Hoge, 1966
Bothrops pauloensis Amaral, 1925
Crotalus durissus Linnaeus, 1758

Elapidae
Micrurus altirostris (Cope, 1860)
Micrurus baliocoryphus (Cope, 1862)
Micrurus corallinus (Merrem, 1820)
Micrurus frontalis (Duméril, Bibron & Duméril, 1854)
Micrurus lemniscatus (Linnaeus, 1758)
Micrurus pyrrhocryptus (Cope, 1862)
Micrurus silviae Di-Bernardo, Borges-Martins & Silva, 2007

Colubridae-Colubrinae
Chironius bicarinatus (Wied, 1820)
Chironius exoletus (Linnaeus, 1758)
Chironius flavolineatus (Jan, 1863)
Chironius maculoventris Dixon, Wiest & Cei, 1993
Chironius quadricarinatus (Boie, 1827)
Drymarchon corais (Boie, 1827)
Drymoluber brazili (Gomes, 1918)
Leptophis ahaetulla (Linnaeus, 1758)
Mastigodryas bifossatus (Raddi, 1820)
Simophis rhinostoma (Schlegel, 1837)
Spilotes pullatus (Linnaeus, 1758)
Tantilla melanocephala (Linnaeus, 1758)

Colubridae-Dipsadinae
Atractus paraguayensis Werner, 1924
Atractus reticulatus (Boulenger, 1885)
Atractus thalesdelemai Passos, Fernandes & Zanella, 2005

Colubridae-Dipsadinae-Imantodini
Imantodes cenchoa (Linnaeus, 1758)

Colubridae-Dipsadinae -Dipsadini
Dipsas bucephala (Shaw, 1802)
Dipsas cisticeps (Boettger, 1885)
Sibynomorphus lavillai Scrocchi, Puorto & Rey, 1993
Sibynomorphus mikanii (Schlegel, 1837)
Sibynomorphus turgidus (Cope, 1868)
Sibynomorphus ventrimaculatus (Boulenger, 1885)

Colubridae-Dipsadinae-Psomophiini
Psomophis genimaculatus (Boettger, 1885)

Psomophis obtusus (Cope, 1864)

Colubridae-Dipsadinae-Elapomorphini
Apostolepis ambiniger (Peters, 1869)
Apostolepis assimilis (Reinhardt, 1861)
Apostolepis barrioi Lema, 1978
Apostolepis dimidiata (Jan, 1862)
Apostolepis intermedia Koslowsky, 1898
Phalotris lemniscatus (Duméril, Bibron & Duméril, 1854)
Phalotris matogrossensis Lema, D′Agostini & Cappellari, 2005
Phalotris nigrilatus Ferrarezzi, 1993
Phalotris normanscotti Cabral & Cacciali, 2015
Phalotris tricolor (Duméril, Bibron & Duméril, 1854)

Colubridae-Dipsadinae-Tachymenini
Thamnodynastes chaquensis Bergna & Álvarez, 1993
Thamnodynastes hypoconia (Cope, 1860)
Thamnodynastes lanei Bailey, Thomas & da Silva Jr., 2005
Thamnodynastes strigatus (Günther, 1858)
Tomodon dorsatus Duméril, Bibron & Duméril, 1854
Tomodon ocellatus Duméril, Bibron & Duméril, 1854

Colubridae-Dipsadinae-Echinantherini
Taeniophallus occipitalis (Jan, 1863)

Colubridae-Dipsadinae-Pseudoboini
Boiruna maculata (Boulenger, 1896)
Clelia clelia (Daudin, 1803)
Clelia plumbea (Wied, 1820)
Mussurana bicolor (Peracca, 1904)
Mussurana quimi Franco, Marques & Puorto, 1997
Oxyrhopus guibei Hoge & Romano, 1977
Oxyrhopus petolarius (Linnaeus, 1758)
Oxyrhopus rhombifer Duméril, Bibron & Duméril, 1854
Phimophis guerini (Duméril, Bibron & Duméril, 1854)
Phimophis vittatus (Boulenger, 1896)
Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)
Rhachidelus brazili Boulenger, 1908

Colubridae-Dipsadinae-Phylodryadini
Philodryas aestiva (Duméril, Bibron & Duméril, 1854)
Philodryas agassizii (Jan, 1863)
Philodryas baroni Berg, 1895
Philodryas erlandi Lönnberg, 1902
Philodryas livida (Amaral, 1923)
Philodryas mattogrossensis Koslowsky, 1898
Philodryas nattereri Steindachner, 1870
Philodryas olfersii (Lichtenstein, 1823)
Philodryas patagoniensis (Girard, 1858)
Philodryas psammophidea Günther, 1872

Colubridae-Dipsadinae-Hydropsini

Helicops infrataeniatus Jan, 1865
Helicops leopardinus (Schlegel, 1837)
Hydrops caesurus Scrocchi, Ferreira, Giraudo, Avila & Motte, 2005
Pseudoeryx plicatilis (Linnaeus, 1758)

Colubridae-Dipsadinae-Hydrodynastini
Hydrodynastes gigas (Duméril, Bibron & Duméril, 1854)

Colubridae-Dipsadinae-Xenodontini
Erythrolamprus aesculapii (Linnaeus, 1766)
Erythrolamprus albertguentheri Grazziotin, Zaher, Murphy, Scrocchi, Benavides, Zhang & Bonatto, 2012
Erythrolamprus almadensis (Wagler, 1824)
Erythrolamprus frenatus (Werner, 1909)
Erythrolamprus jaegeri (Günther, 1858)
Erythrolamprus miliaris (Linnaeus, 1758)
Erythrolamprus poecilogyrus (Wied, 1825)
Erythrolamprus reginae (Linnaeus, 1758)
Erythrolamprus sagittifer (Jan, 1863)
Erythrolamprus semiaureus (Cope, 1862d)
Erythrolamprus typhlus (Linnaeus, 1758)
Lygophis anomalus (Günther, 1858)
Lygophis dilepis (Cope, 1862d)
Lygophis flavifrenatus Cope, 1862
Lygophis meridionalis (Schenkel, 1901)
Lygophis paucidens Hoge, 1953
Xenodon dorbignyi (Duméril, Bibron & Duméril, 1854)
Xenodon histricus (Jan, 1863)
Xenodon merremi (Wagler, 1824)
Xenodon neuwiedii (Günther, 1863)
Xenodon pulcher (Jan, 1863)

Colubridae-Dipsadinae-Incertae Sedis
Xenopholis undulatus (Jensen, 1900)

Anomalepidae
Liotyphlops beui (Amaral, 1924)
Liotyphlops ternetzii (Boulenger, 1896)

Leptotyphlopidae
Epictia albipuncta (Burmeister, 1861)
*Epictia vellardi (*Laurent 1984)

Typhlopidae
Amerotyphlops brongersmianus (Vanzolini, 1972)

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List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees.

Species	Voucher	Locality	Lat/Long
Ameiva ameiva	ALA 3502	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Ameiva ameiva	ALA 3518	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Ameiva ameiva	GK 3611	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Ameiva ameiva	MNHNP 11823	Cabaña Pindú, San Pedro Department	-24,2036 / -56,4249
Ameiva ameiva	MNHNP 11825	Asentamiento San Ramón, Concepción Department	-22,8828 / -57,4041
Ameiva ameiva	MNHNP 11828	Estancia Kumaré, Concepción Department	-22,8975 / -57,4013
Ameiva ameiva	MNHNP 11829	Estancia Kumaré, Concepción Department	-22,8975 / -57,4013
Ameiva ameiva	MNHNP 11834	Retiro de Estancia Kumaré, Concepción Department	-22,9377 / -57,3954
Ameiva ameiva	PCS 300	Estancia Achira, Concepción Department	-25,1270 / -57,2263
Ameiva ameiva	PCS 306	Estancia La Susanita, Ñeembucú Department	-26,1545 / -58,0653
Ameiva ameiva	PCS 407	Retiro 7 de Junio, Estancia Garay Cue, Concepción Department	-22,5830 / -57,3278
Ameiva ameiva	PCS 572	Ocampo Cué, San Pedro Department	-23,8802 / -57,1050
Ameiva ameiva	PCS 595	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Ameivula ocellifera	MNHNP 11826	Asentamiento San Ramón, Concepción Department	-22,8828 / -57,4041
Ameivula ocellifera	MNHNP 11830	Estancia Kumaré, Concepción Department	-22,8975 / -57,4013
Ameivula ocellifera	PC 17	Quinta Las Andreas, Cordillera Department	-25,2250 / -57,2410

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Ameivula ocellifera	PCS 403	Retiro 7 de Junio, Estancia Garay Cue, Concepción Department	-22,5830 / -57,3278
Amerotyphlops brongersmianus	GK 3675	Estancia Schoder, Concepción Department	-23,4724 / -57,2363
Amerotyphlops brongersmianus	GK 3813	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Amerotyphlops brongersmianus	GK 3908	Areguá, Central Department	-25,3156 / -57,3771
Amphisbaena bolivica	GK 3687	Estancia Amistad, Boquerón Department	-22,4077 / -60,7259
Amphisbaena bolivica	PCS 542	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Amphisbaena prunicolor	PCS 521	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Amphisbaena sp.	PCS 314	Tobich, Alto Paraguay Department	-20,2913 / -58,5636
Boiruna maculata	GK 3722	Filadelfia, Boquerón Department	-22,3512 / -60,0544
Boiruna maculata	Rel 11 2 12	Paraguay	
Bothrops alternatus	PCS 333	Belén, Concepción Department	-23,4445 / -57,3544
Bothrops neuwiedii	PCS 302	Estancia Achira, Concepción Department	-25,1277/ -57,2263
Bothrops neuwiedii	PCS 318	Tobich, Alto Paraguay Department	-20,2913 / -58,5636
Bothrops neuwiedii	PCS 331	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Bothrops neuwiedii	PCS 504	Laguna Blanca, San Pedro Department	-23,7572 / -56,3226
Bothrops neuwiedii	PCS 530	Guairá Department	

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Bothrops neuwiedii	R 14	Quinta Las Andreas, Cordillera Department	-25,2250 / -57,2410
Cercosaura schreibersii	PCS 410	Estancia Garay Cué, Concepción Department	-22,7096 / -57,3778
Cercosaura sp.	ALA 3498	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Chironius maculoventris	GK 3720	Filadelfia, Boquerón Department	-22,3512 / -60,0544
Chironius maculoventris	PCS 319	Ruta 9, Km 398, Boquerón Department	-22,8645 / -59,5876
Clelia clelia	PCS 468	Estancia Ka'i Rague, Amambay Department	-23,2643 / -56,2038
Colobosaura kraepelini	GK 3852	San Bernardino, Cordillera Department	-25,2588 / -57,2850
Epicrates alvarezi	GK 3678	Filadelfia, Boquerón Department	-22,3389 / -60,0349
Epicrates crassus	PCS 517	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Erythrolamprus aesculapii	PCS 327	Belén, Concepción Department	-23,4445 / -57,3544
Erythrolamprus poecilogyrus	GK 3734	Estancia Amistad, Boquerón Department	-22,4077 / -60,7259
Erythrolamprus poecilogyrus	PCS 309	Estancia Agatapé, Ñeembucú Department	-26,1102 / -58,0180
Erythrolamprus poecilogyrus	PCS 315	Tobich, Alto Paraguay Department	-20,2913 / -58,5636
Erythrolamprus poecilogyrus	PCS 599	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Erythrolamprus reginae	PCS 529	Granja SHS, Guairá Department	-25,7209 / -56,2512
Erythrolamprus sagittifer	GK 3747	Filadelfia, Boquerón Department	-22,3470 / -60,0489

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Erythrolamprus semiaureus	GK 3789	Laguna Capitán, Presidente Hayes Department	-22,5352 / -59,6791
Helicops leopardinus	GK 3612	Río Paraguay, Concepción Department	-23,4174 / -57,4526
Helicops leopardinus	PCS 600	Ruta 9, Km 68,5, Presidente Hayes Department	-24,8460 / -57,7440
Hemidactylus maebouia	PCS 597	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Homonota septentrionalis	MNHNP 11873	Mariscal Estigarribia, Boquerón Department	-22,0365 / -60,6168
Homonota septentrionalis	MNHNP 12238	Fortín Mayor Infante Rivarola, Boquerón Department	-21,6790 / -62,4010
Homonota septentrionalis	SMF 101984	Fortín Mayor Infante Rivarola, Boquerón Department	-21,6790 / -62,4010
Homonota marthae	GK 3731	Filadelfia, Boquerón Department	-22,3470 / -60,0489
Hydrodynastes gigas	PC 29	Ruta 3, km 121, San Pedro Department	-24,7432 / -56,6285
Kentropyx viridistriga	PCS 495	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Leptophis ahaetulla	GK 3594	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Leptophis ahaetulla	GK 3809	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Leptophis ahaetulla	PCS 532	Granja SHS, Guairá Department	-25,7209 / -56,2512
Liolaemus azarai	GK 3919	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318
Liolaemus azarai	GK 3927	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318
Lygodactylus wetzeli	GK 3689	Estancia Amistad, Boquerón Department	-22,4077 / -60,7259

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Lygophis dilepis	GK 3808	Road Pozo Colorado - Concepcion, Presidente Hayes Department	-23,4709 / -57,6245
Manciola guaporicola	CZPLT 567	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Mastigodryas bifossatus	GK 3993	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Mastigodryas bifossatus	PCS 325	Ruta Nueva Colombia – Altos, Cordillera Department	-25,1453 / -57,2598
Micrablepharus maximiliani	PCS 519	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Micrurus frontalis	GK 3932	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318
Micrurus pyrrhocryptus	PCS 310	5 km de N of Toro Pampa, Alto Paraguay Department	-20,9282 / -58,5968
Micrurus sp.	PCS 334	Belén, Concepción Department	-23,4445 / -57,3544
Micrurus sp.	PCS 337	Belén, Concepción Department	-23,4445 / -57,3544
Mussurana bicolor	GK 3621	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Mussurana bicolor	PCS 330	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Notomabuya frenata	ALA 3516	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Notomabuya frenata	GK 3846	San Bernardino, Cordillera Department	-25,2944 / -57,3126
Notomabuya frenata	PCS 313	Tobich, Alto Paraguay Department	-20,2913 / -58,5636
Notomabuya frenata	PCS 496	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Notomabuya frenata	PCS 538	Granja SHS, Guairá Department	-25,7209 / -56,2512

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Ophiodes intermedius	GK 3854	San Bernardino, Cordillera Department	-25,3074 / -57,3029
Ophiodes intermedius	GK 3864	Areguá, Cordillera Department	-25,3145 / -57,3753
Ophiodes intermedius	PCS 332	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Ophiodes intermedius	PCS 608	Near Estancia La Rural, Presidente Hayes Department	-24,8168 / -57,7781
Ophiodes intermedius	PCS 615	Near Estancia La Rural, Presidente Hayes Department	-24,8168 / -57,7781
Ophiodes sp.	PCS 374	Estancia Garay Cué, Concepción Department	-22,7096 / -57,3778
Oxyrhopus guibei	ALA 3517	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Oxyrhopus guibei	PCS 618	Estancia Achira, Cordillera Department	-25,1277 / -57,2263
Phalotris matogrossensis	PCS 329	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Phalotris matogrossensis	PCS 335	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Philodryas olfersii	GK 3937	Granja El Roble, Concepción Department	-23,4560 / -57,2803
Philodryas patagoniensis	PCS 311	Road Loma Plata - Tobich, Alto Paraguay Department	-21,8695 / -58,6221
Philodryas patagoniensis	PCS 408	Estancia Garay Cué, Concepción Department	-22,7096 / -57,3778
Philodryas patagoniensis	PCS 605	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Philodryas patagoniensis	PCS 622	27 km ESE of 25 de Diciembre, Cordillera Department	-24,9084 / -56,9742
Phimophis guerini	GK 3934	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Phimophis vittatus	GK 3728	Filadelfia, Boquerón Department	-22,3470 / -60,0489
Phimophis vittatus	GK 3775	12 km E Filadelfia, Boquerón Department	-22,3347 / -60,1578
Phyllopezus przewalskii	MNHNP 11957	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Phyllopezus przewalskii	MNHNP 11958	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Phyllopezus przewalskii	SMF 100495	Estancia Amistad, Boquerón Department	-22,4077 / -60,7259
Phyllopezus sp. nov.	MNHNPTH 2 39	Cerro de Tobatí, Cordillera Department	-25,2797 / -57,0925
Phyllopezus sp. nov.	MNHNPTH 2 40	Cerro de Tobatí, Cordillera Department	-25,2797 / -57,0925
Polychrus acutirostris	GK 3802	Near Granja El Roble, Concepción Department	-23,4551 / -57,2858
Polychrus acutirostris	GK 3807	1 km E Concepción, Presidente Hayes Department	-23,4541 / -57,4676
Polychrus acutirostris	PCS 612	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Psomophis genimaculatus	GK 3791	E of Filadelfia, Boquerón Department	-22,5585 / -59,4885
Salvator merianae	PCS 437	Estancia Ka'i Ragüe, Amambay Department	-23,2469 / -56,2099
Sibynomorphus turgidus	PCS 528	Melgarejo, Guairá	-25,7193 / -56,2346
Stenocercus caducus	PC 43	Dr. Juan E. Estigarribia, Caaguazú Department	-25,3672 / -55,7127
Stenocercus caducus	PCS 498	Laguna Blanca, San Pedro Department	-23,8103 / -56,2954
Teius oculatus	MNHNP 11802	Distrito Itakyry, Alto Paraná Department	-24,9830 / -55,0827

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Teius oculatus	MNHNP 11803	Distrito Itakyry, Alto Paraná Department	-24,9830 / -55,0827
Teius oculatus	MNHNP 11836	Parque Nacional San Rafael, Itapúa Department	-26,5115 / -55,7924
Teius teyou	MNHNP 11813	Agroganadera Solito, Presidente Hayes Department	-24,2908 / -58,8372
Teius teyou	PCS 215	Quinta Las Andreas, Cordillera Department	-25,2250 / -57,2410
Teius teyou	PCS 563	Rancho 068, San Pedro Department	-23,7089 / -56,3596
Thamnodynastes chaquensis	GK 3638	Road Concepción/Pozo Colorado, Presidente Hayes Department	-23,4664 / -57,6071
Thamnodynastes hypoconia	PCS 610	Near Estancia La Rural, Presidente Hayes Department	-24,8023 / -57,7882
Thamnodynastes hypoconia	PCS 611	Estancia La Rural, Presidente Hayes Department	-24,8290 / -57,7670
Tropidurus catalanensis	GK 3918	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318
Tropidurus catalanensis	GK 3921	Isla Yacyretá, Misiones Department	-27,4306 / -56,7318
Tropidurus etheridgei	GK 3726	Filadelfia, Boquerón Department	-22,3470 / -60,0489
Tropidurus etheridgei	GK 3727	Filadelfia, Boquerón Department	-22,3470 / -60,0489
Tropidurus guarani	GK 3845	San Bernardino, Cordillera Department	-25,3074 / -57,3029
Tropidurus guarani	GK 3850	San Bernardino, Cordillera Department	-25,3074 / -57,3029
Tropidurus lagunablanca	PCS 545	Laguna Blanca, San Pedro Department	-23,8174 / -56,2910
Tropidurus spinulosus	GK 3790	Estancia Amistad, Boquerón Department	-22,4069 / -60,7285

List of species and vouchers (with details on locality data) from Paraguay used for genetic analyses. Latidude (Lat) and Longitude (Long) are given in decimal degrees. CONT.

Species	Voucher	Locality	Lat/Long
Tropidurus spinulosus	NN	Estación Biológica Tres Gigantes, Alto Paraguay Department	-20,0810 / -58,1651
Tropidurus spinulosus	PCS 312	Tobich, Alto Paraguay Department	-20,2913 / -58,5636
Tropidurus tarara	PCS 412	Retiro 7 de Junio, Estancia Garay Cue, Concepción Department	-22,5830 / -57,3278
Tropidurus tarara	PCS 413	Retiro 7 de Junio, Estancia Garay Cue, Concepción Department	-22,5830 / -57,3278
Tropidurus tarara	PCS 426	Estancia Garay Cué, Concepción Department	-22,7096 / -57,3778
Tropidurus teyumirim	AMNHFS 20281	Parque Nacional Ybycuí, Paraguarí Department	-26,0954 / -56,8387
Vanzosaura rubricauda	ALA 3513	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Vanzosaura rubricauda	ALA 3514	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Vanzosaura rubricauda	ALA 3528	Parque Nacional Cerro Cora, Amambay Department	-22,6502 / -56,0141
Vanzosaura rubricauda	GK 3801	Estancia Amistad, Boquerón Department	-22,4069 / -60,7285
Xenodon merremi	GK 3821	San Bernardino, Cordillera Department	-25,3074 / -57,3029
Xenodon merremi	PCS 531	Guairá Department	
Xenodon pulcher	GK 3773	Filadelfia, Boquerón Department	-22,3470 / -60,0489
Xenodon pulcher	GK 3778	Kleefeld, Boquerón Department	-22,3406 / -60,0088

APPENDIX II

Declaration on the contributions of authors

to the publication: Revision of the phylogeny and chorology of the tribe Iphisini with the revalidation of *Colobosaura kraepelini* Werner, 1910 (Reptilia, Squamata, Gymnophthalmidae).
Status: Published (2017).
Name of the journal: ZooKeys 669
Authors involved: Pier Cacciali (PC), Nicolás Martínez (NM), Gunther Köhler (GK).

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor NM: 5%
Coauthor GK: 25%

(2) to the implementation of the respective studies and experiments

PhD candidate: 80% – molecular analyses, morphological analyses, revision of museum vouchers.
Coauthor NM: 5% – revision of museum vouchers.
Coauthor GK: 15% – field work (collecting and documenting specimens).

(3) to the creation of the data collection and figures

PhD candidate: 80% – created database, sequenced DNA barcodes, created figures, created maps.
Coauthor NM: 10% – provided photographs.
Coauthor GK: 10% – provided photographs.

(4) to the analysis and interpretation of the data

PhD candidate: 80% – analysis and interpretation of molecular, morphological, and biogeographical data.
Coauthor NM: 5% – contributed to data analysis and interpretation.
Coauthor GK: 15% – contributed to data analysis and interpretation.

(5) to writing the manuscript

PhD candidate: 90%
Coauthor GK: 10%

Date/place: _____

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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RESEARCH ARTICLE

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Revision of the phylogeny and chorology of the tribe Iphisini with the revalidation of *Colobosaura kraepelini* Werner, 1910 (Reptilia, Squamata, Gymnophthalmidae)

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Abstract

The family Gymnophthalmidae contains nearly 235 species with a distribution range from southern Mexico to central Argentina as well as in the Antilles. Among gymnophthalmids, the genus *Colobosaura* is a member of the tribe Iphisini, and currently is considered monotypic (*C. modesta*). The diversity of the tribe was studied recently, with the erection of several new genera. In this work genetic and morphological data of specimens of *Colobosaura* recently collected in Paraguay were analyzed. Genetic (16S barcode) data indicate that these samples are not conspecific with *C. modesta* and they are allocated to the nominal species *C. kraepelini*. Because the original primary type of the latter taxon is considered to be lost, a neotype (SMF 101370) is designated for this species and a redescription provided based on our material. *Colobosaura kraepelini* is distributed in the Humid Chaco, being the only member of the whole tribe in this ecoregion.

Keywords

16S barcodes, Humid Chaco, neotype, Paraguay, taxonomy

Introduction

Gymnophthalmids are among the least known Neotropical lizards given their secretive habits and small size, and some of them are known only from the original description (Castoe et al. 2004). Currently, 232 species of gymnophthalmid lizards are recognized (Goicochea et al. 2016) with a geographic distribution ranging from Argentina widely across South America to southern Mexico, including some Caribbean islands (Doan 2003, Vitt and Caldwell 2009), with several recently described taxa from the Caatinga and the Cerrado (Ribeiro Delfim et al. 2006). In fact, Cacciali (2010) pointed out the high diversity of gymnophthalmid lizards in the Paraguayan Cerrado with respect to other ecoregions in the country.

In the last decade, this family has been analyzed from a molecular perspective, leading to some changes in phylogenetic hypotheses (Castoe et al. 2004, Rodrigues et al. 2007, Peloso et al. 2011).

One of the genera that underwent taxonomic modifications is *Colobosaura*, which was established by Boulenger (1887) to include *Perodactylus modestus* Reinhardt & Lütken, 1862 described from Morro da Garça, Minas Gerais, Brazil. Somewhat later, Werner (1910) described *Perodactylus kraepelini* from Puerto Max, Concepción, Paraguay. Amaral (1933) considered *C. kraepelini* to be a synonym of *C. modesta* attributing the observed morphological variation to sexual dimorphism. In that contribution the author described *Colobosaura mentalis* which was later transferred to the genus *Acratosaura* by Rodrigues et al. (2009a). Burt and Burt (1933) recognized *C. kraepelini* as a valid species, a view followed by Peters and Donoso-Barros (1970) and Talbot (1979). Vanzolini and Ramos (1977) stated that the description of *C. kraepelini* is brief and not very informative so they suggested that the type specimen must be carefully analyzed to reach more solid taxonomic decisions. However, the type specimen of *C. kraepelini* (originally deposited in the Hamburg Zoological Museum) is considered to be lost (Rodrigues et al. 2007).

In this work, and in the framework of a DNA barcoding project of the Paraguayan herpetofauna, genetic and morphology data of recently collected specimens of *Colobosaura* tentatively assigned to *C. kraepelini* were analyzed, providing a redescription of its external morphology and information on its taxonomic status.

Materials and methods

Tissue samples for genetic analyses were extracted and stored as recommended by Gamble (2014). The protocol for DNA extraction follows Ivanova et al. (2006). Samples were washed in 50 µl of diluted PBS buffer (1:9 of buffer and water respectively) for 14 h. A solution of vertebrate lysis buffer and proteinase K (60:6 µl respectively), kept at 56°C for 14 h was used for digestion. After extraction, DNA samples were eluted in 50 µL TE buffer. Amplification of mitochondrial 16S rRNA gene fragments was performed using the eurofins MWG Operon primers L2510 (forward: 5'–CGCCT-

GTTTATCAAAAACAT–3') and H3056 (reverse: 5'–CCGGTCTGAACTCAGATCACGT–3') in an Eppendorf Mastercycler pro. The PCR conditions were: denaturation 2 min (94°C) – denaturation 35 sec (94°C)×40 – hybridization 35 sec (48.5°C) – elongation 60 sec (72°C) – final elongation 10 min (72°C). The examination of DNA chromatograms and development of consensus sequences were performed with SeqTrace 0.9.0 (Stucky 2012).

The mtDNA 16S sample was compared with sequences available in GenBank for species of the most closely related clade (Iphisini: Gymnophthalminae, according to Colli et al. 2015), and a sample of *Cercosaura ocellata* (Cercosaurinae) as an outgroup. GenBank accession numbers and localities of genetic samples are provided in Appendix. It is important to note that currently the tribe Iphisini is composed of four monotypic genera (*Alexandresaurus*, *Colobosaura*, *Iphisa*, and *Stenolepis*) and two genera with two species (*Acratosaura* and *Rondonops*) (Colli et al. 2015), but we only had access to five of the eight species, missing *Acratosaura spinosa*, *Rondonops biscutatus*, and *R. xanthomystax*.

Sequences were aligned with Clustal W (Larkin et al. 2007) followed by a visual inspection and edition if necessary. Final sequence length was 512 bp. The best substitution model was chosen according to the corrected Akaike Information Criterion (AICc) (Burnham and Anderson 2002). We estimated the uncorrected genetic pairwise distances for our dataset, and performed a Maximum Likelihood (ML) analysis for a phylogenetic inference with 10,000 replicates. All these steps were executed in MEGA 6 (Tamura et al. 2013). We used FigTree v1.3.1 for tree editing (http://tree.bio.ed.ac.uk/software/figtree/).

Additionally, the external morphology of specimens of *Colobosaura* was examined (Appendix 2). We scored the following morphometric characters: snout–vent length (SVL) from the tip of the snout to the anterior edge of the cloaca; head length (HL) from the tip of the snout to the anterior edge of the ear opening; head width (HW) measured at the widest section of the head; eye diameter (ED); and ear opening (EO), both taken at the widest section. These measures (except SVL taken with a ruler) and other standard measurements were taken with digital calipers. Paired structures are presented in left/right orientation. In the color descriptions, the capitalized colors and the color codes (in parentheses) are those of Köhler (2012).

A distribution map was generated for the species of the tribe Iphisini to compare ecoregional affinities of the two species of *Colobosaura* and its closest relatives. Ecoregional information is based on Olson et al. (2001), downloaded from the web site of The Nature Conservancy (http://maps.tnc.org/gis_data.html). All coordinates are in decimal degrees and WGS 84 datum, and all the elevations are in meters above sea level. Geographic imagery produced using ArcMap 10.3. Minimum convex polygons were produced upon about 200 bibliographic records based on Brito et al. (2012) for *Acratosaura mentalis*; Rodrigues et al. (2009a) and Freitas et al. (2012) for *A. spinosa*; Freire et al. (2013) and Freitas (2014) for *Alexandresaurus camacan*; Nogueira (2001), Rodrigues et al. (2007), Cuoto-Ferreira et al. (2011), Cardozo Ribeiro et al. (2012), Freire et al. (2012), Cavalcanti et al. (2014), López Santos et al. (2014), da Silva et al.

(2015), Cacciali et al. (2016), and De Alcantara et al. (2016), for *Colobosaura modesta*; Avila-Pires (1995) and Castoe et al. (2004) for *Iphisa elegans*; Colli et al. (2015) for *Rondonops biscutatus* and *R. xanthomystax*; and Rodrigues et al. (2007) for *Stenolepis ridleyi*.

Acronyms of institutions used in the text are **SMF** (Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Frankfurt am Main, Germany), **LG** (Laboratorio de Citogenetica de Vertebrados, Universidade de São Paulo, Brazil), and **MNHNP** (Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay).

Results

The best substitution model was GTR+G, and the phylogeny recovered is shown in Figure 1. Our genetic sample of *Colobosaura* (SMF 101370) is sister to, but deeply divergent from *C. modesta*. A similar arrangement is observed between *Acratosaura mentalis* and *Stenolepis ridleyi* which constitute the sister clade of *Colobosaura*. *Iphisa elegans* is recovered as a sister clade of the above mentioned groups, and *Alexandresaurus camacan* as the most basal representative of the tribe.

The pairwise distance shows a divergence of ~7.7% between *C. modesta* and SMF 101370, which is even higher than the divergence between SMF 101370 and *I. elegans* (~7.1%), SMF 101370 and *S. ridleyi* (~5.5%), *C. modesta* and *S. ridleyi* (~4.7%), or *A. mentalis* and *S. ridleyi* (~3.1%) (Table 1).

From the distribution it is possible to identify two groups within the tribe Iphisini: one strongly related to Amazonian ecoregions (*Iphisa* and *Rondonops*), and another linked to the Dry Diagonal (*Acratosaura*, *Alexandresaurus*, *Colobosaura*, and *Stenolepis*). Two monotypic genera (*Alexandresaurus* and *Stenolepis*) and *Acratosaura spinosa* are mainly associated to Caatinga environments, whereas *Acratosaura mentalis* have some records in Cerrado. *Colobosaura modesta* together with *Iphisa elegans* has the widest distribution, and it is strongly linked to Caatinga and Cerrado. The collecting site of SMF 101370 is in the Humid Chaco (Fig. 2).

The genetic data presented above demonstrate that our sample SMF 101370 is not conspecific with *C. modesta*. The only other available nominal species that SMF 101370 could be assigned to is *Colobosaura kraepelini* Werner, 1910. Unfortunately, the holotype and only known specimen of this taxon is considered to be lost (see above) and its original description is brief. Therefore, there is no morphological basis to support our claim that SMF 101370 is conspecific with *C. kraepelini* which leaves us with two options: The more conservative option is to assign SMF 101370 to *C. kraepelini* whereas the alternative would be to describe a new species based on our sample. Since we know of no diagnostic character that would differentiate between SMF 101370 and *C. kraepelini*, we think that the better option is to assign SMF 101370 to *C. kraepelini.* Thus, we herewith designate SMF 101370, a subadult male from 2.5 km E of Altos (25.2588°S, 57.2850°W, ca 280 masl), Cordillera Department, Paraguay, collected

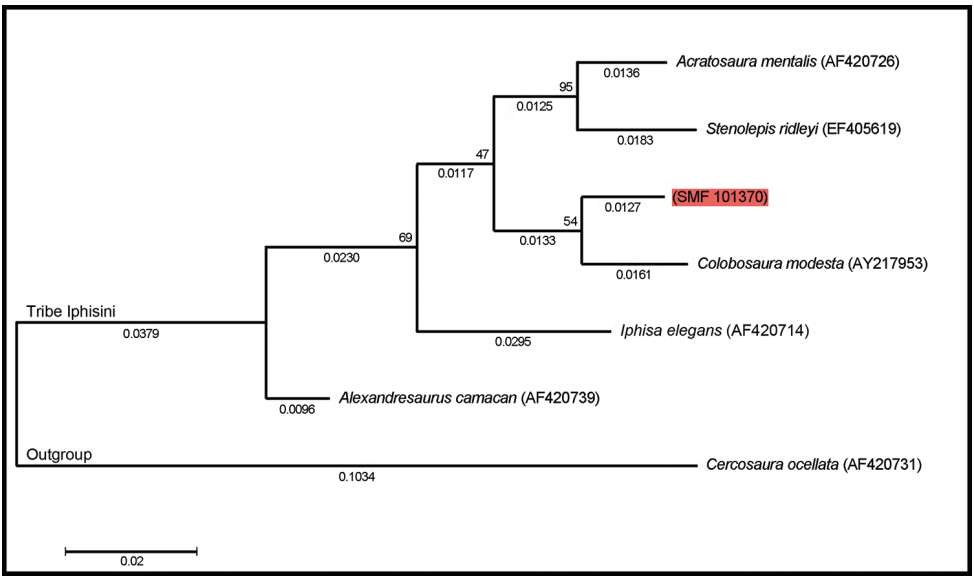

Figure 1. Maximum Likelihood tree obtained from 16S mtDNA for the tribe Iphisini (Gymnophthalmidae). Numbers on the nodes represent the bootstrap values and numbers below branches (and scale bar at the bottom left corner) denote branch length (substitutions/site). Specimen highlighted in red indicates our sample. See Appendix 1 for details of specimens used in the analysis.

Table 1. Pairwise genetic distances (lower-left diagonal), and SD (upper-right diagonal) among species of Iphisini: Gymnophthalminae.

	A. mentalis	*A. camacan*	(SMF 101370)	*C. modesta*	*I. elegans*	*S. ridleyi*
Acratosaura mentalis		0.016	0.016	0.013	0.015	0.001
Alexandresaurus camacan	0.122		0.015	0.015	0.013	0.011
Colobosaura (SMF 101370)	0.122	0.101		0.013	0.012	0.011
Colobosaura modesta	0.079	0.103	0.077		0.013	0.010
Iphisa elegans	0.103	0.089	0.071	0.087		0.011
Stenolepis ridleyi	0.031	0.055	0.055	0.047	0.060	

on 27 February 2012 by Gunther Köhler, as the neotype of *C. kraepelini*. Thereby we clarify and stabilize this taxonomic situation and link the name *kraepelini* to a voucher specimen and a genetic sample which will help to avoid taxonomic uncertainties in the future. We provide a species account and description of the neotype as well as data on individual variation below.

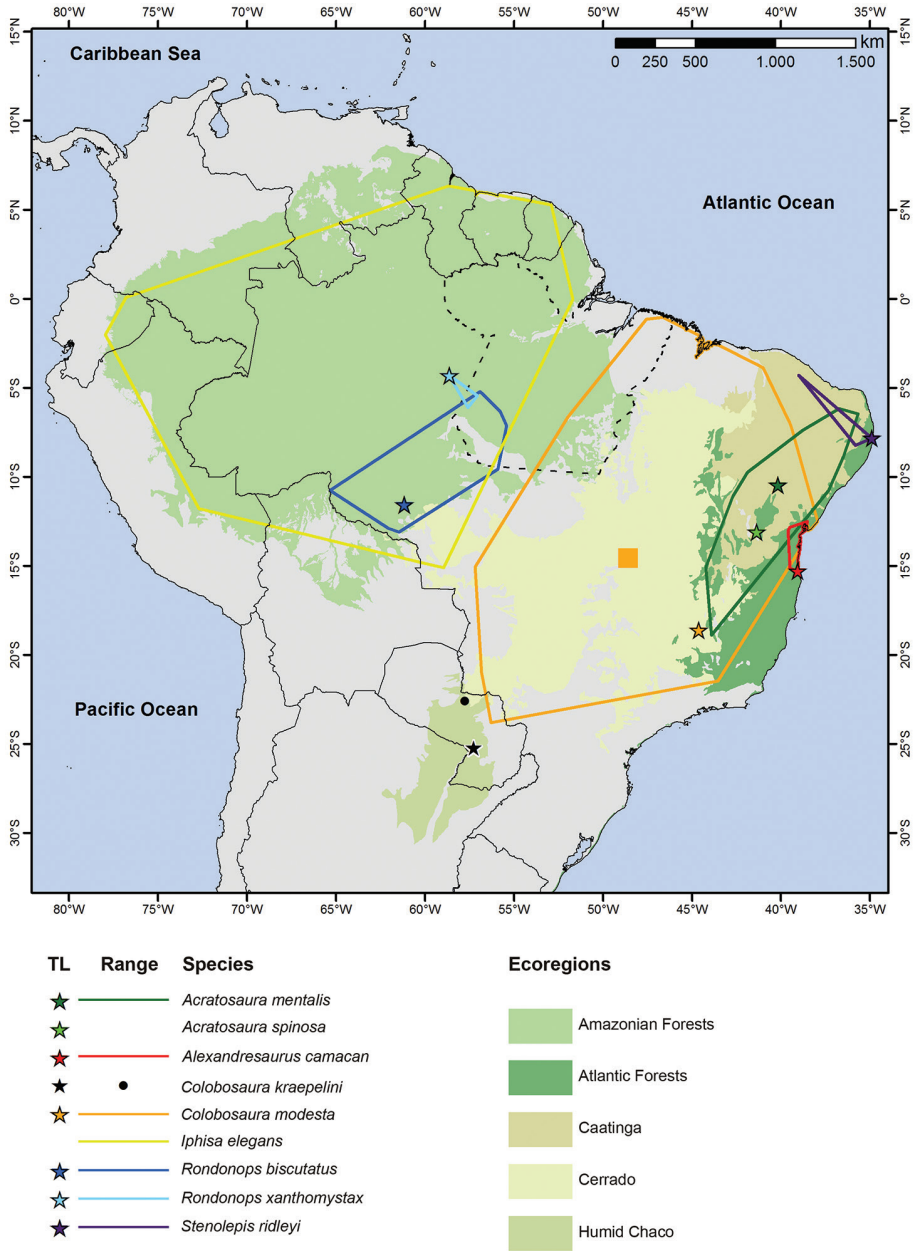

Figure 2. Central and northern region of South America showing the distribution (ranges in color) of the members of the tribe Iphisini. LT indicate type localities. Note that type locality for *I. elegans* is not shown since is referred as the whole Brazilian State of Pará. Range for *A. spinosa* is not shown because records come from vicinities of type locality. *Colobosaura kraepelini* is known only from two areas: the locality mentioned in the original description (black dot) and the neotype locality (black star); the second specimen of *C. kraepelini* reported here is from near the neotype locality. Orange square represents locality of the genetic sample of *C. modesta* (Niquelândia, GO, Brazil). Data for ecoregions according to Olson et al. (2001).

Colobosaura kraepelini Werner, 1910

Colobosaura kraepelini Werner, 1910: 32 (neotype, SMF 101370 [by present designation] (Fig. 3); type locality: 2.5 km E of Altos (25.2588°S, 57.2850°W, ca 280 masl), Cordillera Department, Paraguay by neotype selection). Original type locality: Puerto Max, San Pedro Department, Paraguay.

Diagnosis. *Colobosaura kraepelini* differs from the other species of the family Gymnophthalmidae except for *C. modesta*, by a combination of the following characters: limbs short but well developed; Finger I vestigial, not clawed; dorsal and lateral body scales keeled; four longitudinal series of ventral scales; prefrontal present; occipital present; two pairs of chin shields. *Colobosaura kraepelini* differs from *C. modesta* by having two mid-central rows of immaculate scales (vs. four immaculate ventral rows in *C. modesta*); flanks completely dark (Fig. 3) (vs. clear mottling in that area in *C. modesta*, Fig. 4); and gular shields profusely suffused with dark reaching the midline (vs. dark mottling restricted to the external edge of the shields, Fig. 5).

Description of the neotype. Subadult male. Body elongated; neck not well differentiated; SVL 29 mm; tail (clipped) stump 14 mm; HL 6.55 mm; HW 4.52 mm; ED 1.42 mm; EO (oblique) 0.66 mm. Head with juxtaposed scales, except posterior edge of interparietal and parietals imbricate with occipital and first row of nuchal scales.

Rostral broad, wider (1.81 mm) than high (0.72 mm), contacting frontonasal, nasals, and first supralabials; frontonasal heptagonal, wider (1.81 mm) than long (1.30 mm), contacting rostral, nasals, loreals, and prefrontals; prefrontals wider (1.07 mm) than long (0.70 mm) with a 0.29 mm contact line between them, and contacting frontonasal, loreals, first and second supraocular, and frontal; frontal hexagonal, longer (1.67 mm) than wide (1.11), contacting prefrontals, second supraocular, and frontoparietals; frontoparietals regular pentagonal, with a 0.67 mm mid contact line between them, and contacting frontal, second (slightly) and third (broad contact) supraoculars, parietals, and interparietal; interparietal longer (2.15 mm) than wide (1.18 mm), contacting frontoparietals, parietals, first row of nuchals, and occipital; parietals broad, wider than interparietal, contacting the interparietal, frontoparietals, third supraocular, three rows of temporals, and the first row of nuchals; occipital pentagonal and small (0.57×0.83 mm) located between the interparietal and the first and second row of nuchals; nasal elongated (0.95×0.72 mm), with nares located in the mid-lower region, contacting the rostral, frontonasal, loreals, and first supralabial; loreal curved, higher (0.67 mm) than wide (0.41 mm), in contact with nasal, frontonasal, first supraocular, first superciliary, preocular (narrowly), frenocular, and first (slightly) and second superciliaries; of which the middle one is the shortest; three supraoculars, the first smaller than the other two; three elongated superciliars, being the middle scale shorter than the first and third; eleven upper palpebrals and ten lower palpebrals surrounding the orbit; semitransparent eyelid; four elongated suboculars, second and third longer than first and fourth; seven supralabials, first contacting rostral, nasal, and loreal narrowly; second contacting loreal, frenocular, and the first subocular; third and

Figure 3. Neotype of *Colobosaura kraepelini* (SMF 101370) from the vicinity of Altos, Cordillera Department, Paraguay.

fourth supralabials in contact with suboculars; fifth supralabial (largest) contacting third and fourth subocular, lower postocular, and lower first temporal, sixth contacting the lowermost scale of the second temporal row, and other scales in the temporal region, and seventh supralabial reaching the border of the ear opening; two postoculars, the upper (in contact with the two last upper palpebrals, third superciliary, third supraocular, and upper temporal) slightly larger than the lower (in contact with the last upper palpebral, fourth subocular, fifth supralabial, and the first row of temporals); two first temporals, the upper twice the size of the lower; three second temporals, the upper twice longer than the two lower.

Mental broad, wider (mm) than long (mm); postmental pentagonal, wider (mm) than long (mm), in contact with mental, first and second infralabials, and first pair of chin shields; two pairs of chin shields, the second larger than the first pair, and followed by elongated and oblique scales that separate the second pair of chin shields from the scales of the gular region; seven infralabials, the first the widest, and the fifth the longest.

Nuchal region with seven rows of paired imbricate scales; lateral sides of the neck with three to four irregular series of juxtaposed scales, and two imbricate located in the lowermost portion; seven paired rows of gular scales, first two rows irregular, and homogeneously arranged in pairs from the third to the seventh row.

Dorsal scales imbricate, 21 transversal rows between axilla and groin, wider at neck level, and narrower and homogeneously arranged in longitudinal rows on trunk; lateral scales similar to dorsals in the upper flanks, becoming wider towards the ventral

Figure 4. Specimen of *Colobosaura modesta* showing lateral coloration patter. Image given by Paul Smith (Fauna Paraguay). Additional photographs available at http://www.faunaparaguay.com/colobosauramodesta.html

region; sternal scale triangular, flanked by large rectangular scales in the clavicular region; four longitudinal rows of ventral scales; 26 scales around midbody; scales at insertion of limbs granular, except in the ventral region; all of tail with imbricate, elongated, hexagonal, and keeled scales.

Forelimbs covered with large, imbricate and smooth scales on the dorsal and lateral surfaces, being smaller on the ventral region of the limb; carpal region covered with large imbricate scales; palmar surface covered with granular juxtaposed scales; scales on fingers from I to V: 1/1-4/5-6/6-7/7-4/4; infradigital single lamellae under fingers from I to V: 2/2-8/8-10/10-11/12-6/5; fingers clawed except vestigial finger I.

Hind limbs medium-sized, imbricate, moderately keeled scales on the dorsal surface; anterior and posterior parts of the hind limbs with large, imbricate, and smooth scales; posterior part of hind limbs covered with granular juxtaposed scales on the thigh, and smooth medium-sized imbricate scales on the shank; tarsal region covered with large imbricate scales; plantar surface covered with granular juxtaposed scales; scales on toes from I to V: 3/3-4/4-8/8-10/10-6/(toe clipped as tissue sample); infradigital single lamellae under toes from I to V: 4/4-8/7-14/12-15/17-9/(toe clipped); toes clawed.

Coloration in life of the neotype. Dorsal surface of head Olive Clay Color (85) with Vandyke Brown (282) mottling on frontal and second supraocular and posteri-

Figure 5. Ventral view of the head showing the different coloration pattern between *Colobosaura modesta* (MNHNP 8521, left) and *C. kraepelini* (MNHNP 11726, right).

orly, and a diffuse Vandyke Brown (282) line edging anterior margin of frontal and second supraocular and anterior scales; lateral parts of the head homogeneous Vandyke Brown (282); supralabials with Medium Neutral Gray (298) bars in the center interleaved with Cyan White (155) in the sutures; background color of mandibular region Cyan White (155) with Medium Neutral Gray (298) blotches on infralabials (one per scale) and second pair of chin shields; iris Burnt Umber (48); dorsal scales Mikado Brown (42), anteriorly (before forelimbs level) with Vandyke Brown (282) suffusions more concentrated near the laterals, and posteriorly (after forelimbs level) with faint irregular suffusions of Warm Sepia (40), more regularly present on the scales margins; lateral sides of the neck and body Vandyke Brown (282) with irregular Mikado Brown (42) speckles and blotches after forelimbs level, grading into a reticulated Vandyke Brown (282) and Mikado Brown (42) pattern near the groin; background ventral color Cyan White (155) with intrusions of Vandyke Brown (282) on the throat, and a faint mottling of Vandyke Brown (282) on the lateral rows of ventral scales; forelimbs mostly Vandyke Brown (282), Cyan White (155) restricted to the anteroventral regions; hind limbs Mikado Brown (42) with suffusion of Vandyke Brown (282) on the scales margins, and Cyan White (155) on the ventral region of the limb; tail background color Plumbeous (295) with Brownish Olive (292) suffusions on the anterior third of the organ, and Pale Greenish White (97) paravertebral spots located every two scales; iridescent hue all along the body.

Coloration in preservative of the neotype. (After five years in 70% ethanol): The general pattern remains the same, and the background Mikado Brown (42) color also remains; the darker parts of the body (lateral sides of neck and body) turned to Sepia

(279); tail turned to Hair Color (277) on the dorsum, with the paravertebral spots faintly visible; ventral side of the head Smoky White (261); ventral side of the body Pale Buff (1).

Variations. MNHNP 11726 agrees well in most aspects of the scalation to those observed in the neotype, with the following differences: two superciliaries; 21 transversal rows between axilla and groin; 27 scales around midbody; 11 infradigital lamellae under IV finger; 16 infradigital lamellae under IV toe. Background color of MNHNP 11726 slightly clearer (Sayal Brown 41) than SMF 101370, and the dark (Fuscous 283) lateral suffusions are less dense. Ventrally Pale Buff (1). The coloration pattern is the same in both specimens with some differences: MNHNP 11726 has dark blotches also on the first pair of chinshields; posterior margin of dorsal scales strongly marked; caudal spots absent.

Distribution and habitat. The species is distributed in the Humid Chaco. The environment is basically a savanna composed of palms (*Copernicia alba*), native bunch grasses, and scattered islands of semideciduous temperate forest. The area is adapted to periodical floods from the Paraguay River. The locality of Puerto Max (former type locality of *C. kraepelini*) consists of a small village and cattle farm with intense anthropic pressure. The new specimens (SMF 101370 and MNHNP 11726) came from the vicinities of the capital city, about 280 km (airline) southwards from the original type locality, also in Humid Chaco.

Discussion

The tribe Iphisini was described recently by Rodrigues et al. (2009b) which was before merged within the tribe Heterodactylini. Nevertheless, Rodrigues et al. (2007) already discovered that the genera *Acratosaura*, *Alexandresaurus*, *Colobosaura*, *Iphisa*, and *Stenolepis* exhibit a strong sexual dimorphism, absent in other Heterodactylini. Our ML phylogenetic hypothesis of the tribe Iphisini based on the mtDNA 16S gene recovered *Acratosaura mentalis* and *Stenolepis ridleyi* as sister taxa which was also inferred by Rodrigues et al. (2007) and Colli et al. (2015). The position of *Iphisa* differs from the phylogeny presented by Colli et al. (2015), being the sister clade of *Acratosaura*+*Colobosaura*+*Stenolepis* in our analysis. It is important to note that sequences of *Rondonops biscutatus* used by Colli et al. (2015) were not available at GenBank. The placement of *Iphisa* as a basal clade in relation to *Acratosaura* and *Colobosaura* was also shown by Pellegrino et al. (2001) and Castoe et al. (2004). And *Alexandresaurus camacan* is shown as the most basal taxon in the group (Fig. 1) as also exposed by Pellegrino et al. (2001), Castoe et al. (2004) (referred in these two publications as *Colobosaura* spn), Rodrigues et al. (2007), and Colli et al. (2015).

From the genetic point of view there is no doubt that the neotype of *Colobosaura kraepelini* is different from *C. modesta*. The high genetic distance between these two species compared with the even lower genetic distance between some related genera (Table 1) could indicate that a new taxonomic arrangement should be proposed. Never-

theless, based on the little morphological differentiation in *Colobosaura* we keep a conservative approach. In our phylogeny, the divergence between *Colobosaura modesta* and *C. kraepelini* is as deep as the divergence between the genera *Acratosaura* and *Stenolepis.*

The only previously known reference to a specimen of *Colobosaura kraepelini* was in the original description based on an individual from Puerto Max, and the species was never found again. Given the brevity of the original description the species was considered as synonym of *C. modesta* (Vanzolini and Ramos 1977, Rodrigues et al. 2007). Vanzolini and Ramos (1977) additionally stated that maybe the specimen used for the description of *C. kraepelini* was not even a *Colobosaura* because in the description the author referred to some oblique folds on the tongue of the specimen, which is a character that does not occur in the group. Our specimen differs morphologically from *C. modesta* in some aspects of coloration, and it was found in the Humid Chaco (as is the original type locality of *C. kraepelini*) whereas *C. modesta* is restricted to Caatinga and Cerrado in areas adjacent to Atlantic Forest (Fig. 2). All three known localities for *C. kraepelini* are located in the drainage system of the Paraguay River sharing some topographical traits.

Biogeographically, Rodrigues et al. (2007) hypothesized that *Stenolepis* should have originally a wider distribution followed by a major constriction, resulting in its current restricted range associated with the Atlantic Forest. The basal location of *Alexandresaurus* in the tribe's phylogeny could suggest that it probably also had a wider distribution, although it is currently restricted to a small patch of Atlantic Forest on the coast of Bahia. In the remaining taxa it is possible to distinguish a major phylogenetic split of eastern (only *Iphisa* in our phylogeny) and western (*Acratosaura*, *Colobosaura*, and *Stenolepis*) clades, which was also noted by Colli et al. (2015). Whereas the western clade is strictly related to Amazonian forests, the eastern clade is present mainly in the Dry Diagonal, although *S. ridleyi* is also present in Atlantic Forest and Caatinga (Fig. 2). According to this biogeographical perspective and based on the distribution of the whole tribe, *C. kraepelini* could be the most derived member of the clade.

Rodrigues et al. (2007) highlighted the importance of analyzing the wide distribution ranges of *Colobosaura* and *Iphisa* and, in fact, more recently Nunes et al. (2012) revealed that *Iphisa* is actually composed of five different species, and Colli et al. (2015) suggest that a detailed analysis of *Colobosaura* could indicate a similar pattern. Here we provide evidence that at least the genus *Colobosaura* is composed of two species. The morphological traits proposed by Peters and Donoso-Barros (1970) to differentiate between *C. modesta* and *C. kraepelini* (shape of the interparietal) are useless. Instead, we show that coloration can differentiate between these two taxa. Following, we present a key for the identification of species in the tribe Iphisini.

Key to species of Iphisini

1	Two longitudinal rows of ventral scales	**2**
–	Four or six longitudinal rows of ventral scales	**4**

Appendix 1

Genetic samples

Species	Voucher	GBAN	Locality
Acratosaura mentalis	MRT 906448	AF420726	Morro do Chapéu, BA, Br
Alexandresaurus camacan	MD 1106	AF420739	Una, BA, Br
Colobosaura kraepelini	SMF 101370	KY782646	Altos, Cordillera, Pa
Colobosaura modesta	LG 1145	AY217953	Niquelândia, GO, Br
Iphisa elegans	MRT 977426	AF420714	Aripuanã, MT, Br
Stenolepis ridleyi	-?-	EF405619	-?-
Cercosaura ocellata OG	MRT 977406	AF420731	Aripuanã, MT, Br

For each species the voucher specimen, GenBank accession number (GBAN), and the locality (Br = Brazil, Pa = Paraguay) are presented for samples used in the genetic analysis. Outgroup marked with OG. See Materials and methods section for indication of institutional acronyms. MRT and MD indicate Miguel Trefaut Rodrigues (Museu de Zoologia, Universidade de São Paulo, Brazil) and Marianna Dixo (Instituto de Biociências, Universidade de São Paulo, Brazil) voucher specimens, respectively. Data for the sample of *S. ridleyi* are missing in the original publication (Rodrigues et al. 2007).

Appendix 2

Examined specimens

Colobosaura kraepelini

PARAGUAY: Cordillera: San Bernardino, 50 metros del Lago Ypacarai (MNHNP 11726).

Colobosaura modesta

PARAGUAY: Amambay: Parque Nacional Cerro Corá (MNHNP 8454–56, 8521). San Pedro: Reserva Natural Laguna Blanca (MNHNP 11684, 11596, 11652).

2 One pair of enlarged chin shields ..... ***Iphisa elegans***
– Two pairs of enlarged chin shields ..... (***Rondonops***) **3**
3 Lateral neck scales smooth; 16–20 infradigital lamellae under toe IV.....
..... ***R. biscutatus***
– Lateral neck scales keeled; 20–26 infradigital lamellae under toe IV.....
..... ***R. xanthomystax***
4 Prefrontals absent ..... ***Stenolepis ridleyi***
– Prefrontals present **5**
5 Occipitals absent..... ***Alexandresaurus camacan***
– Occipitals present **6**
6 Three pairs of chin shields..... (***Acratosaura***) **7**
– Two pairs of chin shields ..... (***Colobosaura***) **8**
7 Lateral neck scales smooth and juxtaposed; dorsal scales slightly keeled (keel covers half of the scale) at midbody..... ***A. mentalis***
– Lateral neck scales keeled and imbricate; dorsal scales strongly keeled at midbody ..... ***A. spinosa***
8 Ventrals immaculate; dark mottling on the external edge of gular shields .....
..... ***C. modesta***
– Two central rows of ventral scales immaculate, and dark mottling on the two external rows; gular shields profusely mottled with dark ..... ***C. kraepelini***

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APPENDIX III

Declaration on the contributions of authors

to the publication: Diversity of *Tropidurus* (Squamata: Tropiduridae) in Paraguay—an integrative taxonomic approach based on morphological and molecular genetic evidence
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What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 50%
Coauthor GK: 50%

(2) to the implementation of the respective studies and experiments

PhD candidate: 80% – field work (collecting and documenting specimens), molecular analyses, morphological analyses, revision of museum vouchers.
Coauthor GK: 20% – field work (collecting and documenting specimens)

(3) to the creation of the data collection and figures

PhD candidate: 90% – created database, sequenced DNA barcodes, created figures, created maps.
Coauthor GK: 10% – provided photographs.

(4) to the analysis and interpretation of the data

PhD candidate: 70% – analysis and interpretation of molecular, morphological, and biogeographical data.
Coauthor GK: 30% – contributed to data analysis and interpretation.

(5) to writing the manuscript

PhD candidate: 85%
Coauthor GK: 15%

Date/place: _____

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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Diversity of *Tropidurus* (Squamata: Tropiduridae) in Paraguay—an integrative taxonomic approach based on morphological and molecular genetic evidence

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Abstract

Tropidurus is a Neotropical genus of iguanoid lizards characterized by a conspicuously enlarged interparietal plate, the presence of gular folds, presence of infradigital keels, and the absence of femoral pores. Currently, 29 species are recognized within the genus, seven of which are present in Paraguay: *T. etheridgei*, *T. torquatus*, *T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara*, and *T. teyumirim*. We generated genetic data based on two DNA mitochondrial markers (16S and COI) and one nuclear (PRLR) marker for all the seven Paraguayan species with the goal to identify the taxonomic relationships among taxa based on the intra- and interspecific genetic variation and the construction of molecular clusters. ML and BI analyses match in the recognition of two main clusters: groups *torquatus* and *spinulosus*, and within the *torquatus* group the differentiation between *T. catalanensis* and *T. etheridgei* is highly supported. Nevertheless, there is a complete lack of congruence between mitochondrial and nuclear genes in the topology within the *spinulosus* group. *Tropidurus guarani* and *T. spinulosus* are more differentiated from the remaining species of the *spinulosus* group with genetic p-distances from 4.0 to 6.0. Low distances were found between *T. lagunablanca* and *T. tarara* (1.0–1.1%), and slightly higher, among *T. teyumirim*, *T. lagunablanca*, and *T. tarara* (2.0–2.6% respectively). From a morphological perspective, species of the *Tropidurus torquatus* group are easily distinguished; but we found strong overlaps of scalation characters in the *spinulosus* group. We interpret the low genetic distances documented among the nominal taxa *Tropidurus lagunablanca*, *T. tarara*, and *T. teyumirim* as evidence for conspecificity. This hypothesis is supported by the lack of morphological characters that would diagnose any of the three taxa. Similarly, we found low genetic distances among populations assigned to the nominal taxa *T. guarani* and *T. spinulosus*, including samples from near the type locality of the former, and therefore we recognize only two species of the *T. spinulosus* complex in Paraguay: *T. spinulosus* and *T. lagunablanca.*

Key words: Iguanoidea, 16S, COI, PRLR, South America, ring species

Introduction

Tropidurus is a Neotropical genus of iguanoid lizards characterized by a conspicuously enlarged interparietal plate, the presence of gular folds, presence of infradigital keels, and the absence of femoral pores (Cei 1993; Carreira *et al*. 2005). Currently, 29 species are recognized within the genus, divided into four groups: the *Tropidurus bogerti* group (monotypic), diagnosed by lateral gular scales, that are imbricate posteriorly, and a color pattern of white dots on a black background; the *T. semitaeniatus* group (four species), characterized by extreme flattening body and head; the *T. spinulosus* group (eight species), characterized by yellow or white marks on thighs; and the *T. torquatus* group (16 species), diagnosed by the absence of an enlarged vertebral scale row (Frost *et al*. 2001; Carvalho 2016; Carvalho *et al*. 2016).

This genus is distributed widely across South America, from Venezuela southeastwards through the Guianas to Brazil, and along the Dry Diagonal (Caatinga-Cerrado-Chaco) across southern Brazil, Bolivia and Paraguay, to central Argentina (Carvalho 2013). Along their distribution, these lizards inhabit mostly dry environments (only few records in humid forests) in open areas, with both rocky outcrops dwellers and sandy soils inhabitants (Rodrigues 1987; Carvalho 2013).

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In relation with other taxa, *Tropidurus* received little attention from a molecular perspective. Frost *et al*. (1998) used the mitochondrial gene COI to analyze patterns of variation and evolution within the *T. spinulosus* group. Based on the assumption that specimens west of The Paraguay River (Bolivia and occidental region of Paraguay) were *T. spinulosus* and specimens east of the river (Brazilian state of Mato Grosso do Sul and oriental region of Paraguay) were *T. guarani*, the authors claimed that the latter species is composed of two lineages (Frost *et al*. 1998). Later, Frost *et al*. (2001) with a more extensive representation of taxa, and using three mitochondrial genes (12S, 16S, and valine tDNA), resurrected some genera (*Plica*, *Strobilurus*, and *Uracentron*) from the synonymy of *Tropidurus*, identifying apomorphies and providing diagnoses for the different groups of the genus. More recently, Werneck *et al*. (2015) investigated the diversity of the *T. semitaeniatus* group documenting a high cryptic diversity. Finally, Carvalho *et al*. (2016) applied the use of four mitochondrial and six nuclear markers to the *T. torquatus* group, recognizing a new species (*T. sertanejo*) from the State of Bahia (Brazil) in the Caatinga ecoregion.

FIGURE 1. Distribution of species of the *Tropidurus torquatus* group in Paraguay.

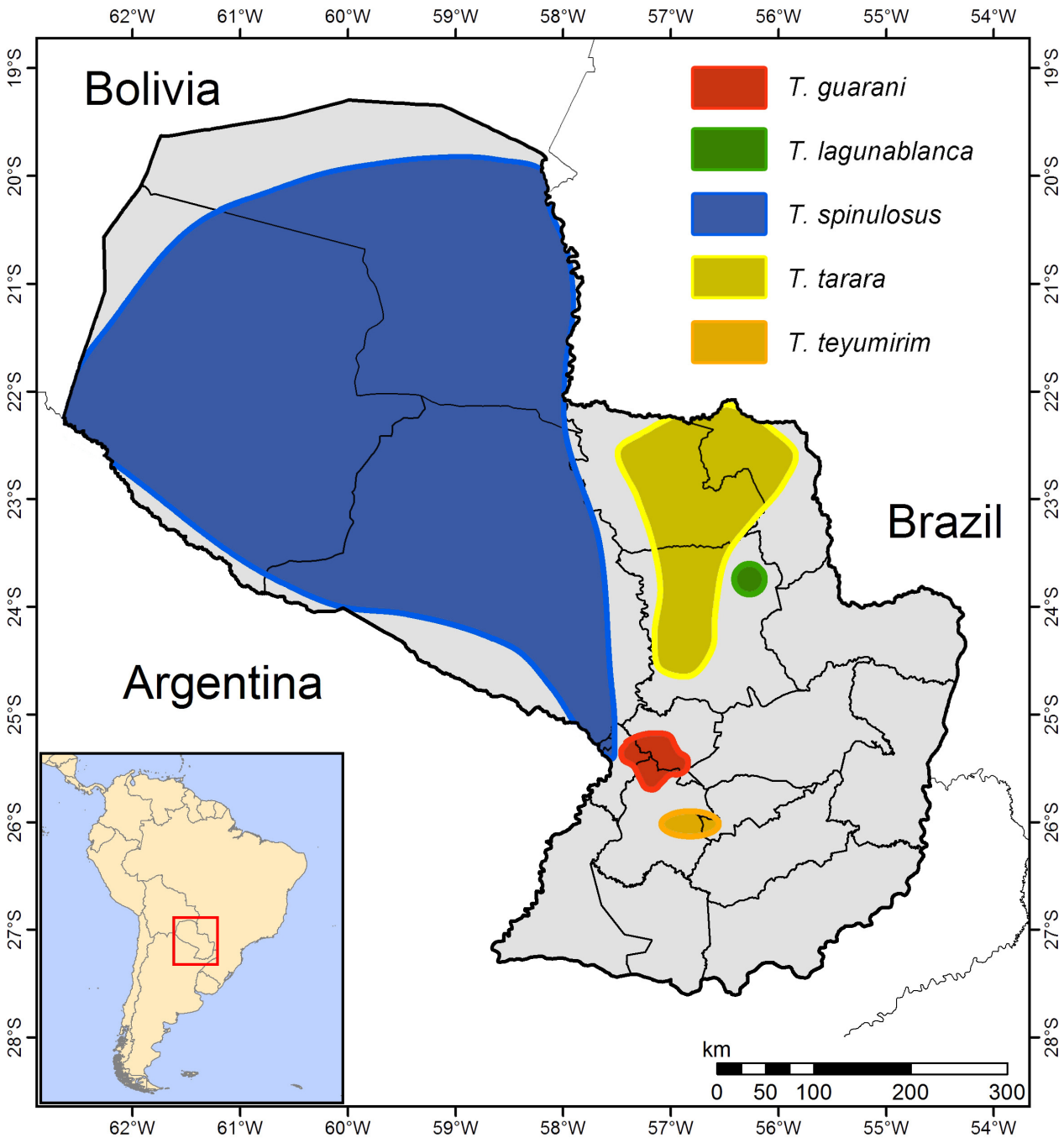

FIGURE 2. Distribution of species of the *Tropidurus spinulosus* group in Paraguay.

For the present work we analyzed samples of *Tropidurus* from Paraguay, since this country received little attention (from a genetic perspective) by Frost *et al*. (1998) who recognized two species in the *T. spinulosus* group. Traditionally, four species of *Tropidurus* were recognized for Paraguay: *T. etheridgei* and *T. torquatus* (*T. torquatus* group) and *T. guarani* and *T. spinulosus* (*T. spinulosus* group) (Aquino *et al.* 1996); and in fact, the type locality for these two species of the *T. spinulosus* group is in Paraguay. Following the recognition of two taxa related to *T. guarani* east of Paraguay River by Frost *et al.* (1998), Cacciali *et al*. (2016) restricted the name *T. guarani* to populations in the surroundings of the type locality (Cordillera, Paraguarí, and Guariá departments) referring as "*Tropidurus* sp. 1" to the populations in the northern portion of the country, east of Paraguay River (Amambay, Concepción, and San Pedro departments). In that same year, Carvalho (2016) made a morphological analysis of Paraguayan populations of *Tropidurus* and described three new species. In that work the author found

morphological differences among specimens from the northern portion east of Paraguay River ("*Tropidurus* sp. 1" of Cacciali *et al*. 2016) leading to the recognition of *Tropidurus lagunablanca* (San Pedro Department) and *T. tarara* (Amambay and Concepción departments) (Carvalho 2016). Additionally, *T. guarani* was also split into two species, with the recognition of the new species *T. teyumirim* from the Parque Nacional Ybycui and surroundings (Paraguarí Department).

Thus, currently seven species of *Tropidurus* are recognized in Paraguay: *T. etheridgei* Cei, 1982 (Fig. 1); *T. torquatus* (Wied-Neuwied, 1820) (Fig. 1); *T. guarani* Álvarez, Cei & Scolaro, 1994 (Fig. 2); *T. lagunablanca* Carvalho, 2016 (Fig. 2); *T. spinulosus* (Cope, 1862) (Fig. 2); *T. tarara* Carvalho, 2016 (Fig. 2); *T. teyumirim* Carvalho, 2016 (Fig. 2). We generated genetic data based on two DNA mitochondrial markers (16S and COI) and one nuclear (PRLR) marker for all the seven Paraguayan species with the goal to identify the taxonomic relationships among taxa based on the intra- and interspecific genetic variation and the construction of molecular clusters. To complement the molecular inference, we carried out a morphological analysis in order to verify the usefulness of the proposed diagnostic characters and to explore the potential taxonomic value of additional morphological data.

Materials and methods

Paraguayan samples of *Tropidurus* used for analyses are presented in Figure 3, and listed in Appendix 1, indicating the collecting locality, and the GenBank accession number for each sequence. Institutional codes in Appendix 1 follow Sabaj Pérez (2016). For comparison, we downloaded available sequences of the species present in Paraguay, as well as sequences of *Plica plica* used as outgroup (also detailed in Appendix 1) from the same online repository, based on Carvalho *et al*. (2016) and Oliveira *et al*. (2016).

We used tail clips (when regenerated) or muscle (thigh) for DNA analyses. Protocol for DNA extraction follows Ivanova *et al*. (2006). Washing of samples was made in 50 µl of diluted PBS buffer (1:9 of buffer and water respectively) for 14 h. For digestion was used a solution of vertebrate lysis buffer and proteinase K (60:6 µl respectively), kept at 56°C for 14 h. After extraction, DNA samples were eluted in 50 µL TE buffer.

For amplification we used Eurofins MWG Operon primers. Primer sequences for mitochondrial 16S rRNA gene we follow Palumbi (1991): 16sar-L2510 (forward: 5'– CGCCTGTTTATCAAAAACAT –3') and 16sar-H3056 (reverse: 5'– CCGGTCTGAACTCAGATCACGT –3'); and for Cytochrome Oxidase I (COI) gene we follow Meyer (2003): dgLCO-1490 (forward: 5'– GGTCAACAAATCATAAAGAYATYGG –3') and dgHCO-2198 (reverse: 5'– TAAACTTCAGGGTGACCAAARAAYCA –3'). The nuclear gene Prolactin Receptor (PRLR) was amplified with the primers PRLR_f1 (forward: 5'– GACARYGARGACCAGCAACTRATGCC –3') and PRLR_r3 (reverse: 5'– GACYTTGTGRACTTCYACRTAATCCAT –3') based on Townsend *et al*. (2008). Amplifications were performed in an Eppendorf Mastercycler® pro. PCR conditions follow Lotzkat *et al*. (2013) for 16S, a modification of Meyer (2003) for COI, and Noonan & Yonder (2009) for PRLR, and are detailed in Appendix 2.

Consensus sequences were generated through assembling of bidirectional trace files and the inspection of their chromatograms in SeqTrace 0.9.0 (Stucky 2012). The dataset for the mtDNA genes 16S and COI consist of 17 and 18 samples, respectively (Appendix 1). For the nuclear gene PRLR, 17 samples were available (Appendix 1). The alignments of the sequences were performed in MAFFT 7 (Katoh *et al*. 2002; Katoh & Standley 2013) through the webserver (available at http://mafft.cbrc.jp/alignment/server/). For the alignment of the 16S gene fragment, we include the Q-INS-i search strategy for the secondary structure of that gene (Katoh & Toh 2008). The alignments were visualized and exported as fasta files with MSA Viewer (Yachdav *et al*. 2016).

The best substitution model was selected using the Bayesian Information Criterion (BIC) parameter, with ModelFinder (Kalyaanamoorthy *et al*. 2017) in the IQ-Tree web server. For phylogenetic hypotheses, we used Maximum Likelihood (ML) and Bayesian inference (BI) to compare the concordance between these two algorithms. The phylogenetic analyses with ML were run in IQ-Tree web server (Trifinopoulos *et al*. 2016), using 10,000 non-parametric bootstrap replicates plus 10,000 replicates of Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT) (Anisimova *et al*. 2011) and 10,000 ultrafast bootstrap (UFBoot) approximation replicates (Minh *et al*. 2013). Missing data were treated as unknown characters, providing no information. Alignments were converted to nexus format in the online server Alter (Glez-Peña *et al*. 2010) available at http://sing.ei.uvigo.es/ALTER/. Bayesian inferences were performed in MrBayes v3.2 (Huelsenbeck & Ronquist 2001; Ronquist &

Huelsenbeck 2003) in independent duplicates, each with 1,000,000 generations for MCMC with a sampling frequency of 500 generations. Concatenated sequences (only mitochondrial and mitochondrial plus nuclear genes together) were analyzed under the same parameters as the Single-gene sequences.

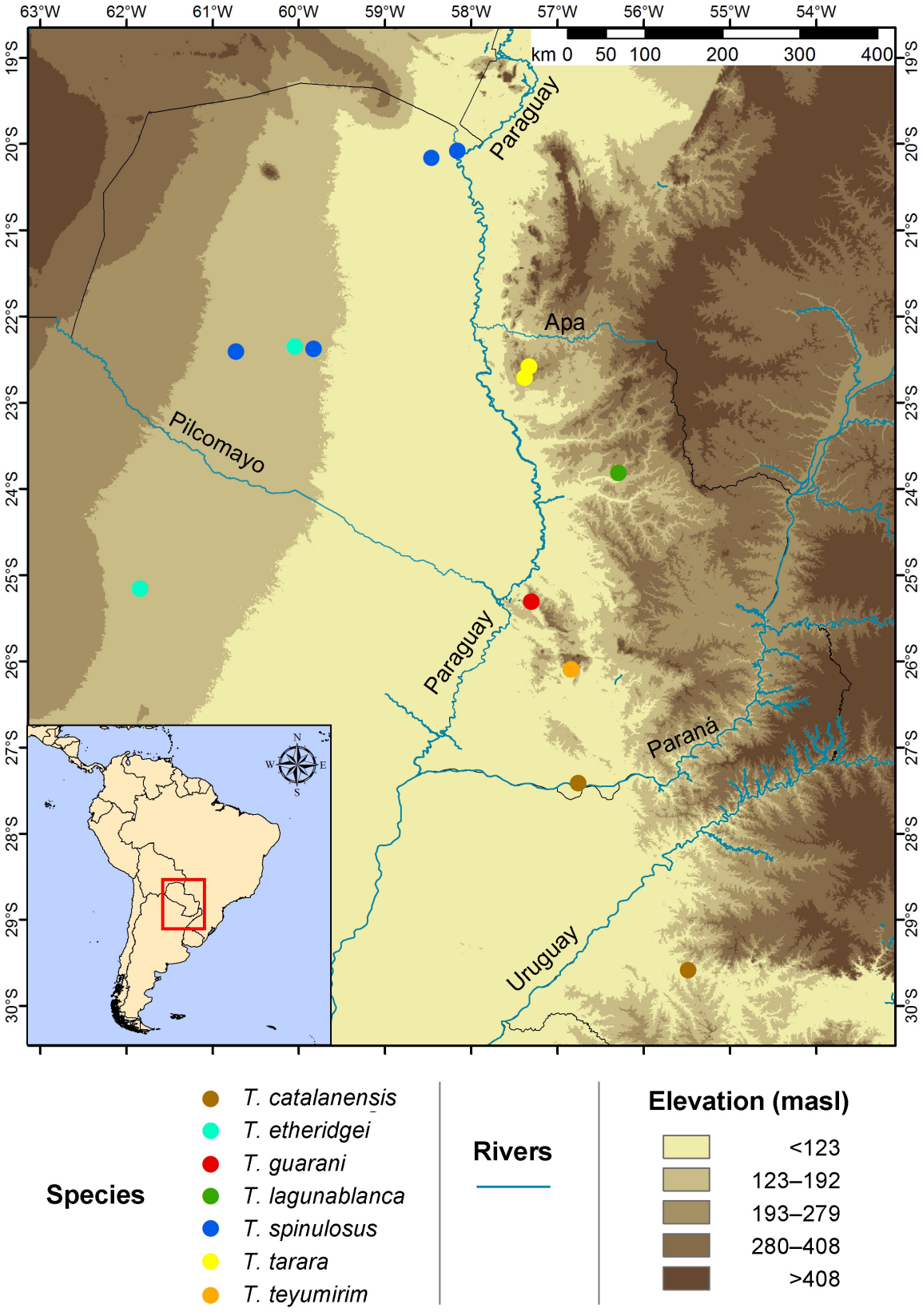

FIGURE 3. Location of genetic samples used for the analyses. See Appendix 1 for information on the specimens.

All trees were visualized and exported in FigTree v1.4.3 (available at http://tree.bio.ed.ac.uk/software/figtree/), and were stored in TreeBase along with the alignments (ID 21797).

Additionally, we estimated the genetic distance for the mitochondrial gene 16S given that it evolves faster than nuclear genes (Brown *et al*. 1979; Lopez *et al*. 1997) providing good resolution for contrast of results and its wide use in the literature allows comparability. For this, we computed the uncorrected pairwise distance (p-distance) in MEGA 6 (Tamura *et al*. 2013) with 10,000 bootstrap replicates, and excluding the outgroup. We considered a range of 2.4–4% as acceptable interspecific genetic distance, as recorded for other Iguanoids (Batista *et al*. 2015; Köhler *et al*. 2012; Köhler *et al*. 2016).

We analyzed the phenotypic variation through morphological examination of available specimens, detailed in Appendix 3, according to terminology of Frost (1992). We analyzed some meristic characters used by Rodrigues (1987), Kunz & Borges-Martins (2013), and Carvalho (2016): DOR (dorsal scales, counted along the vertebral region, from the first scale behind the occipital, to the level of the vent), SAB (scales around body, counted transversally around the body in a mid-section between axilla and groin) and GUL (gular scales, counted along the midline from the first scale contacting the mental, to the level of the scapular region). Whenever possible, we studied the hemipenis morphology in those males with everted copulatory organs.

Geographic references were based on Hellmich 1960; Gallardo 1969; Gallardo *et al.* 1985; Álvarez *et al*. 1994; Cruz et al. 1997; Lions & Álvarez 1998; Céspedez *et al.* 2001; Álvarez *et al*. 2002; Avila & Carrizo 2003; Carreira *et al*. 2005; Kacoliris *et al.* 2006; Pelegrin & Leynaud 2006; Pelegrin 2007; Carvalho 2013; Kuntz & Borges-Martins 2013; Cacciali *et al*. 2016; and Pérez-Iglesias *et al.* 2017.

Results

The length of the final alignment for 16S was 538 bp, and the best substitution model was TPM2u+G. The ML tree for this gene clearly clusters species of the *spinulosus* group separated from the *torquatus* group (Fig. 4A). Within these clades, the tree shows a high support for recognizing two clades: the *torquatus* group with *T. catalanensis* and *T. etheridgei*; and the *spinulosus* group with *T. guarani* + *T. spinulosus*, and *T. lagunablanca* + *T. tarara* + *T. teyumirim*. In this tree, *T. spinulosus* is paraphyletic. The relations within the clade *T. lagunablanca* + *T. tarara* + *T. teyumirim* are poorly supported. The topology of the BI tree is rather consistent with that shown by the ML, with only one variation: there is a trichotomy in the clade *T. lagunablanca* + *T. tarara* + *T. teyumirim*, but again the node support in this node is weak (Fig. 4A).

The final alignment of the COI gene sequences consisted of 566 bp. The best substitution model identified was HKY+G. The topology of the ML analysis matches with that observed for the 16S gene, but with stronger support rendering *T. spinulosus* as paraphyletic; and with weaker values for the recognition of the clade *T. lagunablanca* + *T. tarara* + *T. teyumirim*, where this last (also observed in the 16S topology) indicated to be sister to *T. lagunablanca* + *T. tarara* (Fig. 4B). The tree inferred with BI shows a similar arrangement observed in previous trees, but with robust support values in most of the nodes (Fig. 4B). A main difference with other topologies is that BI shows a strongly supported trichotomy with the following clades: *T. guarani* + *T. spinulosus*, *T. lagunablanca* + *T. tarara*, and *T. teyumirim*.

According to the ML analysis for concatenated mitochondrial genes, the tree has the same topology (only slightly different within the cluster of *T. tarara*) as the one produced by the ML of the COI sequences, but with stronger support for the recognition of *T. teyumirim* as the sister clade to *T. lagunablanca* + *T. tarara* (Fig. 4C). The concatenated analysis with BI of the mitochondrial sequences retrieved a topology similar to the COI tree (Fig. 4C), but the clusters *guarani* + *T. spinulosus* and *T. lagunablanca* + *T. tarara* + *T. teyumirim* are differentiated in a dichotomy.

The sequence alignment of the nuclear DNA fragment PRLR consisted of 451 bp. The substitution model according to the BIC criteria was K3P. Both trees using ML and BI match in the strong recognition of two clusters: groups *torquatus* and *spinulosus*, and within the *torquatus* group the differentiation between *T. catalanensis* and *T. etheridgei* is highly supported (Fig. 4D). Nevertheless, there is a complete lack of congruence between mitochondrial and nuclear genes in the topology within the *spinulosus* group. The inferred phylogenies based on concatenated sequences of all three markers (16S, COI, and PRLR) have similar topologies as observed for mitochondrial genes, but with stronger support values (Fig. 5).

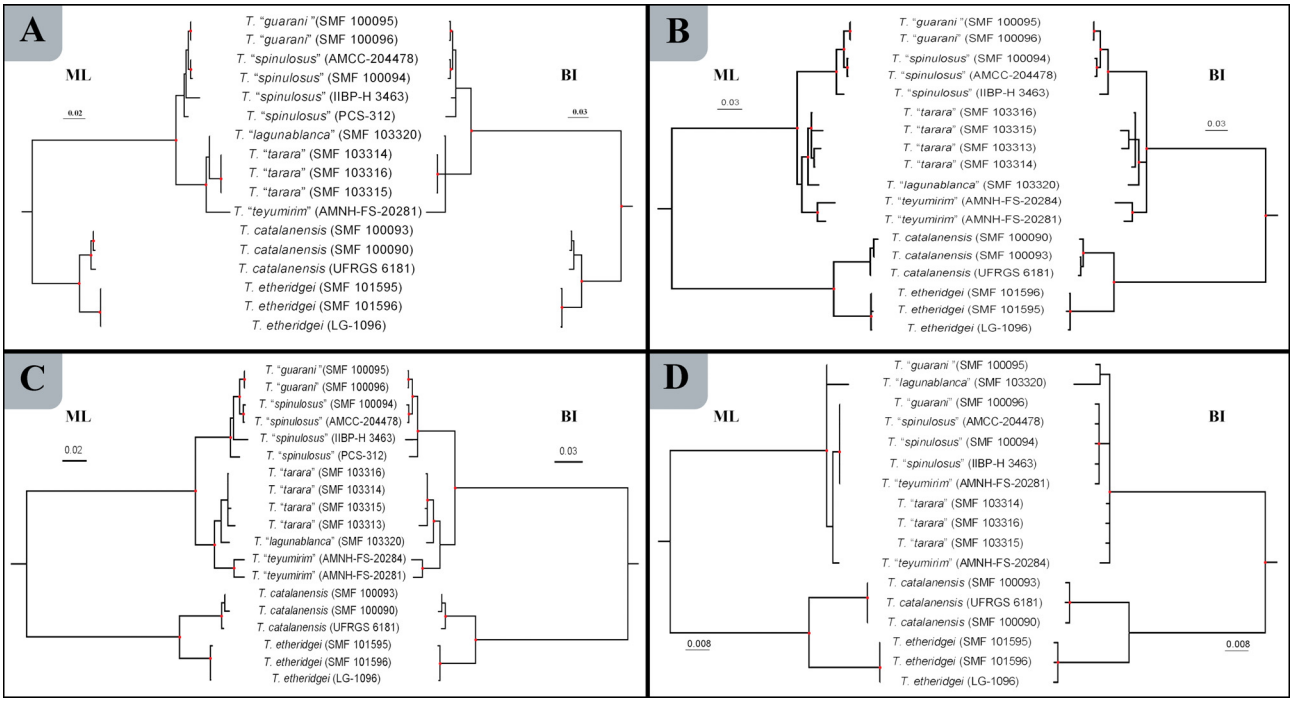

FIGURE 4. Phylogenetic trees of Paraguayan samples of *Tropidurus* inferred from 16S (A), COI (B), 16S+COI (C), and PRLR (D) partial gene sequences. For each analysis we present maximum likelihood (ML, left) and Bayesian inference (BI, right) trees. Red dots indicate support values (basedon on SH-aLRT/UFBoot for ML and posterior probability for BI) equal or superior to 80 for ML and 0.85 for BI. Roots to outgroup *Plica plica* (AMCC-106953). Reference bar represents substitutions per site.

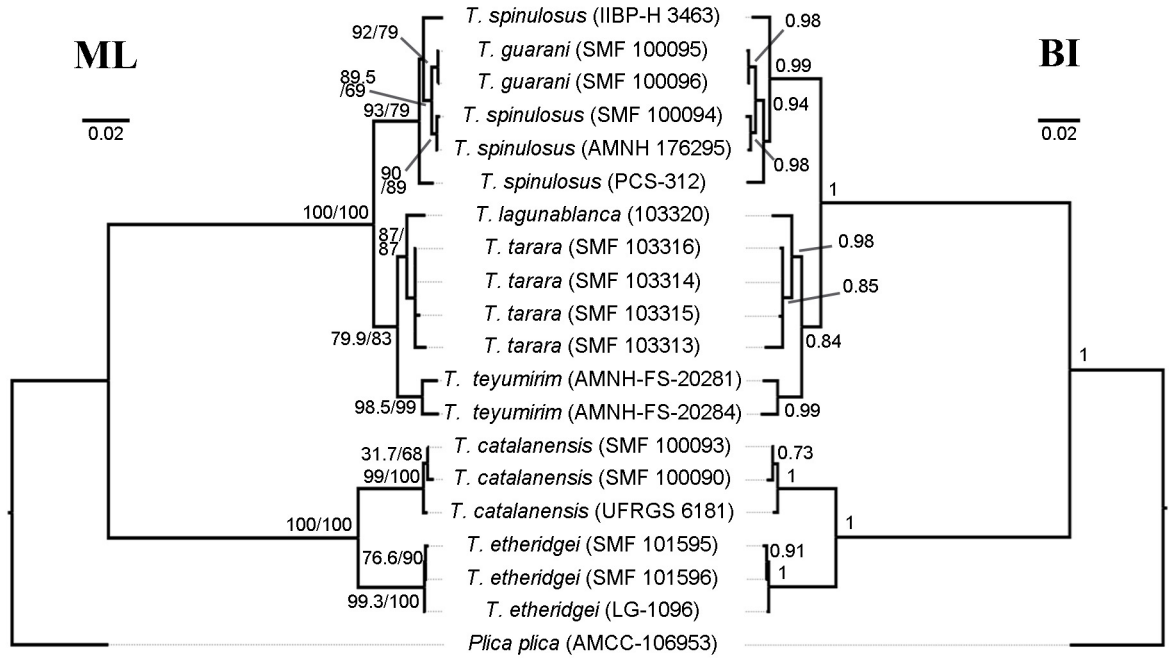

FIGURE 5. Maximum Likelihood (left) and Bayesian (right) trees inferred using concatenated mitochondrial (16S and COI) and nuclear (PRLR) DNA genes for samples of *Tropidurus* from Paraguay. Support values on nodes represent SH-aLRT/ UFBoot (in percentages) for ML (only values above 65 are shown), and posterior probability for BI (only values above 70 are shown). See Appendix 3 and Figure 1 for geographic location of samples. Reference bar represents substitutions per site.

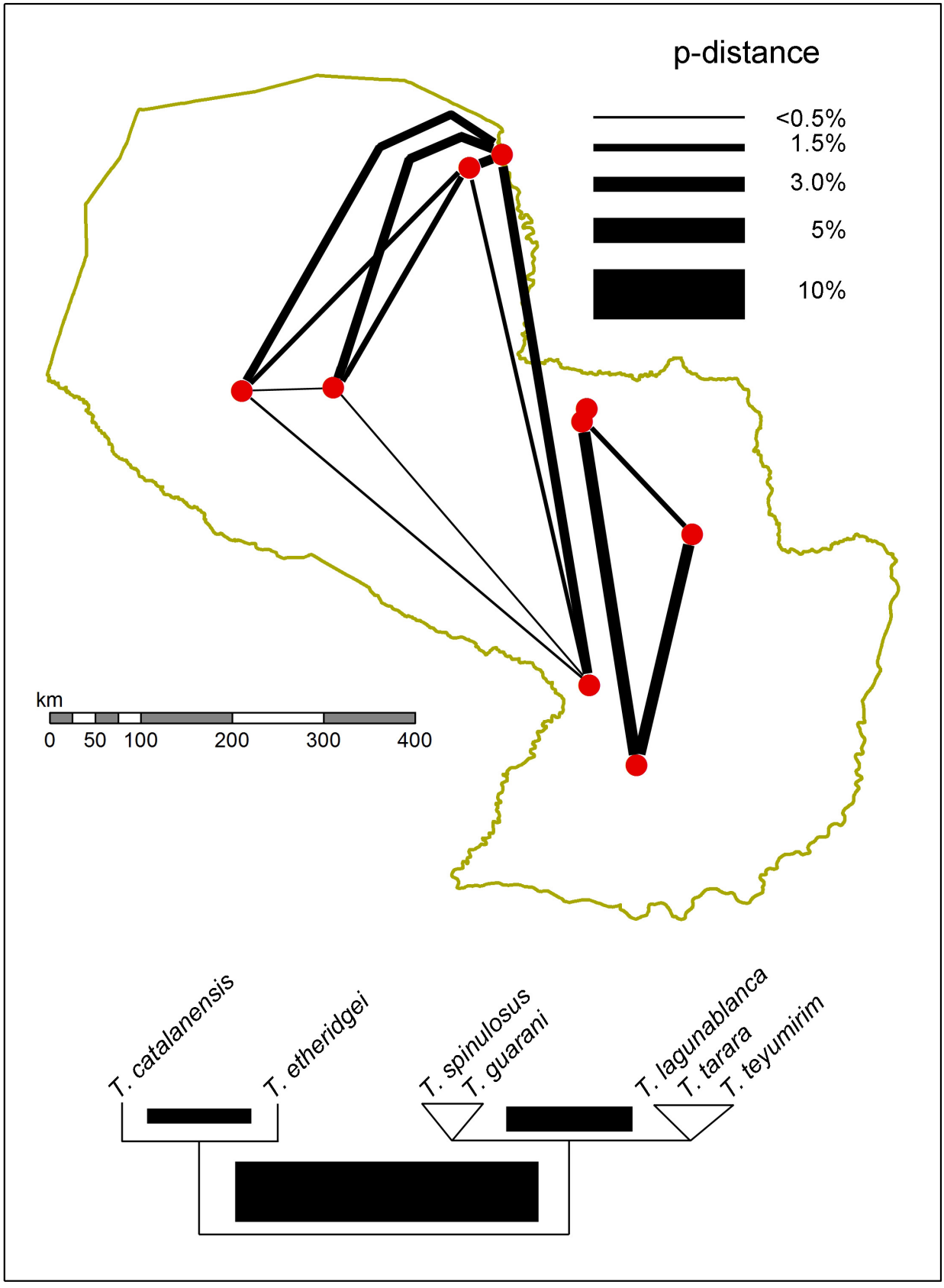

FIGURE 6. Graphic visualization of genetic distances among specimens of the *Tropidurus spinulosus* group (red dots). The width of the lines refer to the p-distance between specimens (reference at the upper right corner). At the bottom is presented the mean p-distance between species of the *torquatus* group (*T. catalanensis* and *T. etheridgei*) and the two bigger clades of the *spinulosus* group.

The estimated genetic p-distance for 16S between the *spinulosus* (*T. guarani*, *T. lagunablanca*, *T. spinulosus*, *T. tarara*, and *T. teyumirim*) and *torquatus* (*T. catalanensis* and *T. etheridgei*) groups varies from 11.7 to 13.7% (Fig. 5, Table 1). The p-distance between *T. catalanensis* and *T. etheridgei* in the *torquatus* group range between 3.1 and 3.4% (Table 1), and the intraspecific variation in this group is higher in *T. catalanensis* with a maximum of 0.6% between samples from Brazil (UFRGS 6181) and southern Paraguay (SMF 100090, 100093) (Table 1, Appendix 4). The samples of *T. guarani* and *T. spinulosus* exhibit low distances (0.2–1.5%) (Table 1), and the most differentiated specimen of *T. spinulosus* is one from the Pantanal area (IIBP-H 3463) (Fig. 6, Table 1), which clusters as a basal lineage within the clade *T. guarani* + *T. spinulosus* according to the COI tree (Fig. 4B). *Tropidurus guarani* and *T. spinulosus* are more differentiated from the remaining species of the *spinulosus* group with genetic p-distances from 4.0 to 6.0% (Fig. 6, Table 1). Low distances were found between *T. lagunablanca* and *T. tarara* (1.0–1.1%) (Table 1, Appendix 4), and, although slightly higher, among *T. teyumirim*, *T. lagunablanca*, and *T. tarara* (2.0–2.6%, respectively) which is lower than the distance between *T. catalanensis* and *T. etheridgei* mentioned before.

TABLE 1. Ranges of uncorrected pairwise genetic distances (in percentages) based on mtDNA genes COI (upper right section) and 16S (lower left section) among samples of *Tropidurus* from Paraguay.

COI 16S	*T. catalanensis*	*T. etheridgei*	*T. "guarani"*	*T. "lagunablanca"*	*T. "spinulosus"*	*T. "tarara"*	*T. "teyumirim"*
T. catalanensis	0.3–0.8 0.6	7.2–8.2	19.8–20.3	18.8–19.2	19.8–20.8	18.9–20.2	19.0–20.0
T. etheridgei	3.1–3.4	0.1–0.5 <0.01	20.8–21.2	19.1–19.3	20.5–21.0	19.4–20.6	19.7–20.8
T. "*guarani*"	12.1–12.5	12.5	<0.01 <0.01	7.1	1.2–2.5	6.2–7.7	7.0–8.4
T. "*lagunablanca*"	12.5–13.0	12.9	4.0	- -	7.1–7.4	1.6–1.9	4.6–5.4
T. "*spinulosus*"	12.1–12.8	12.5–12.7	0.2–1.5	4.0–4.6	0.3–2.3 0.2–2	6.2–8.1	7.5–9.1
T. "*tarara*"	11.7–12.6	12.1–13.0	4.6–4.9	1.0–1.1	4.4–5.4	0.7–1.6 <0.01	3.9–6.1
T. "*teyumirim*"	13.3–13.5	13.7	5.5	2.2	5.3–6.0	2.0–2.6	2.8 -

The coloration of *Tropidurus catalanensis*, *T. etheridgei*, and *T. torquatus* is similar, consisting of grayish background color with clear and black speckling, sometimes forming intermittent transversal lines. The three species have dark reticulations in the pectoral, gular, and chin regions, sometimes formed by convergent irregular lines; and dark marks under thighs and precloacal regions. Nevertheless, there are some differences in the color of the claws. We found that *T. torquatus* has light brown claws, whereas *T. catalanensis* has dark brown claws (Fig. 7) and this is a character that persists also in preserved specimens.

With respect to the scalation, we found a smaller number of SAB and GUL in *T. catalanensis* than in *T. torquatus* (Table 2), although our sample size was small for these species. Additionally, we found strongly mucronate dorsal scales in *T. catalanensis*, whereas less evident mucronation in *T. torquatus* (Fig. 8).

Tropidurus etheridgei is the most different species within the group, given that this species lacks axillary and inguinal folds, whereas *T. catalanensis* has two or three axillary folds and one inguinal fold (Fig. 9). Additionally,

T. etheridgei is a species with large scales, which can be noted by the low number of scales, compared to the previous species (Table 2).

In the *spinulosus* group, we found a strong sexual dimorphism in the number of dorsal scales, due to the presence of the vertebral crest in males, which causes lower counts (Table 2). We have not recorded sexual dimorphism in other than that character. The ranges of *T. spinulosus* (in both sexes) are largely the same as those recorded for *T. guarani* (Table 2). Equally, the counts of the only specimen of *T. lagunablanca* are contained in the variation of *T. tarara* (Table 2).

TABLE 2. Pholidotic ranges of *Tropidurus* from Paraguay. DOR: dorsal scales, SAB: scales around body, GUL: gulars. Numbers in parentheses indicate sample size. We were not able to assess the gender of two juveniles (J).

Species	DOR	SAB	GUL	Sex
T. catalanensis	108–115	7–106	51–55	M (2)
	112–121	97–116	54–57	F (2)
T. etheridgei	86	79	36	M (1)
	94	82	39	F (1)
	82–97	80–92	43–44	J (2)
T. torquatus	121	129	62	M (1)
	102–114	124–139	58–64	F (2)
T. guarani	76	113	71	M (1)
	111	109	69	F (1)
T. lagunablanca	75	111	68	M (1)
T. tarara	70–79	87–106	68–74	M (2)
	90–103	99–111	58–71	F (6)
T. spinulosus	64–88	99–103	52–61	M (5)
	96–110	97–122	54–60	F (4)

The species of the *spinulosus* group exhibit a great variation in coloration. The examined specimens show a basic dorsal background color of gray and greenish suffusions, with black marks (triangular blotches, rhombs, or irregular lines) disposed transversally, which are absent in the larger specimens. Most of the specimens (except SMF 100095) have light green speckles. Nevertheless, we found that all females of *T. tarara* (n=6) have a black band on the lateral areas of the neck, which is absent in females of *T. guarani* and *T. spinulosus*.

In conclusion, we interpret the low genetic distances documented among the nominal taxa *Tropidurus lagunablanca*, *T. tarara*, and *T. teyumirim* as evidence for conspecificity. This hypothesis is supported by the lack of morphological characters that would diagnose any of the three taxa. Therefore, we consider these three nominal taxa to be conspecific. Since *T. lagunablanca* has page priority over the other two names (Principle of Priority, Article 23, International Code of Zoological Nomenclature), this is the valid species name for this taxon. Similarly, we found very low genetic distances among populations assigned to the nominal taxa *T. guarani* and *T. spinulosus*, including samples from near the type locality of the former. Again, we were unable to find any morphological characters that would differentiate between the two taxa and therefore, we synonymize *T. guarani* with *T. spinulosus*. Thus, we recognize two species of the *T. spinulosus* complex in Paraguay (i.e., *T. spinulosus* and *T. lagunablanca*).

Unfortunately, the only fully everted hemipenis we had access to, was of *T. lagunablanca* (SMF 103316, Fig. 10), and it was recorded as follows: A medium-sized, bilobate organ with well-developed lobes; truncus mostly without ornamentation except for two concave areas on asulcate side at bifurcation level of sulcus spermaticus; sulcus spermaticus barely visible, bifurcating at level of base of lobes and its branches continuing to tip of lobes; lobes extensively covered with calyces; tips of lobes with a crown (ca. 1.08 mm Ø) of papillae of ~0.5 mm.

Following we present species accounts for the Paraguayan species of the genus *Tropidurus*, according to our taxonomic conclusions.

***Tropidurus catalanensis* Gudynas & Skuk, 1983**

Taraguira torquata: Cope 1862.

Tropidurus torquatus: Koslowsky 1898; Bertoni 1914, 1939; Schouten 1929; Giraudo & Contreras 1994; Duré Rodas 1995; Aquino *et al*. 1996; Motte *et al*. 2004; Motte *et al*. 2009; Cabral & Weiler 2014; Cacciali *et al*. 2016.

Tropidurus catalanensis Gudynas & Skuk, 1983. Type locality: 1 km ESE of Route 30 and Arroyo Catalan Grande, Artigas, Uruguay. Álvarez *et al*. 1995; Kunz & Borges-Martins 2013.

Tropidurus torquatus torquatus: Burt & Burt 1933.

Tropidurus torquatus hispidus: Hellmich 1960.

Geographic distribution. The distribution of *T. catalanensis* ranges from the southern region of the Brazilian State of Mato Grosso do Sul, through eastern Paraguay and the State of Paraná (Brazil) to the provinces of Chaco and Corrientes (Argentina), and Uruguay (Fig. 11A).

***Tropidurus etheridgei* Cei, 1982**

Tropidurus hispidus: Koslowsky 1898; Bertoni 1914, 1939; Talbot 1978, 1979

Tropidurus etheridgei Cei, 1982. Type locality: Mina Claveros, Córdoba, Argentina. Aquino *et al*. 1996; Motte *et al*. 2009; Carvalho 2013; Cabral & Weiler 2014; Cacciali *et al*. 2016

Tropidurus torquatus hispidus: Hellmich 1960

Geographic distribution. Central and southern Bolivia, some areas in western Brazil, Paraguayan Chaco (west of Paraguay River), and a narrow area in the Dry Chaco of Argentina (Fig. 11B)

***Tropidurus lagunablanca* Carvalho, 2016**

Tropidurus spinulosus: Peracca 1895; Schenkel 1901; Bertoni 1939; Hellmich 1960; Elter 1981.

Tropidurus sp.: Aquino *et al*. 1996 [part.]

Tropidurus guarani: Frost *et al*. 1998 [part.]; Carvalho 2013 [part.]; Cacciali *et al*. 2016 [part.].

Tropidurus lagunablanca Carvalho, 2016. Type locality: Reserva Natural Laguna Blanca, Santa Rosa del Aguaray, San Pedro, Paraguay.

Tropidurus tarara Carvalho, 2016. Type locality: Reserva Natural Cerrados del Tagatiya, Concepción, Paraguay.

Tropidurus teyumirim Carvalho, 2016. Type locality: Parque Nacional Ybycui, Paraguarí, Paraguay.

Tropidurus sp. 1: Cacciali *et al*. 2016.

Geographic distribution. Broadly distributed in Mato Grosso and Mato Grosso do Sul (Brazil), narrowing the distribution southwards, reaching the Ybycuí National Park (Paraguarí, Paraguay) as the southernmost record for the species (Fig. 11D).

***Tropidurus spinulosus* (Cope, 1862)**

Microlophus spinulosus Cope, 1862. Type locality: Paraguay.

Tropidurus spinulosus: Boulenger 1885, 1894, 1898; Koslowsky 1898; Bertoni 1914, 1939; Burt & Burt 1933; Cochran 1961; Scott & Lovett 1975; Talbot 1979; Aquino-Shuster *et al*. 1991; Aquino *et al*. 1996; Frost *et al*. 1998; Ziegler *et al*. 2002; Motte *et al*. 2009; Carvalho 2013; Cacciali *et al*. 2016.

Tropidurus (*Microlophus*) *spinulosus*: Boettger 1885.

Tropidurus spinulosus guarani Álvarez *et al*., 1994. Type locality: Cerro Hũ, Paraguarí, Paraguay.

Tropidurus sp.: Aquino *et al*. 1996 [part.].

Tropidurus guarani: Frost *et al*. 1998 [part.]; Motte *et al*. 2004 [part.]; Motte *et al*. 2009 [part.]; Carvalho 2013 [part.]; Cabral & Weiler 2014; Cacciali *et al*. 2016 [part.].

Geographic distribution. *Tropidurus spinulosus* is mainly associated with xeric areas in southern Bolivia, western Paraguay, with records in rocky outcrops east of the Paraguay River, and northeastern Argentina (Fig. 11C)

Discussion

As Frost *et al*. (2001) stated, the systematics and taxonomy of *Tropidurus* was static until some contributions of Rodrigues (Rodrigues 1981, 1984, 1986, 1988) with an exhaustive revision of the genus (Rodrigues 1987), which

helped to increase the knowledge (mostly in the *T. torquatus* group) on its diversity. Based on evidence from integrative approaches (molecular and morphological data), here we synonymized three species, with which the current documented diversity of the genus is 26 species, 4 of them known to occur in Paraguay.

Tropidurus torquatus was considered a taxon with a wide distribution (see Carvalho 2013) until Kunz & Borges-Martins (2013), based on morphological data, revalidated *T. catalanensis*. Cacciali *et al.* (2016) did not considered *T. catalanensis* as a valid species arguing that *T. torquatus* is a wide spread species and differentiating characters proposed by Kunz & Borges-Martins (2013) are interpreted as intraspecific variation. The distinction between *T. catalanensis* and *T. torquatus* was strongly supported later by Carvalho *et al*. (2016) based on molecular genetic evidence. According to Kuntz & Borges-Martins (2013), *T. catalanensis* can be differentiated from *T. torquatus* by the yellow coloration of the chest of *T. catalanensis*, which is white in *T. torquatus*, but this trait is better observed in adult males, and easily lost in preserved animals, and thus we failed to detect the mentioned coloration differences in museum vouchers. However, our morphological examination provided evidence that the coloration of the claws can also be used as a diagnostic character (Fig. 7).

FIGURE 7. Detailed view of the left hind claws of *T. torquatus* (A, SMF 100097) showing a paler color than observed in *T. catalanensis* (B, SMF 100093). This coloration is also present in the fore claws.

The other species of the *torquatus* group present in Paraguay is *T. etheridgei*, which phylogenetically is the sister clade to *T. catalanensis*, and all the analyzed gene fragments show a clear genetic distinction between these two species (Fig. 5). In Paraguay these two species are parapatric, with *T. catalanensis* occupying the eastern region of Paraguay and *T. etheridgei* being restricted to the Chaco, west of the Paraguay River (Cacciali *et al*. 2016) (Fig. 1). *Tropidurus catalanensis* can be distinguished from *T. etheridgei* by the skin folds of the armpit and the groin. According to Rodrigues (1987) and Kuntz & Borges-Martins (2013), *T. catalanensis* has two or three axillary folds and one inguinal fold, whereas *T. etheridgei* lacks these folds (Fig. 9). Nevertheless, there are additional differences such as the low number of scales in the three meristic variables analyzed by us (Table 2).

Carvalho (2013) provided a general map of the distribution of *T. etheridgei* showing its wide range from almost the Atlantic coast in Brazil to the Andes. In Paraguay and Argentina *Tropidurus etheridgei* is strongly associated with the Dry Chaco, with a few records in the Humid Chaco (Álvarez *et al*. 2002; Cacciali *et al*. 2016) (Fig. 11B). This species has two Paraguayan records east of the Paraguay River: ZSM 242/1933 from Estancia

Centurión, Concepción Department (22º18'S, 57º33'W) based on Hellmich (1960), and MZUSP 95202 from San Lorenzo, Central Department (25º17'S, 57º37'W) as reported by Carvalho (2013), but a verification of these records is needed, and we did not include them in the distribution map of the species.

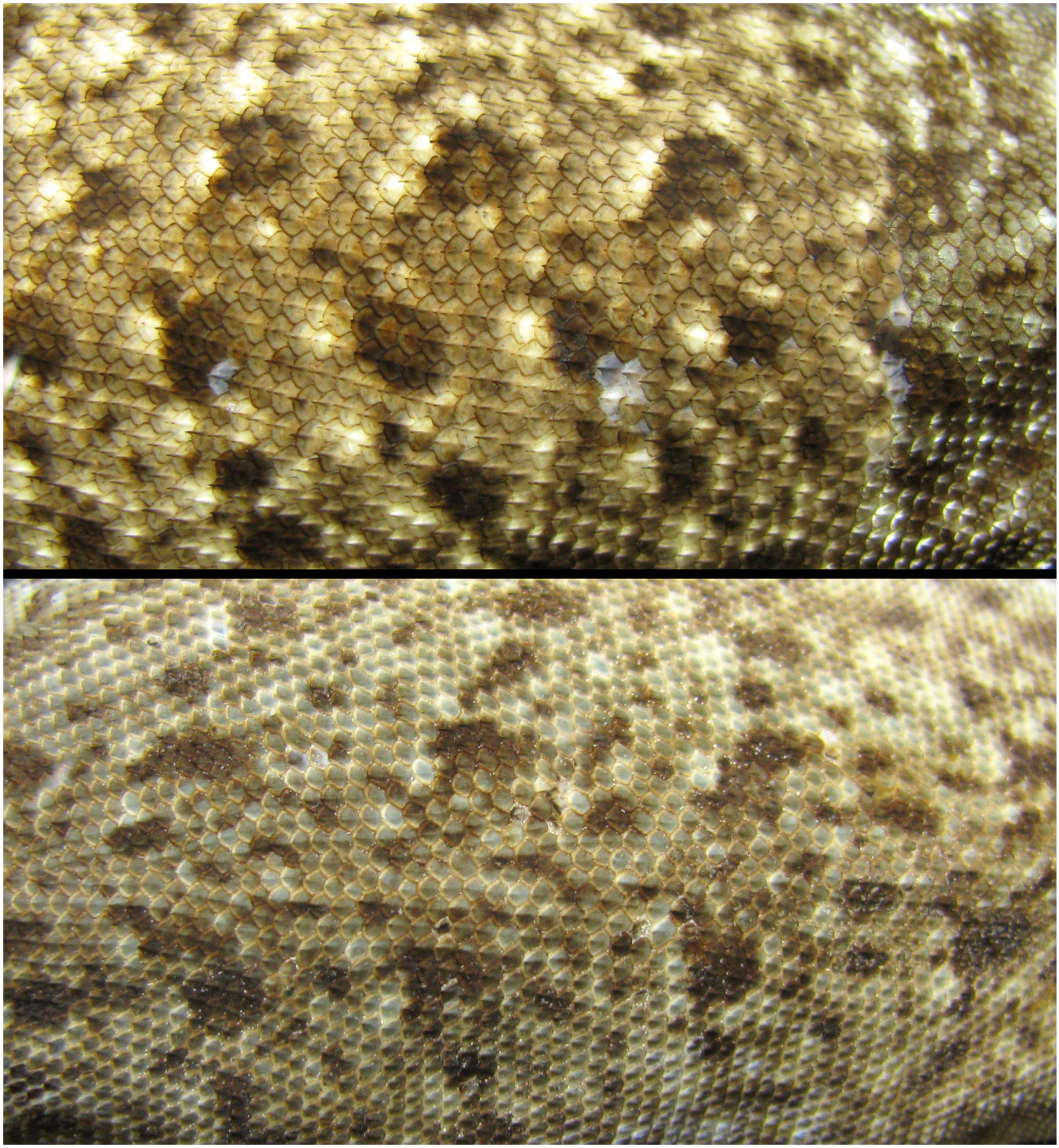

FIGURE 8. Differences in the mucronation of dorsal scales of *Tropidurus catalanensis* (above, SMF 100091) and *T. torquatus* (below, SMF 100097).

The *spinulosus* group in Paraguay proved to be taxonomically more dynamic. *Tropidurus spinulosus* was described from "Paraguay" (without more specific locality data) by Cope (1862) and more than a century later, Álvarez *et al*. (1994) described the subspecies *T. s. guarani* which was later elevated to species status by Harvey & Gutberlet (1998), referring to the species concept of Frost & Hills (1990). Based on molecular and morphological analyses, Frost *et al*. (1998) provided evidence for the distinction between "*T. guarani*" and *T. spinulosus*. Nevertheless, these authors did not use topotypic samples of "*T. guarani*" for the analyses, and here we present the

first analysis including actual populations of "*T. guarani*" from near the type locality of this taxon. The reason for the erroneous taxonomic allocation of the samples labeled as "*T. guarani*" by Frost *et al*. (1998) was that they referred to *T. guarani* all specimens that came from the east of the Paraguay River and their eastern genetic samples covered the departments of Concepción (i.e., "*T. tarara*" of Carvalho 2016) and Paraguarí (i.e., "*T. teyumirim*" of Carvalho 2016), but lacking the critical population from Cordillera (type locality of *T. guarani*). Given the geographic proximity of Cordillera with Paraguarí it is natural to think that they belong to the same clade, although they do not (Fig. 5–6). Our analysis based on the mitochondrial genes 16S and COI revealed that specimens from the regions occupied by the nominal species "*T. guarani*" and *T. spinulosus* are very closely related (Fig. 4A–B), rendering *T. spinulosus* paraphyletic if "*T. guarani*" is accepted as a valid species. Additionally, our estimation of the genetic distances with 16S, showed little differentiation between "*T. guarani*" and *T. spinulosus* (p-distance 0.2–0.4%) from central Chaco, and a larger distance among these two populations and samples of *T. spinulosus* (p-distance 0.8–1.5%) from the Paraguayan Pantanal (Appendix 4) where no natural barriers are observed. Thus, contrary to what Frost *et al*. (1998) suggested, our genetic data do not provide evidence for the River Paraguay being a relevant barrier for the differentiation of *Tropidurus*, nor for a vicariant event between *T. spinulosus* (west of Paraguay river) and "*T. guarani*" (east of Paraguay River) at some point in the past. There is no genetic evidence to consider "*T. guarani*" distinct from *T. spinulosus*.

FIGURE 9. Dextral lateral views of specimens of *T. catalanensis* (A, SMF 100093) indicating inguinal (left arrow) and axillary (right arrow) folds, compared with *T. etheridgei* (B, SMF 87389), which lacks these folds.

In the original description of *T. guarani*, the authors suggested that it differs from *T. spinulosus* in having a smaller body size, a reduced dorsal crest, and less mucronate scales (Álvarez *et al*. 1994). Additionally, "*T. guarani*" supposedly tends to have a higher number of body scales (scales around midbody, vertebral scales, paravertebral scales, scales between axillae, gular scales, etc.), although with the exception of scales around midbody, the ranges are all overlapping. Morphologically, Frost *et al*. (1998) recorded differences between *T. spinulosus* and "*T. guarani*" such as well-developed dorsal crest, white flash marks under thighs, and mottling bars

in the gular region in the former species, against reduced dorsal crest, yellow flash marks under thighs, and convergent bars in the gular region in "*T. guarani*". These authors claimed these morphological traits for "*T. guarani*" to be constant within populations from Cordillera (Piraretá) and Paraguarí (Cerro Hũ and Ybycuí National Park), even when the specimens from Cerro Hũ (northern Paraguarí) and Ybycuí National Park (southeastern Paraguarí) belong to different clades. Then, it is evident that those morphological traits are variable and can respond to different lifestyles: *T. spinulosus* is a tree-dweller west of the Paraguay River (where there are no rocky outcrops) and a rocky-dweller east of the river. When the river separated *T. spinulosus* is difficult to infer given the absence of temporal references about diversification of the genus (Carvalho *et al*. 2013).

It is important to note that Frost *et al*. (1998) recorded a variation range of scales around midbody for Paraguayan specimens of *T. spinulosus* (Álvarez *et al*. 1994 did not have samples from Paraguay, using only specimens from Argentina for comparison) of 80–103 (81–92 in Álvarez *et al*. 1994, and 97–122 in this study) which falls in the variation range of "*T. guarani*" (97–106 according to Álvarez *et al*. 1994, and 109–113 in this study). Thus, clinal variation is expected, especially in populations with different ecological adaptations.

FIGURE 10. Asulcate (left) and sulcate (right) views of the left hemipenes of *T. lagunablanca* (SMF 103316). White bar = 5 mm.

Thus, considering "*T. guarani*" as a synonym of *T. spinulosus*, this species would be present on both sides of the Paraguay River (Fig. 11C). In fact, there is at least one old record (NHMUK 94.3.14 according to Cacciali *et al*. 2016) of *T. spinulosus* in Asunción (capital city of Paraguay) which could be an error in the location of origin, or a local extinction given that the capital of the country is rather urbanized. In an addendum, Álvarez *et al*. (1994) considered a record from San Bernardino (25°16'S, 57°19'W) in Cordillera Department based on a specimen in the Museum Alexander Koenig (no voucher provided) as belonging to "*T. guarani*".

Interesting is the fact that Frost *et al*. (2001) identified "*T. guarani*" as the sister taxon of the clade *T. spinulosus* + *T. xanthochilus* (Fig. 6 in Frost *et al*. 2001). The synonymization of "*T. guarani*" with *T. spinulosus* as we propose would render this last species as paraphyletic if *T. xanthochilus* is a valid species. Studies addressing this taxonomic issue are clearly needed.

Tropidurus spinulosus is strongly supported as the sister species to the clade containing the nominal taxa *T. lagunablanca* + "*T. tarara*" + "*T. teyumirim*", but the phylogenetic relations among these three species is poorly resolved. In some tree topologies "*T. teyumirim*" is placed in its own clade in a trichotomy with *T. spinulosus* and *T. lagunablanca* + "*T. tarara*" (BI in Fig. 4B), but in most of the cases is inferred as the basal species of the clade *T. lagunablanca* + "*T. tarara*" + "*T. teyumirim*" (Fig. 4A–C). The genetic distances among samples, help to interpret the results. The p-distance for the mtDNA gene 16S between *T. lagunablanca* and "*T. tarara*" is low (1.0–1.1%) (Table 1), and even lower than the interspecific variation of *T. spinulosus* (up to 1.9%). For the same gene, the uncorrected p-distance between potential species was found to be at least 2% between candidate species of the

related genus *Plica* (Oliveira *et al.* 2016), although usually the expected distance between species is of 2.4–4% for other Iguanoids (Batista *et al*. 2015; Köhler *et al*. 2012; Köhler *et al*. 2016). This threshold coincides with that observed for the *torquatus* group. So, 3% of genetic distance between species seems to be reasonable, and the nominal taxa here synonymized do not show that level of differentiation (Table 1).

In morphology, *T. lagunablanca* supposedly differs from "*T. tarara*" by the pattern of cephalic and cervical orange coloration in males, and by well separated ranges of pholidosis in females (Carvalho 2016). However, the sample size of females covered to detect diagnostic characters between the species was low in the original description (two for *T. lagunablanca* and four for "*T. tarara*"), and for example, those scalation ranges are totally or partially overlapped with the females' variation of "*T. teyumirim*" which has a bigger sample size. Furthermore, we found larger scalation ranges for females of "*T. tarara*", and then the level of overlap is high.

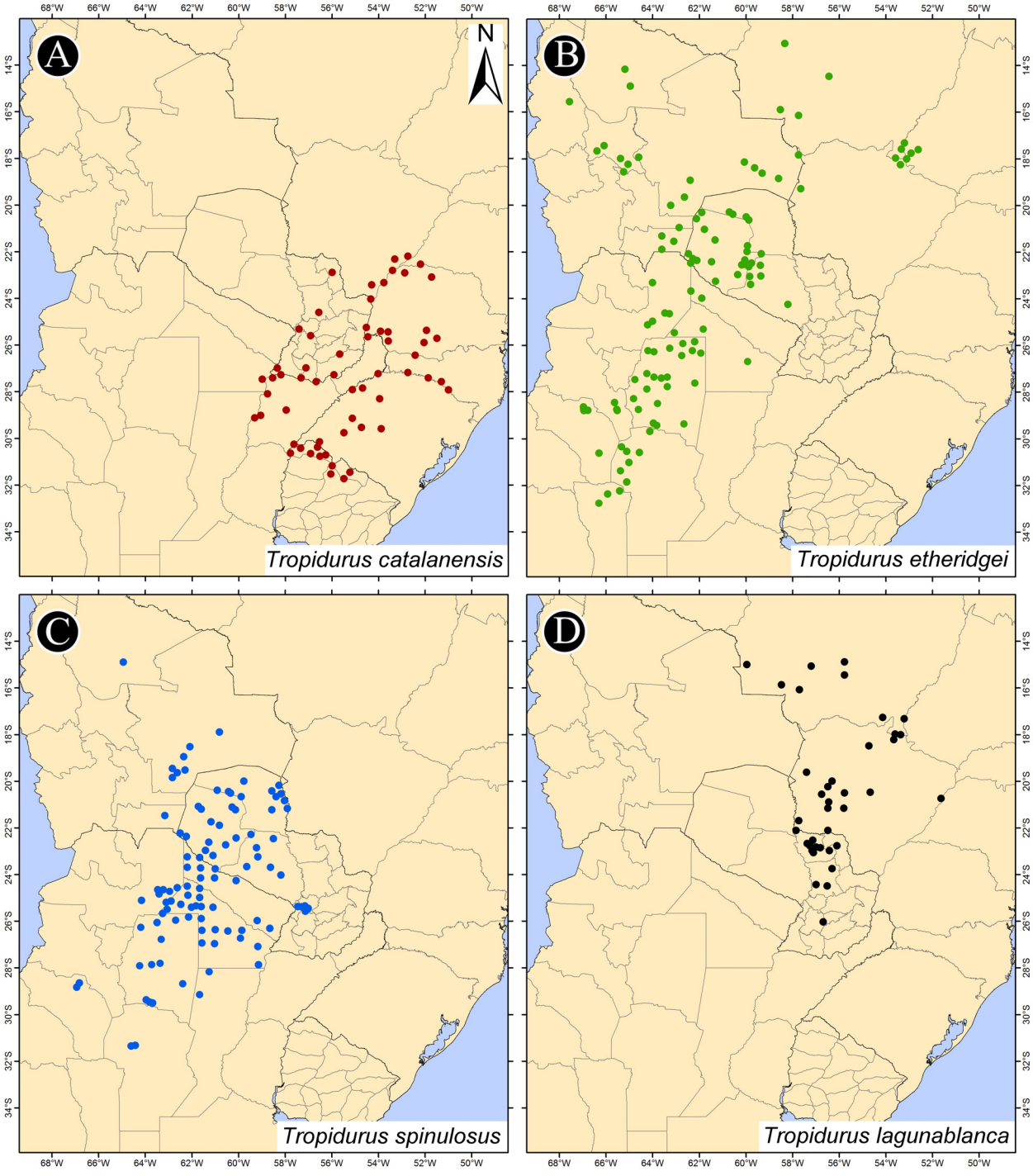

FIGURE 11. Distribution maps of *Tropidurus catalanensis* (A), *T. etheridgei* (B), *T. spinulosus* (C), and *T. lagunablanca* (D).

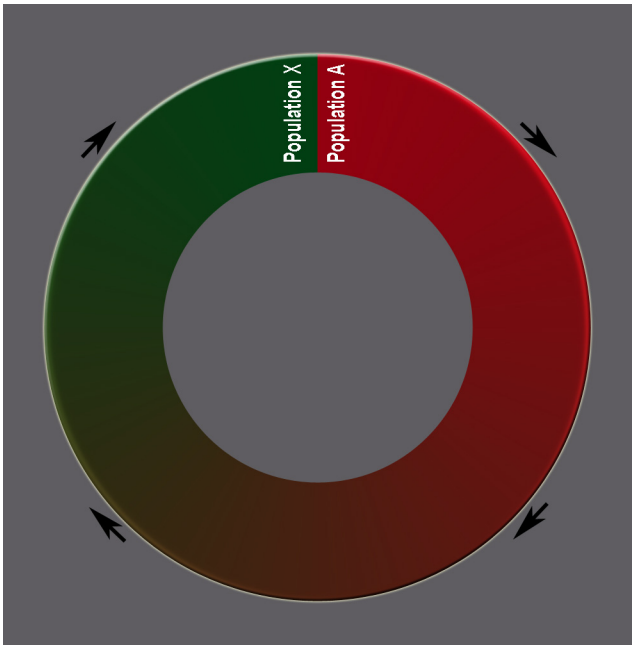

FIGURE 12. Diagram showing the theory of a ring species, where populations accumulate gradual changes along temporal and spatial scales (represented here by black arrows), originating different species.

With respect to "*Tropidurus teyumirim*", this was estimated as sister clade of *T. lagunablanca* but in some phylogenies it is observed at the same deep position with *T. spinulosus* and *T. lagunablanca* in a trichotomy. Frost *et al*. (1998) recognized a deep phylogenetic divergence (based on COI) between samples of "*T. guarani*" from Paraguarí (later recognized as "*T. teyumirim*" by Carvalho 2016) and "*T. guarani*" from Concepción and Amambay (posteriorly recognized as "*T. tarara*" by Carvalho 2016), which suggests an early divergence. It is important to note that we tried to use the COI sequences of Frost *et al*. (1998) (GenBank accession numbers: AFO40130–41), but due to the use of different primers, the sequences were completely different. The p-distance for the mtDNA gene 16S between "*T. teyumirim*" and *T. lagunablanca* (including the synonym "*T. tarara*") is 2.0–2.6%, which is lower than the distance observed between *T. etheridgei* and *T. catalanensis* (3.1–3.4%) (Table 1). This is a difficult threshold on which to base decisions because the p-distances are not very high, but the nuclear gene can shine a light on this problem. The PRLR sequences robustly separate *T. catalanensis* from *T. etheridgei*, but completely fail in the distinction of clusters in the *spinulosus* group (Fig. 4D), leading to an important conclusion: the *spinulosus* group in Paraguay had recent divergence events. From a nuclear point of view, only three lineages are recognized.

Without diving in the complex speciation process and the taxonomic decisions (Mayr & Ashlock 1991), the molecular genetic data presented here could be interpreted as evidence for the hypothesis of the *spinulosus* group being a sort of "ring species". Ring species are populations that gradually accumulate mutations along its dispersal range around a geographic barrier, and that at the end, the initial and final populations are very different due to the accumulation of changes (Fig. 12) (Cain 1954; Irwin *et al*. 2001). Of course this is a theoretical example, because the dispersion process is rather intricate than unidirectional, and many breaks in gene flow can be found along a gradient (Alcaide *et al*. 2014; Irwin *et al*. 2016). However, this could be an explanation for the pattern of genetic distances observed in *Tropidurus* from Paraguay, where two populations geographically close are genetically very distant from each other. Thus, a big question concerning this geographic closeness but genetic remoteness is how they achieved the current distribution without contact. The department of Paraguarí is full of hills and small mountain chains that can barely represent a true geographic barrier for these animals adapted to live in rocky environments. The explanation of the ring species provided before could explain the pattern, since Monahan *et al*. (2012) demonstrated that many geographic systems (usually understudied) provide continuous divergence towards speciation. More studies are required for a better understanding of the genetic variation of *Tropidurus*, and without doubt, the *spinulosus* group is a good model for evolutionary analyses.

Regarding morphology, "*T. teyumirim*" is a small *Tropidurus* lizard with low vertebral crest (Carvalho 2016) which is not a strong morphological gap as expected for a species, and it would rather correspond to subspecies

differences according to Mayr & Ashlock (1991). Frost *et al*. (1998) mentioned that gular coloration of *Tropidurus* populations from Cordillera and Paraguarí have convergent bars and a dark gular patch, and yellow thigh flash marks. In that case, that is an indication of a strong morphological convergence or parallelism in the evolution of these two clades, since these two populations are phylogenetically very distinct (Fig. 5), with a high degree of genetic distance (5.6%).

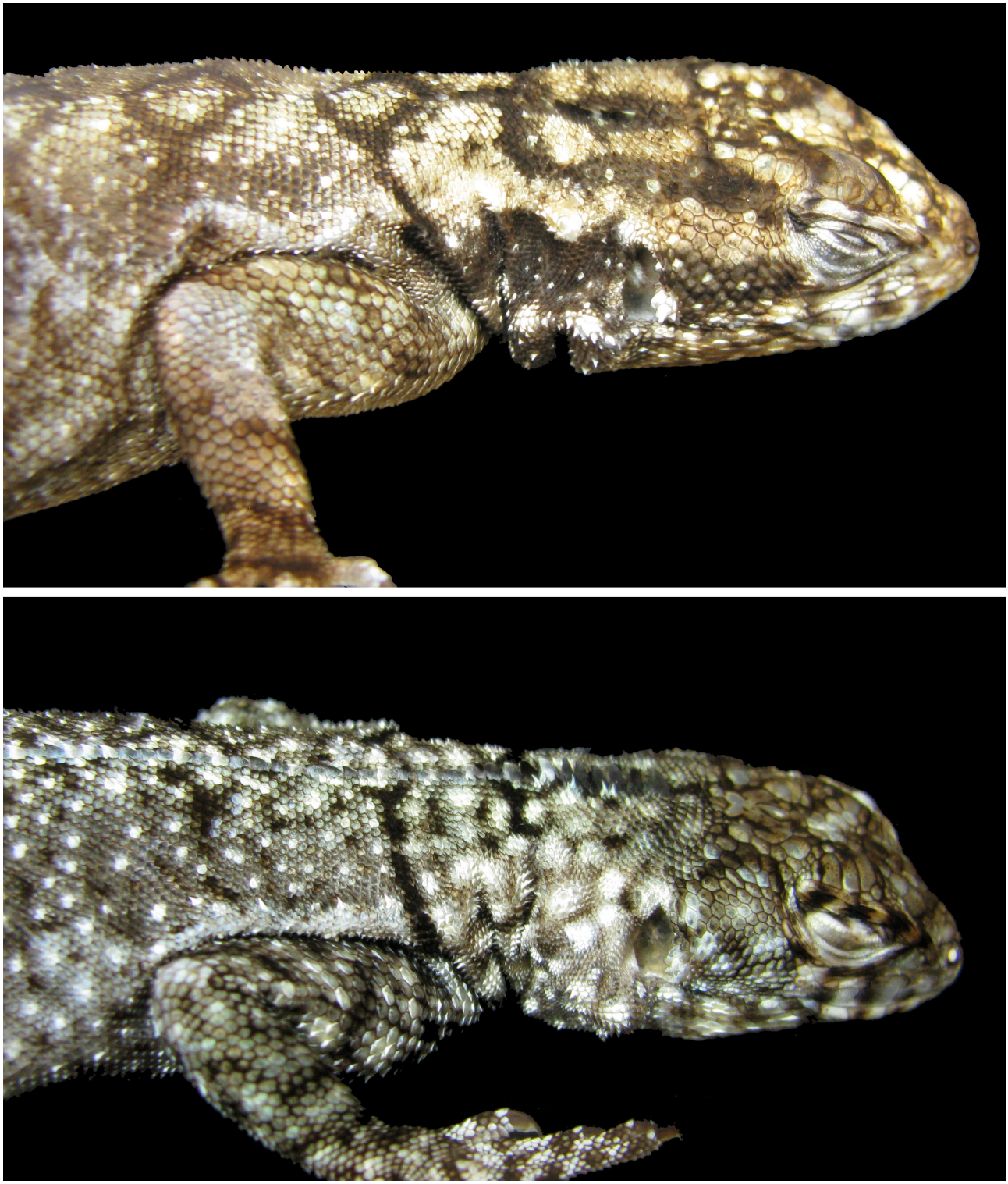

FIGURE 13. Differences in the color of females of *T. lagunablanca* (above, SMF 103315) and *T. spinulosus* (below, SMF 103322). Note the black stripes (the upper one behind the eye, and the lower beyond the ear opening) of *T. lagunablanca*, absent in *T. spinulosus*.

In conclusion, the evidence presented here justifies the recognition of only two species in the *spinulosus* group in Paraguay: *T. spinulosus* and *T. lagunablanca*. In fact, it is still difficult to differentiate between these two taxa using external morphology. Our morphological analysis revealed that females of these two species can be distinguished by the coloration of the lateral sides of the neck (Fig. 13).

It is important to note that in our species account, we considered the population of *Tropidurus* from Guairá (based on the record of Cacciali *et al*. 2016) as *T. lagunablanca* grounded on the proximity with samples from Ybycuí National Park. Morphologically, the specimens from Guairá (image available at http://www.faunaparaguay.com/tropidurusguarani.html) show significant dimorphism where the juvenile has no dorsal crest and a brownish coloration, whereas the adult male has a large vertebral crest on the neck and intense bluish coloration. This is an additional evidence of the extremely high variation in coloration within the genus.

The genus *Tropidurus* in Paraguay is well represented in scientific collections (especially at the Museo Nacional de Historia Natural del Paraguay) and abundant. Then, the morphological variation can be well studied. But on the other hand, genetic data are not well represented, and still there are some gaps that have to be filled in Paraguay. We hope in the future more data can build a better understanding of the Paraguayan species and their relations with neighbor regions.

Finally, it is important to highlight that this study does not show that the diversity of the genus *Tropidurus* is less rich than considered previously. It is well known that the genetic diversity is a major component of the biological diversity (Vold & Buffett 2008), and we provided evidence that the *spinulosus* group has a high diversity; and in this respect, the Ybycuí National Park protects a unique lineage of *T. lagunablanca* that is product of the long evolutionary process.

Acknowledgments

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APPENDIX 1. Localities and GenBank accession numbers (GBAN) of samples used in this study. GBAN generated through this work, are presented in bold. MD indicates missing data. Paraguayan samples highlighted in bold. Field numbers (or tissue numbers) separated by hyphen. Institutional codes follow Sabaj Pérez (2016).

Species	Voucher	Locality	Country	GBAN		
				COI	16S	PRLR
T. catalanensis	SMF 100093	Isla Yacyretá, Misiones	**Paraguay**	**MG459292**	**MG438491**	**MG457741**
T. catalanensis	SMF 100090	Isla Yacyretá, Misiones	**Paraguay**	**MG459293**	**MG438492**	**MG457742**
T. catalanensis	UFRGS 6181	Manoel Viana, Rio Grande do Sul	Brazil	KU245091	KU245311	KU245190
T. etheridgei	SMF 101595	Filadelfia, Boquerón	**Paraguay**	MD	**MG438493**	**MG457743**
T. etheridgei	SMF 101596	Filadelfia, Boquerón	**Paraguay**	MD	**MG438494**	**MG457744**
T. etheridgei	LG-1096	Fuerte Esperanza, Chaco	Argentina	KU245088	KU245298	KU245187
T. guarani	SMF 100095	San Bernardino, Cordillera	**Paraguay**	**MG459294**	**MG438495**	**MG457745**
T. guarani	SMF 100096	San Bernardino, Cordillera	**Paraguay**	**MG459295**	**MG438496**	**MG457746**
T. lagunablanca	SMF 103320	Laguna Blanca, San Pedro	**Paraguay**	**MG459296**	**MG438497**	**MG457747**
T. spinulosus	AMNH 176295	Loma Plata, Boquerón	**Paraguay**	KU245103	KU245314	KU245204
T. spinulosus	SMF 100094	Estancia La Amistad, Boquerón	**Paraguay**	**MG459297**	**MG438498**	**MG457748**
T. spinulosus	IIBP-H 3463	Estación Biológica Tres Gigantes, Alto Paraguay	**Paraguay**	**MG459298**	**MG438500**	**MG457749**
T. spinulosus	PCS-312	Reserva Natural Tobich, Alto Paraguay	**Paraguay**	MD	**MG438499**	MD
T. tarara	SMF 103313	Estancia Garay Cué, Concepción	**Paraguay**	**MG459299**	MD	MD
T. tarara	SMF 103314	Estancia Garay Cué, Concepción	**Paraguay**	**MG459300**	**MG438501**	**MG457750**
T. tarara	SMF 103315	Estancia Garay Cué, Concepción	**Paraguay**	**MG459301**	**MG438502**	**MG457751**
T. tarara	SMF 103316	Estancia Garay Cué, Concepción	**Paraguay**	**MG459302**	**MG438503**	**MG457752**
T. teyumirim	AMNH-FS-20281	Parque Nacional Ybycuí, Paraguarí	**Paraguay**	**MG459303**	**MG438504**	**MG457753**
T. teyumirim	AMNH-FS-20284	Parque Nacional Ybycuí, Paraguarí	**Paraguay**	**MG459304**	MD	**MG457754**
Plica plica	AMCC-106953	NW bank of the Konawaruk River, Potaro-Siparuni	Guyana	KU245104	KU245313	KU245203

APPENDIX 2. PCR conditions for each gene, used for amplification.

16S			
Initial denaturation:	94.0ºC	2 min	
Denaturation:	94.0ºC	35 sec	×40
Annealing:	48.5ºC	35 sec	
Extension:	72.0ºC	1 min	
Final extension:	72.0ºC	10 min	

COI			
Initial denaturation:	94.0ºC	90 sec	
Denaturation:	94.0ºC	40 sec	×40
Annealing:	45.0ºC	40 sec	
Extension:	72.0ºC	40 sec	
Final extension:	72.0ºC	6 min	

PRLR			
Initial denaturation:	95.0ºC	90 sec	
1st Denaturation:	95.0ºC	35 sec	×10
1st Annealing:	63.0ºC (↓) -0.5ºC/cycle	35 sec	
1st Extension:	72ºC	2 min	
2nd Denaturation:	95.0ºC	35 sec	×10
2nd Annealing:	58.0ºC	35 sec	
2nd Extension:	72.0ºC	1 min	
3rd Denaturation:	94.0ºC	35 sec	×15
3rd Annealing:	55.0ºC	35 sec	
3rd Extension:	72.0ºC	1 min	
Final extension:	72.0ºC	10 min	

APPENDIX 3. Examined specimens.

Tropidurus catalanensis (4)

PARAGUAY: Misiones: Isla Yacyretá (SMF 100090–3).

Tropidurus etheridgei (10)

BOLIVIA: Santa Cruz: Caballero (SMF 87393); Comarapa (SMF 87394); San Sebastián (SMF 87390–1); Villa Merced (SMF 87389, 87392). PARAGUAY: Boquerón: Filadelfia (SMF 101595–7). Presidente Hayes: Laguna Capitán (SMF 101598).

Tropidurus lagunablanca (9)

PARAGUAY: Concepción: Estancia Garay Cué (SMF 103313–9); Estancia Kumaré (MNHNP 11832). San Pedro: Laguna Blanca (SMF 103320).

Tropidurus spinulosus (11)

BOLIVIA: Tarija: Villamontes (SMF 24922–7). PARAGUAY: Boquerón: Estancia La Amistad (SMF 100094). Cordillera: Cerro Pedregal (SMF 103321–2); San Bernardino (SMF 100095–6).

Tropidurus torquatus (3)

BRAZIL: Petrópolis: Fazenda Inglesa (SMF 100097–9).

APPENDIX IV

Declaration on the contributions of authors

to the publication: Taxonomic analysis of Paraguayan samples of *Homonota fasciata* Duméril & Bibron (1836) with the revalidation of *Homonota horrida* Burmeister (1861) (Reptilia: Squamata: Phyllodactylidae) and the description of a new species
Status: Published (2017).
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Authors involved: Pier Cacciali (PC), Mariana Morando (MMo), Cintia D. Medina (CDM), Gunther Köhler (GK), Martha Motte (MMe), Luciano J. Avila (LJA).

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 60%
Coauthor MMo: 15%
Coauthor GK: 10%
Coauthor LJA: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 65% – field work (collecting and documenting specimens), molecular analyses, morphological analyses, revision of museum vouchers.
Coauthor MMo: 5% – molecular analyses.
Coauthor CDM: 5% – molecular analyses.
Coauthor GK: 5% – revision of museum vouchers.
Coauthor MMe: 5% – revision of museum vouchers.
Coauthor LJA: 15% – field work (collecting and documenting specimens), morphological analyses.

(3) to the creation of the data collection and figures

PhD candidate: 70% – created database, sequenced DNA barcodes, provided photographs, created figures, created maps.
Coauthor MMo: 15% – created database, sequenced DNA barcodes.
Coauthor CDM: 10% – created database.
Coauthor GK: 5% – provided photographs.

(4) to the analysis and interpretation of the data

PhD candidate: 45% – analysis and interpretation of molecular, morphological, and biogeographical data.
Coauthor MMo: 15% – contributed to data analysis and interpretation.
Coauthor CDM: 5% – contributed to data analysis and interpretation.
Coauthor GK: 15% – contributed to data analysis and interpretation.
Coauthor MMe: 5% – contributed to data analysis and interpretation.
Coauthor LJA: 15% – contributed to data analysis and interpretation.

(5) to writing the manuscript

PhD candidate: 70%
Coauthor MMo: 10%
Coauthor GK: 10%
Coauthor CDM: 5%
Coauthor LJA: 5%

Date/place: _____

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

Taxonomic analysis of Paraguayan samples of *Homonota fasciata* Duméril & Bibron (1836) with the revalidation of *Homonota horrida* Burmeister (1861) (Reptilia: Squamata: Phyllodactylidae) and the description of a new species

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ABSTRACT

Homonota is a Neotropical genus of nocturnal lizards characterized by the following combination of characters: absence of femoral pores, infradigital lamellae not dilated, claws without sheath, inferior lamellae laterally not denticulate, and presence of a ceratobranchial groove. Currently the genus is composed of 10 species assembled in three groups: two groups with four species, and the *fasciata* group with only two species. Here, we analyzed genetic and morphologic data of samples of *Homonota fasciata* from Paraguay; according to Maximum Likelihood and Bayesian inference analyses, the Paraguay population represents an undescribed species. Additionally, morphological analysis of the holotype of *H. fasciata* (MNHN 6756) shows that it is morphologically different from the banded, large-scaled *Homonota* commonly referred to as "*H. fasciata*". Given the inconsistency between morphological characters of the name-bearing type of *H. fasciata* and the species commonly referred to as *H. fasciata*, we consider them as different taxa. Thus, *H. fasciata* is a *species inquirenda* which needs further studies, and we resurrect the name *H. horrida* for the banded, large-scaled *Homonota*. The undescribed species from Paraguay is similar to *H. horrida*, but can be differentiated by the high position of the auditory meatus relative to the mouth commissure (vs. low position in *H. horrida*); and less developed tubercles on the sides of the head, including a narrow area between the orbit and the auditory meatus covered with small granular scales with or without few tubercles (vs. several big tubercles on the sides of the head even in the area between the orbit and the auditory meatus). The new species is distributed in the Dry Chaco in South America. With the formal description of this species, the actual diversity of the genus *Homonota* is increased to 12 species. Furthermore, we infer phylogenetic relationships for 11 of the 12 described species of the genus, based on 11 molecular markers (two mitochondrial and nine nuclear genes), with concatenated and species tree approaches.

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Keywords Chaco, Gekkota, Phylogeny, Nomenclatural acts, South America

INTRODUCTION

The genus *Homonota* is a gecko of Gondwanan origin, distributed in South America, being present in southern Bolivia, northern to southern Argentina, western Paraguay, Uruguay, and the Brazilian state of Rio Grande do Sul (*Gamble et al., 2008a*; *Morando et al., 2014*). Along its distribution it inhabits dry environments like Monte, Chaco, Espinal, Patagonian, Andean, and Pampas (*Morando et al., 2014*). Regardless of the ecoregion, the genus is terrestrial and with the exception of *Homonota fasciata*, all species have a reticulated coloration pattern that imitates lichens on rocky backgrounds (*Avila et al., 2012*: Fig. 1). Unlike other geckos in South America, *Homonota* is adapted to a terrestrial life-style being only infrequently found in trees (*Cei, 1986*).

All species in the genus are nocturnal, oviparous—laying one or two eggs—, insectivorous lizards that can be found frequently in human dwellings feeding on a wide range of arthropods (*Cei, 1986*; *Cei, 1993*; *Abdala, 1997*; *Carreira, Meneghel & Achaval, 2005*; *Ibargüengoytía & Casalinas, 2007*; *Kun et al., 2010*). Members of this genus are characterized by the following combination of characters: absence of femoral pores, infradigital lamellae not dilated, claws without sheath, inferior lamellae laterally not denticulate, and presence of a ceratobranchial groove (*Peters & Donoso-Barros, 1970*; *Cei, 1986*; *Carreira, Meneghel & Achaval, 2005*). Currently, ten species are recognized in this genus (*Cajade et al., 2013*), some of which have small distribution ranges restricted to one or few localities (e.g., *H. andicola*, *H. rupicola*, *H. taragui*, and *H. williamsii*) or medium-sized distributions of less than 400 km from north to south (e.g., *H. uruguayensis* and *H. whitii*), whereas others have wide distribution ranges (e.g., *H. borellii*, *H. fasciata*, *H. underwoodi*, and *H. darwinii*) (*Morando et al., 2014*). In fact, *H. darwinii* reaches 50°S latitude, the southernmost limit for the genus and for any gecko species of the world.

Kluge (1964) proposed a grouping arrangement for *Homonota*, in which he placed *H. borellii*, *H. fasciata*, *H. horrida*, and *H. uruguayensis* in one group, and *H. darwinii*, *H. underwoodi*, and *H. whitii* in another. But a recent molecular analysis carried out by *Morando et al. (2014)* shows a different arrangement dividing the genus into three groups (i.e., the *borellii*, *whitii*, and *fasciata* groups). This last group is the least diverse with only two species, whereas each of the former two contain four species (*Morando et al., 2014*). The two species belonging to the *fasciata* group are *H. underwoodi* described by *Kluge (1964)* and *H. fasciata* with a complex taxonomic history discussed by *Abdala & Lavilla (1993)*.

Duméril & Bibron (1836), based on a single specimen from "Martinique", described *Gymnodactylus fasciatus*. *Burmeister (1861)* described *Gymnodactylus horridus* from Sierra del Challao, in Mendoza Province (Argentina). *Gray (1845)* erected the genus *Homonota* to accomodate the "Guidichaud's [sic] Scaled Gecko" *Gymnodactylus gaudichaudii Duméril & Bibron, 1836* (currently *Garthia gaudichaudii*), but according to *Vanzolini (1968)*, Gray actually used a specimen of *Homonota darwinii* (and not *G. gaudichaudii*), for the description of *Homonota*, so that *Homonota darwinii* is the actual type species of the

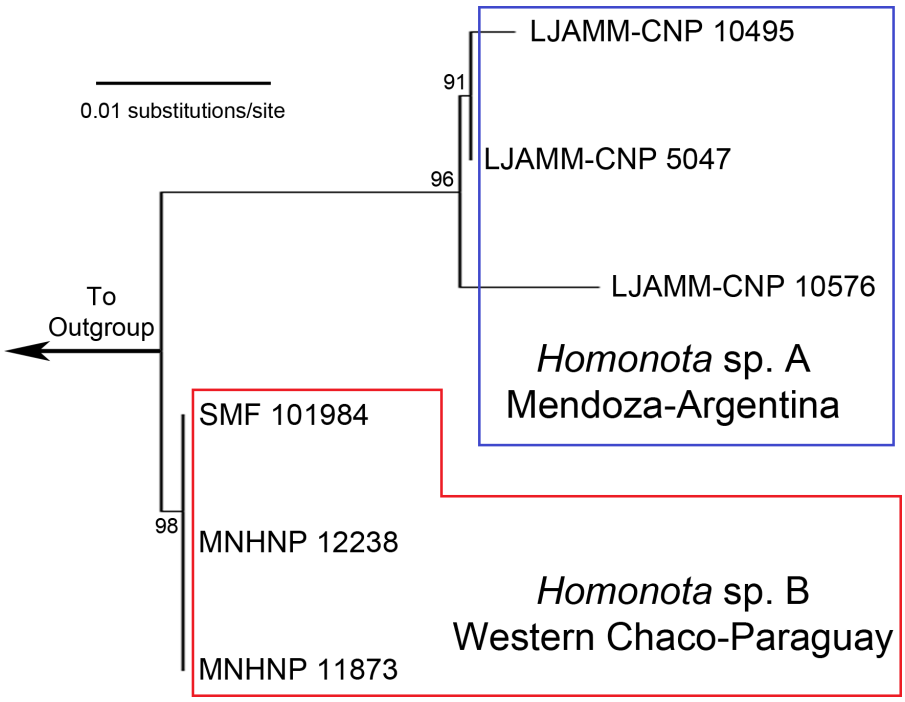

Figure 1 Maximum Likelihood tree. Maximum Likelihood clusters of *Homonota fasciata* from Argentina (blue square) and from Paraguay (red polygon), obtained from 16S mtDNA barcode sequences. Until name assignation, we refer to them as *Homonota* sp. A and *Homonota* sp. B respectively. Outgroup: *Phyllopezus przewalskii*.

genus. In a brief publication, *Berg (1895)* provided a description of a lizard he named *Gymnodactylus mattogrossensis* from Mato Grosso (Brazil, without any specific locality data), referring to a single specimen (not vouchered) given to him by his colleague Julio Koslowsky. *Kluge (1964)* moved these three names to the genus *Homonota* recognizing *H. horrida* and *H. fasciatus* [sic] as a valid species and transferring *Gymnodactylus mattogrossensis* to the synonymy of *H. horrida*. *Kluge (1964)* stated that these species are similar but differ in the number of interorbital scales (10–14 in *H. horrida* vs. 16 in the holotype of *H. fasciata*), the denticulation of ear opening (strongly denticulate all around the opening in *H. horrida* vs. a slight denticulation on the anterior margin in *H. fasciata*), size of postmental scales (moderately enlarged in *H. horrida* vs. greatly enlarged in *H. fasciata*), and size and shape of gular scales (large and plate-like in *H. horrida* vs. small and granular in *H. fasciata*). According to this author, *H. horrida* is present in southern Bolivia and Brazil, Paraguay, and northwestern Argentina, whereas the distribution of *H. fasciata* is unknown because its type locality "Martinique" is apparently based on a mistake, and no more additional locality records were available. *Abdala & Lavilla (1993)* suggested that diagnostic characters between *H. horrida* and *H. fasciata* as proposed by *Kluge (1964)* were intraspecific variation, and they synonymized *H. horrida* with *H. fasciata*. Since then the name *H. fasciata* was applied to the banded, large-scaled *Homonota* distributed from northern Paraguay and southern Bolivia, to Río Negro Province (central Argentina).

An almost complete molecular phylogenetic analysis was performed by *Morando et al. (2014)* including topotypes of all the recognized species. For *H. fasciata* the authors used specimens from Mendoza, since the original type locality (Martinique) is a mistake, and *Abdala & Lavilla (1993)* restricted the type locality of *H. fasciata* to Mendoza (in den Schluchten der Sierra bei Challao), which is actually the type locality for *Homonota horrida*.

In Paraguay, *Homonota fasciata* is distributed mainly in the Dry Chaco, with only one record in a transition zone of Dry Chaco with Humid Chaco (*Cacciali et al., 2016*). Given that *H. fasciata* has a complex taxonomic history, is one of the widest distributed members of the genus, and the almost complete absence of samples from Paraguay in previous publications, here we follow an integrative approach to assess the taxonomic status of samples from this country. First, within the framework of a barcoding project of Paraguayan herpetofauna, we generated molecular data and inferred a first round of hypotheses. Second, based on 11 genes, we inferred the taxonomic position of the Paraguayan populations in a phylogenetic tree that includes all the described species. Lastly, we analyzed detailed morphological data and also examined the holotype of *H. fasciata*.

MATERIALS AND METHODS

Genetic analyses

We carried out a first genetic inspection of the taxonomic status of Paraguayan populations currently referred to as *Homonota fasciata* using sequences of the mtDNA 16S gene as it was proved to be a useful tool for taxonomic identification (*Jansen & Schultze, 2012*; *Batista et al., 2014*; *Köhler, Vargas & Lotzkat, 2014*) with a desirable relation of cost/benefit. The Paraguayan samples ($N = 3$, GenBank accession numbers presented in Appendix S1, Supplementary Information online) from two localities were compared with available samples of the species from Mendoza, Argentina (used by *Morando et al., 2014*) located ~1.400 km in straight line ($N = 3$). Localities of vouchers used for genetic analyses are shown in Appendix S2. Paraguayan samples were collected with collecting permits SEAM No 04/11 and SEAM No 133/2015 issued by the Secretaría del Ambiente in Paraguay. Specimens were euthanized using anesthetic injections of barbituric acids (Tiopental Sódico® 1 g).

Tissue samples were first washed for 15 h with 50 µl Phosphate-buffered saline (PBS) (diluted of 1:9 PBS: H_2O). They were digested in a solution of Vertebrate lysis buffer (60 µl per sample) and proteinase K (6 µl per sample) at 56 °C for 15 h. Protocol for DNA extraction followed *Ivanova, Dewaard & Hebert (2006)*. After extraction, DNA was eluted in 50 µL Tris-EDTA (TE) buffer. Amplification of mtDNA 16S gene fragments was made using the eurofins MWG Operon primers L2510 (forward: 5′–CGCCTGTTTATCAAAAACAT–3′) and H3056 (reverse: 5′–CCGGTCTGAACTCAGATCACGT–3′) in an Eppendorf Mastercycler® pro. PCR conditions were: 94 °C–2 min, 40 ×[94 °C–35 s, 48.5 °C–35 s, 72 °C–1 min], 72 °C–10 min. Sequencing was performed using a BigDye® Terminator with the following cycling conditions: 95 °C–1 min, 30 ×[95 °C–10 s, 50 °C–10 s, 60 °C–2 min], with 10 µl of reaction volume.

The examination of chromatograms and generation of consensus sequences was performed using SeqTrace 0.9.0 (*Stucky, 2012*). Sequences were aligned first automatically with Clustal W (*Larkin et al., 2007*) followed by a visual inspection and edition if necessary, with the freeware MEGA 6 (*Tamura et al., 2013*). The alignment and the tree are available at TreeBase (ID: 20987). The substitution model for our dataset was identified according to the corrected (for finite sample size) Akaike Information Criterion (AICc) (*Burnham & Anderson, 2002*) and computed in MEGA 6.

We estimated the uncorrected genetic pairwise distances for our dataset, and ran Maximum Likelihood (ML) analysis with 30,000 bootstrap replicates in MEGA 6. We used *Phyllopezus przewalskii* as outgroup (SMF 100495, GenBank accession number MF278834), due to availability of relevant genetic information.

We used species delimitation methods to assess the degree of intraspecific divergences and to support the cluster arrangement suggested by the ML approach. This exploration was performed separately for the alignment and for the tree. The alignment was analyzed with ABGD (*Puillandre et al., 2012*) using simple distances to compare with the uncorrected genetic distance. For the tree based on 16S analysis, we applied the Poisson tree process (PTP) (*Zhang et al., 2013*) conducted through the bPTP web Server (http://species.h-its.org/), using default parameters and the outgroup removed. This algorithm does not require an ultrametric tree as input (*Zhang et al., 2013*), and it is a robust tool to estimate species delimitation from ML phylogenetic reconstructions (*Tang et al., 2014*). To assess the phylogenetic position of the Paraguayan samples within the genus, we used data from the recently published phylogenetic inference by *Morando et al. (2014)* and generated new sequences for all markers for samples from Paraguay (Appendix S3). We followed *Morando et al. (2014)* for amplification of the same two mitochondrial and nine nuclear genes, alignment protocols and gene and species trees approaches. Primers are specified in Appendix S4 .

Consensus sequences for each sample was generated with Sequencher v4.8 (^{TM}Gene Codes Corporation Inc. 2007, Ann Arbor, MI, USA), and aligned with Mafft (*Katoh & Standley, 2013*). Confirmation of open reading frames for protein-coding genes was made by translation into amino acids.

The best evolutionary substitution model for each gene was selected using the AICc (*Burnham & Anderson, 2002*) and ran in jModelTest v2.1.10 (*Darriba et al., 2012*). Recombination was tested and excluded for nuclear genes using RDP: Recombination Detection Program v3.44 (*Martin & Rybicki, 2000*; *Heath et al., 2006*). We conducted Separate Bayesian analyses (BI) for each gene using MrBayes v3.2.2 (*Ronquist & Huelsenbeck, 2003*). Four heated Markov chains (with default heating values) and run for five million generations were used for each analysis. The equilibrium samples (after 25% of burn-in) were used to generate a 50% majority-rule consensus tree, and posterior probabilities (PP) were considered significant when $\geq$0.95 (*Huelsenbeck & Ronquist, 2001*). Maximum Likelihood (ML) analyses for each gene were performed with RAxML v7.0.4 (*Stamatakis, 2006*), based on 1,000 rapid bootstrap analyses for the best ML tree.

We performed concatenated analyses with ML and BI for the following datasets: (1) two mitochondrial genes combined, (2) nine nuclear genes combined, (3) all genes combined.

Likelihood analyses were performed using RAxML v7.0.4, based on 1,000 rapid bootstrap analyses. Bayesian analyses were conducted using MrBayes v3.2.2, with four heated Markov chains (using default heating values) and run for 50 million generations, with Markov chains sampled at intervals of 1,000 generations. Equilibrium samples (after 25% of burn-in) were used to generate a 50% majority-rule consensus tree, and posterior probabilities (PP) were considered significant when $\geq$0.95 (*Huelsenbeck & Ronquist, 2001*).

For construction of a species tree incorporating the multispecies coalescent approach, we used the hierarchical Bayesian model integrated in *Beast v1.8.0 (*Drummond & Rambaut, 2007*). For all genes were run two separate analyses for 100 million generations (sampled every 1,000 generations). Clades with PP >0.95 were considered strongly supported.

To ensure that convergence was reached before default program burn-in values, we evaluated convergence of Bayesian MCMC phylogenetic analyses (MrBayes and *Beast) by examining likelihood and parameter estimates over time in Tracer v1.6 (*Rambaut, Suchard & Drummond, 2009*). All parameters were between 157 and 23,400 effective sample sizes (ESS).

All alignments and trees were stored in TreeBase (ID: 20987); phylip files produced by RAxML were converted to nexus with ALTER (*Glez-Peña et al., 2010*), and trees merged with matrices in Mesquite v3.2 (*Madison & Madison, 2017*).

Morphological approach

Voucher specimens are listed in Appendix S5. Coordinates are presented in decimal degrees and WGS 84 datum, and all the elevations are in meters above sea level (masl). Institution codes follow *Sabaj Pérez (2014)*.

Metric characters were taken following *Avila et al. (2012)*, and include snout-vent length (SVL) from tip of snout to vent; trunk length (TrL) distance from axilla to groin from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; foot length (FL) from tip of claws of the 4th toe to heel; tibial length (TL) greatest length of tibia, from knee to heel; arm length (AL) from tip of claws of the 3rd finger to elbow; head length (HL) distance between anterior edge of auditory meatus and snout tip; head width (HW) taken at level of the temporal region; head height (HH) maximum height of head, at level of parietal area; eye-nostril distance (END) from the anterior edge of the eye to the posterior edge of the nostril; eye-snout distance (ESD) from the anterior edge of the eye to the tip of the snout; eye-meatus distance (EMD) from the posterior edge of the eye to the anterior border of the ear opening; interorbital distance (ID) interorbital shortest distance; internostril distance (IND). Meristic data consist of: number of keeled dorsal tubercles (DT) from occipital area to cloaca level; number of transversal rows of ventral scales (TVS), counted longitudinally at midline from the chest (shoulder level) to inguinal level; number of longitudinal rows of ventral scales (LVS), counted transversally at midbody; number of supralabial scales (SL); number of infralabial scales (IL); number of fourth toe lamellae (4TL); and number of third finger lamellae (3FL). Paired structures are presented in left/right order. In the color descriptions, the capitalized colors and the color codes (in parentheses) are those of *Köhler (2012)*.

Based on the genetic clusters recognized by the barcoding analysis, we performed a discriminant function analysis (DA). As a first step we tested normality with Shapiro–Wilk

Table 1 Pairwise distances for 16S. Uncorrected pairwise genetic distances (in percentages) based on 16S mtDNA among samples of Species A from Argentina (white cells) and Species B from Paraguay (gray cells) formerly referred as *H. fasciata*. Minimum and maximum values between species in bold.

	LJAMM-CNP 5047	LJAMM-CNP 10495	LJAMM-CNP 10576	MNHNP 11873	MNHNP 12238	SMF 101984
LJAMM-CNP 5047	–					
LJAMM-CNP 10495	0.4	–				
LJAMM-CNP 10576	0.6	1.0	–			
MNHNP 11873	**1.8**	2.0	**2.5**	–		
MNHNP 12238	2.0	2.2	2.4	<0.01	–	
SMF 101984	2.0	2.2	2.4	<0.01	<0.01	–

Table 2 Fixed sites in the alignment of 16S. The 11 fixed sites differences on our 16S mtDNA alignment among three samples of Species A from Argentina (Ar) and three of Species B from Paraguay (Pa), formerly referred as *Homonota fasciata*. The numbers indicate nucleotide position.

	007	154	191	216	218	284	302	320	339	405	489
Species A (Ar)	T	G	C	T	–	T	A	A	C	T	T
Species B (Pa)	C	A	–	C	R	C	C	C	T	C	C

(W) test (*Shapiro, Wilk & Chen, 1968*; *Zar, 1999*). Then we performed the DA including variables with normal distribution, analyzing continuous characters (metrics) that are sensitive to ontogeny, separated from discrete (non-sensitive to body growth) characters. All statistical procedures were performed with Past 3.14 (*Hammer, Happer & Ryan, 2001*).

RESULTS

Phylogenetic inference

Following we present the size of each aligned gene (in brackets) and the best substitution model identified: 16S [527 bp]: GTR+G; 12S [951 bp]: GTR+G; cyt-b [794 bp]: TRN+I+G; MXRA5 [961 bp]: TPM1lf+G, NKTR [1074 bp]: TRN+G, SINCAIP [449 bp]: TPM2 lf+G, RBMX [600 bp]: HKY+G, DMXL1 [959 bp]: HKY+G, ACA4 [1218 bp]: HKY+G, PRLR [543 bp]: TRN+G, Homo_30b [664 bp]: TRN+I, Homo_19b [642 bp]: F81+G.

The ML tree based on an initial exploration with 16S mtDNA gene sequences shows two separate clades of geckos, formerly referred to as *Homonota fasciata* (Fig. 1), with uncorrected 16S p-distances ranging between 1.8 and 2.5% (Table 1). In the alignment we identified 11 fixed different sites between these clades (Table 2). We interpret the documented genetic differences as evidence for heterospecificity of these two clades. Thus, we recognize two potential species of geckos formerly referred to as *H. fasciata*: Species A (sampled in Low Monte ecoregion) and Species B (sampled in Dry Chaco, Paraguay).

The ABGD analysis for the 16S dataset resulted in the recognition of three groups (1- Species A, 2- Species B, 3- Outgroup) with a range of intraspecific genetic variation from 0.1 to 0.77%; and two groups (1- *Homonota*, 2- Outgroup) with an intraspecific variation of 1.29% (Appendix S6). This is only slightly higher than the higher intraspecific distance between two of our samples (p-distance=1.0% between LJAMM-CNP 10495 and LJAMM-CNP 10576; Table 1) of Species A, whereas the intraspecific distance among

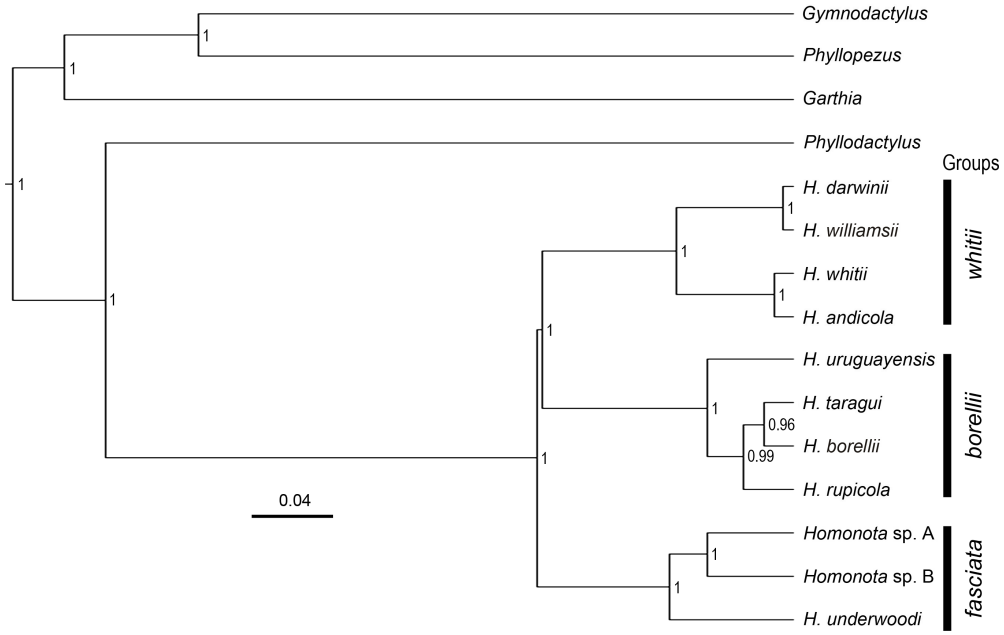

Figure 2 Species tree. Species tree of *Homonota* and related taxa inferred with *Beast, showing the position of the two clades (*Homonota* sp. A and *Homonota* sp. B) formerly referred as *H. fasciata*. Bar represents substitutions per site. Only values ≥0.95 are shown.

specimens of Species B (<0.01%). The PTP also proposed two different clades (both with ML and Bayesian algorithms) grouping separately Argentinean samples (Species A) and Paraguayan samples (Species B) (Appendix S7). Species A was inferred as the sister taxon of Species B in nine of the 11 independent gene trees obtained with both BI and ML (Appendix S8). Exceptions include: 1-the gene Homo_30b (both with BI and ML), which infer Species B as sister of the clade Species A +*H. underwoodi*; 2-DMXL1 inferred the *borelli* group as sister to Species A+Species B (both with BI and ML); 3-the gene SINCAIP (ML only) showed the groups *fasciata* and *whitii* nested together; 4- the gene NKTR with ML inferred *H. underwoodi* as a member of a different group (Appendix S8).

All phylogenies inferred from concatenated datasets of (1) two mitochondrial genes combined, (2) nine nuclear genes combined, (3) all genes combined with both BI and ML showed high support in recognizing Species B from Paraguay as a sister to Species A from Argentina, with *Homonota underwoodi* as sister to these two within the *fasciata* group (Appendix S9). The species tree inferred with *Beast presents the same arrangement within the *fasciata* group as those inferred by BI and ML using concatenated datasets (Fig. 2).

Morphological analyses

All the continuous variables had normal distributions, but two discrete variables (SL and IL) did not (Table 3), thus, they were excluded from further morphological analysis. Convex hulls for metric variables show significant discrimination between Species A and Species B, which support the cluster differentiation inferred from molecular data (Fig. 3). The most contributing variables were SVL and TrL for Axis 1 (Appendix S10). Sexual dimorphism was not recorded for Species A, whereas an evident sexual dimorphism in Species B was

Table 3 Statistical values for mophological analyses. Normality Shapiro–Wilk (W) values for metric (above) and meristic (below) characters showing the *p* value. Values shaded in gray did not reach normality. See Materials and Methods section for reference to the acronyms.

	Continuous												
	SVL	**TrL**	**FL**	**TL**	**AL**	**HL**	**HW**	**HH**	**END**	**ESD**	**EMD**	**ID**	**IND**
W	0.976	0.969	0.955	0.986	0.987	0.960	0.954	0.961	0.975	0.965	0.971	0.979	0.952
p	0.604	0.377	0.377	0.902	0.949	0.223	0.126	0.282	0.602	0.314	0.471	0.688	0.113

	Discrete						
	DT	**TVS**	**LVS**	**SL**	**IL**	**4TL**	**3FL**
W	0.956	0.956	0.967	0.798	0.705	0.943	0.955
p	0.138	0.153	0.349	$9.61E^{-6}$	$2.01E^{-7}$	0.064	0.126

documented (Fig. 3). Nevertheless, the probability ellipse (confidence = 95%) propose a high overlap, and females of Species B is the most different group (Fig. 3).

Regarding meristic data, sexual dimorphism is more pronounced in *H. fasciata* than in *Homonota* sp. "Paraguay" (Fig. 4). Raw data are available in Appendices S11 (metric variables) and S12 (meristic variables).

Taxonomic implications

We take the significant level of genetic differentiation between these two clusters of *Homonota* as evidence for the recognition of two different taxa. In order to correctly assign names to these two species, we examined the relevant primary types of the nominal taxa in this species complex. The holotype of *H. fasciata* is MNHN 6756 (LSID: urn:lsid:zoobank.org:act:14CDAB98-810F-43B3-8F16-B29C830AB80C). As mentioned above, the original type locality of *H. fasciata* was given as "Martinique" and is without doubt erroneous. A detailed analysis of MNHN 6756 (Fig. 5) revealed that it differs in pholidosis in several significant characters from the biological species currently referred to as *H. fasciata* (Table 4), such as margin of auditory meatus (Fig. 6), size of first infralabial scale (Fig. 7), and the arrangement of dorsal scales (Fig. 8). Given these differences in several taxonomically important scalation traits, there is no doubt that MNHN 6756 is not conspecific with the biological species currently referred to as *H. fasciata*. The scalation traits of MNHN 6756 presented above resemble the external morphology of *Homonota uruguayensis* (Vaz-Ferreira & Sierra de Soriano, 1961). However, *H. uruguayensis* does not have transversal bands on the dorsum, and in the original description of *H. fasciata* transversal bands on the dorsum of the type specimen are mentioned. In its current state, the holotype of *H. fasciata* is completely bleached and does not show any trace of banding (Fig. 5). In conclusion, we cannot link the holotype of *H. fasciata* to any of the known populations of *Homonota* which renders this name a *species inquirenda* which needs further studies and cannot be linked to either Species A or Species B. Our examination of the lectotype of *H. horrida* (IZH-R 1) revealed that it is conspecific with our Species A which is supported by the fact that the Argentinian specimens used in our genetic analysis are from the general area of the type locality of *H. horrida*. We therefore resurrect it from synonymy with *H. fasciata* and apply it to our Species A. As mentioned above, the original

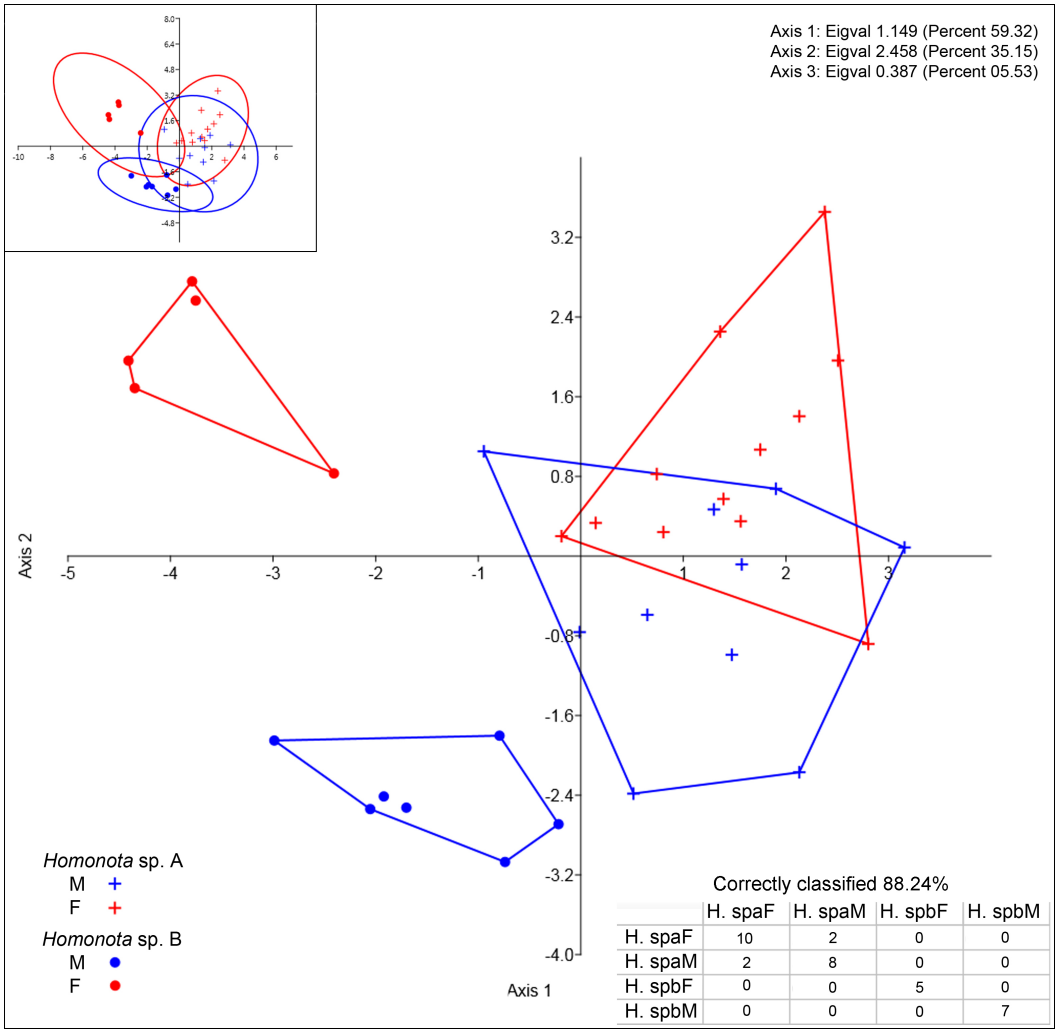

Figure 3 Discriminant analysis of continuous variables. DA scatter plot of individual scores of the three most informative axes for continuous variables (See Appendix S10) of *Homonota* sp. A (Hspa in the table) and *Homonota* sp. B (Hspb in the table). Capital letters "F" and "M" refer to females and males respectively. Inset on upper left corner shows the 95% confidence intervals.

description of *H. mattogrossensis* is very brief, does not provide a precise type locality (and no representative of the genus *Honomota* is known to occur in Mato Grosso do Sul) and no type material or other voucher specimen is known. Therefore this name cannot be applied to any of the known populations of this genus and we consider *Homonota mattogrossensis* to constitute a *nomen dubium*.

No name is available for our Species B and we therefore describe it as a new species below, presenting also a species account and a redescription of *H. horrida*. The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural

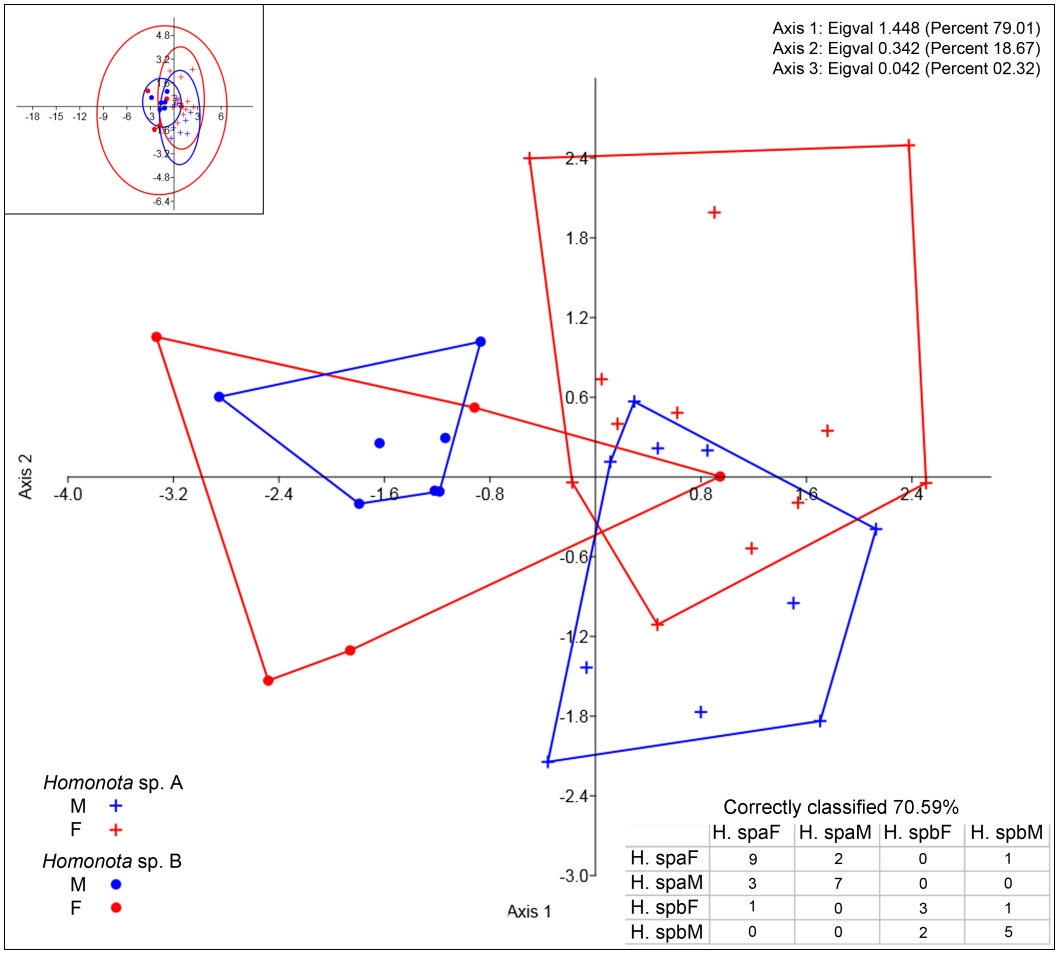

Figure 4 Discriminant analysis of discrete variables. DA scatter plot of individual scores of the three most informative axes for discrete variables (See Appendix S10) of *Homonota* sp. A (Hspa in the table) and *Homonota* sp. B (Hspb in the table). Capital letters "F" and "M" refer to females and males respectively. Inset on upper left corner shows the 95% confidence intervals.

Table 4 Morphological differences. Differences in morphological traits between MNHN 6756 (holotype of *Homonota fascia*) and *Homonota* sp. commonly referred as *H. fascia*.

Trait	MNHN 6756	*Homonota* sp.
Margin of auditory meatus	Smooth	Strongly serrated
Enlarged tubercle on the auditory meatus	Absent	Present
Postmental scale	Exceptionally large	Almost same size of first infralabial
Dorsal scales	Small and widely spaced	Large and juxtaposed

Figure 5 Holotype of *Homonota fasciata*. Dorsal (A) and ventral (B) views of the holotype of *Homonota fasciata* (MNHN 6756). Scale bar = 1 cm.

acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:7233E738-D8B3-424D-B1FC-7CA903BED5A0. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

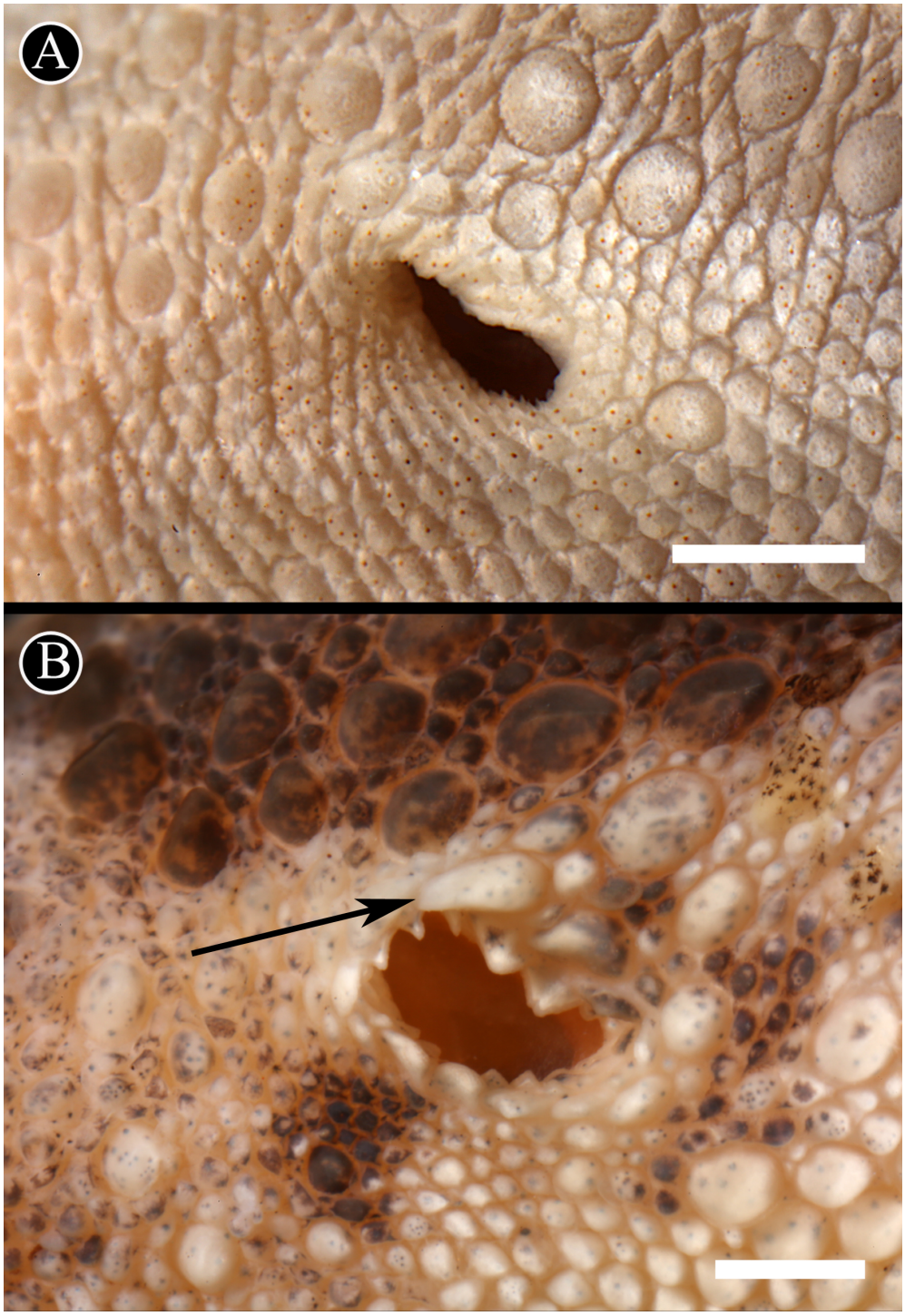

Figure 6 **Auditory meatus.** Detail of the auditory meatus of the holotype of *H. fasciata* (A) showing an even edge, and *Homonota* sp. (B) showing the serrate edge. Black arrow indicates an enlarged tubercle associated to the upper edge of the auditory meatus, absent in the holotype of *H. fasciata*. Head to the right. Scale bar = 1 mm.

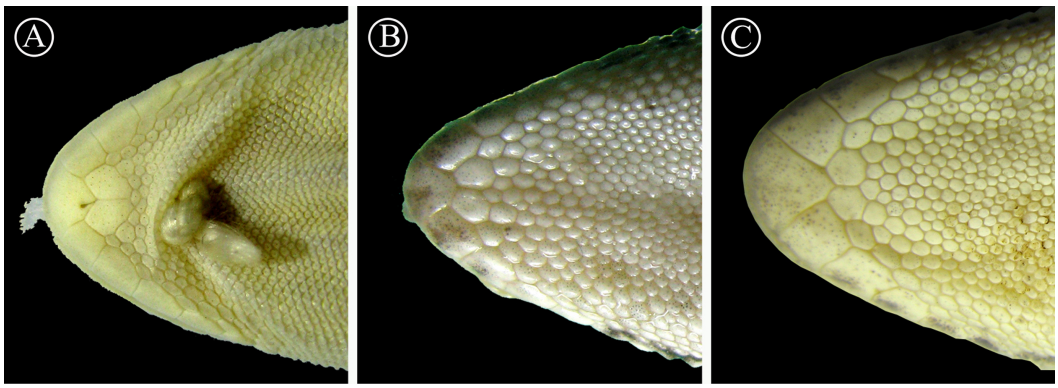

Figure 7 Detailed view of postmental scales. Detail of the mental region, showing the large size of the postmental scales of the holotype of *H. fasciata* (A), compared with *Homonota* sp. A (B) and *Homonota* sp. B (C). Vouchers: A- MNHN 6756; B- MNHNP 12238; C- LJAMM-CNP 6520.

***Homonota horrida* (*Burmeister, 1861*) sp. reval.**

- *Gymnodactylus horridus Burmeister, 1861*

Type locality: "in den Schluchten der Sierra bei Challao", Mendoza, Argentina.

Types: Original description based on three syntypes. Lectotype (IZH-R 1, Fig. 9) and paralectotype (IZH-R 2) designation according to *Müller (1941)*.

- *Wallsaurus horridus Underwood 1954*
- *Gymnodactylus pasteuri Wermuth 1965*

LSID: urn:lsid:zoobank.org:act:27FAE0B5-2E88-46C5-A296-F7BBE0B20AE6

Diagnosis: A large species of *Homonota* with a dark dorsal color (grey or brown) with a pattern of clear transversal bands connected with a vertebral stripe. Additionally, it is differentiated from any other *Homonota* by the large size and development of the keeled scales on the head (including laterals) and dorsum.

Redescription of the lectotype (Fig. 9): Adult male, SVL 44 mm, TrL 19 mm, tail 49 mm, FL 8.0 mm, TL 8.5 mm, AL 12.0 mm, HL 11.1 mm, HW 8.5 mm, HH 6.3 mm, END 3.7 mm, ESD 4.6 mm, EMD 4.1 mm, ID 4.3 mm, IND 1.4 mm; rostral wider than high; nares surrounded by rostral, supranasal, two postnasals, and first SL; SL 9/9; one elongated tubercular scale on the mouth commissure; upper region of the muzzle covered by big homogeneous juxtaposed scales; upper surface of the head covered with medium-sized (smaller than those on the muzzle) homogeneous juxtaposed scales intermixed with small granules; superciliary scales imbricated, associated to spiny-like scales on the posterior half of the orbit; lateral sides of the head heterogeneously covered profusely with large keeled tubercles and small granular (sometimes elongated) scales; auditory meatus oblique and with serrated edge, and one big scale on the upper border; IL 6/6; mental triangular; postmentals big (about twice the size of the following posterior scales) contacting the mental, the first IL, and a row of six posterior scales (the two centrals smaller); scales under the head reducing in size posteriorly; dorsolateral parts of the neck with granular juxtaposed scales mixed with tubercles; throat region covered by imbricated cycloid scales; dorsum covered with 16 strongly keeled scales separated by one or two

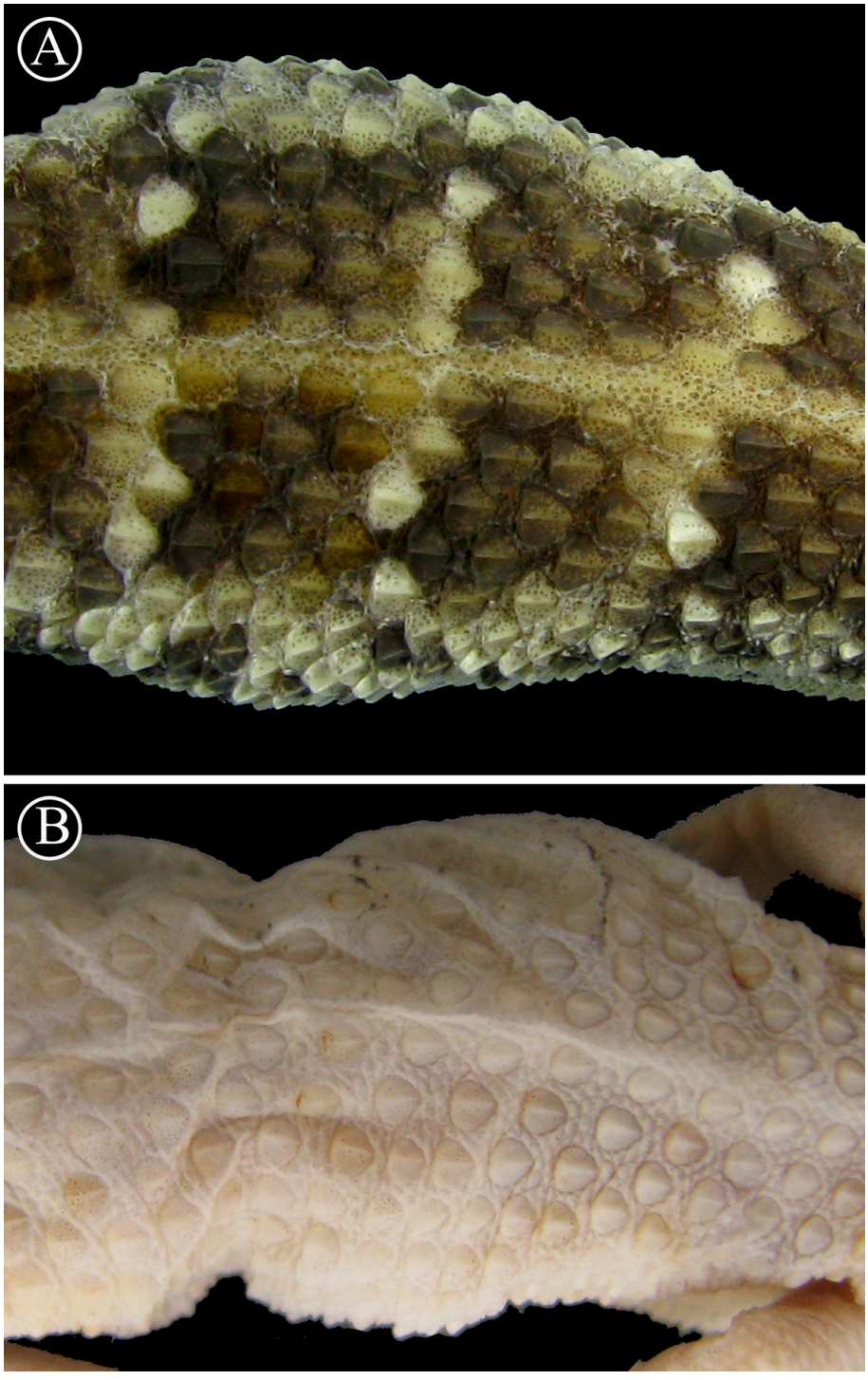

Figure 8 Detailed view of dorsal scales. Lineal arrangement of dorsal scales of *Homonota* sp B. (A) commonly referred to as *H. fasciata*, and the holotype of *H. fasciata* (B). Note the different pattern in the squamation. Head to the right.

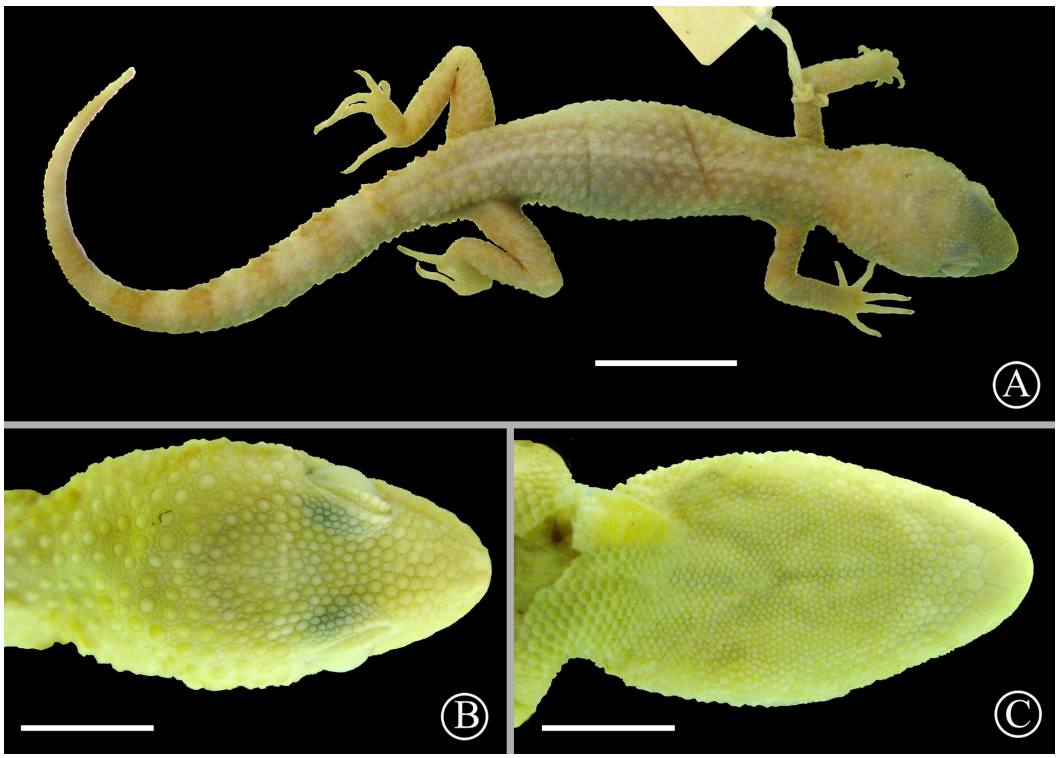

Figure 9 Lectotype of *Homonota horrida*. Dorsal view (A) and details of the head in dorsal (B) and ventral (C) views of the lectotype of *Homonota horrida* (IZH-R 1). Scale bar = 10 mm (A) and 5 mm (B–C).

small granular scales; ventral scales cycloid and imbricated arranged in 18 longitudinal rows at midbody; suprascapular, axillary, and inguinal regions surrounded by small imbricated granules; sides of cloacal opening with two to three conical tubercular scales; anterior and dorsal surfaces of limbs covered by imbricated scales, slightly keeled on the dorsal surface; posterior region of limbs covered by small juxtaposed granules; ventral surface of forelimbs with juxtaposed granules, and ventral surface of hind limbs with large imbricated scales; subdigital lamellae of hands starting from pollex were recorded as follows: 8/8 – 12/12 – 14/14 – 16/16 – 8/11 ; subdigital lamellae of feet starting from hallux were recorded as follow: 17/17 – 21/18 – 17/17 – 13/13 – 7/8 ; large imbricated keeled scales around the tail disposed in rings, separated by two to three series of small scales.

Coloration in preservative of the lectotype: The specimen is at least 147 years old, and coloration is faded in most parts of the animal. The whole body is basically Cream White (52) with vestiges of blotches on the scapular region, pre and postocular lines, and rings around the tail of Salmon Color (58).

Variation: (Based on specimens referred in Appendix S5) SVL 42–64 mm; TrL 16–29 mm (36.9–46.0% of SVL in females, 35.7–46.8% in males); FL 7–11 mm (9.5 ± 0.30) in males, 8–12 mm (10.4 ± 0.41) in females; TL 8.3–11.4 mm (9.7 ± 0.28) in males, 8.3–12.5 mm (10.4 ± 0.35) in females; AL 11.9–14.7 mm (13.3 ± 0.38) in males, 18.8–16.8 mm (13.5 ±0.48) in females; HL 10.5–16.1 mm (12.5 ± 0.73) in males, 9.8–14.6 mm (12.7 ± 0.49) in females; HW 8.2–12.4 mm (65.2–85.5% of HL in females, 77.8–99.0% in

males); HH 4.9–7.8 mm (44.0–62.2% of HL in females, 46.2–55.2% in males); END 2.9–5.0 mm (29.6–40.0% of HL in females, 29.9–34.1% in males); ESD 3.6–6.6 mm (36.7–46.7% of HL in females, 39.0–43.9% in males); EMD 4.2–6.5 mm (35.2–47.9% of HL in females, 38.5–41.9% in males); ID 3.8–5.8 mm (29.7–54.1% of HL in females, 31.7–42.8% in males); IND 1.2–2.3 mm (11.3–23.5% of HL in females, 12.5–17.1% in males); SL 7–9; one or two elongated tubercular scales on the mouth commissure; upper region of the muzzle usually flattened, rarely slightly convex (LJAMM-CNP 6520); auditory meatus with one large scale on the upper border; IL 6–8; 13–20 longitudinal rows of ventral scales at midbody.

The coloration pattern (lost in the type series) consist of a dark and clear reticulation on the dorsal surface of the head, a dark longitudinal stripe from the tip of the snout across the temporal region extending posteriorly and upwards reaching the nuchal region. Dorsal background color usually dark with whitish transversal bands connected with a vertebral stripe of the same color. Limbs with an irregular reticulation. Ventral region of head and body always immaculate clear. Tail with dark and clear rings that can be present only on the dorsal and lateral areas of the organ, or continued to the ventral surface. Some melanic specimens (LJAM-CNP 6532, 6968) lack the vertebral stripe, and the clear transversal bands are inconspicuous.

Distribution: As mentioned before, this is a species complex which needs further analyses. As currently recognized, this clade is distributed from the Argentinean Province of Rio Negro in southern Argentina, to the center of Paraguayan Chaco, according to *Morando et al. (2014)*. Our analyzed samples came from Low Monte ecoregion in southern Argentina.

***Homonota septentrionalis* n. sp.**

LSID: urn:lsid:zoobank.org:act:8AE7D2A8-0D62-4AF2-8CB9-3D4346F63B52

Holotype: MNHNP 12238 (original field number PCS 200), adult female (Fig. 10), collected on 10 December 2014 by P. Cacciali, at Fortín Mayor Infante Rivarola (21.679°S, 62.401°W, 277 masl), Boquerón Department, Paraguay.

Paratypes: MNHNP 2821, 9037–8, 9131, 11406*, 11409*, 11410, 11419, 11421, 11423 (Parque Nacional Teniente Enciso, Boquerón Department, Paraguay; 21.209°S, 61.655°W, 253 masl); MNHNP 11850, 11855, 11860, 11872, 11873* (Cruce San Miguel, in front of Parque Nacional Teniente Enciso, Boquerón Department, Paraguay; 21.203°S, 61.662°W, 254 masl); SMF 101984* (topotype); SMF 29277 (Villamontes, Tarija Department, Bolivia; 21.266°S, 63.451°W, 398 masl). Holotype and specimens marked with an asterisk (*) were used for molecular analyses.

Etymology: The specific name *septentrionalis* is Latin, meaning ''northern'' and refers to the fact that this species has the northernmost distribution of all the *Homonota* species.

Diagnosis: This is the largest species of the genus (max. 65 mm SVL) with robust body, prominent keeled tubercles disposed in four to eight longitudinal rows, and coloration pattern of dark background with one vertebral and six or seven transversal clear bands. It can be distinguished from *H. andicola*, *H. whitii*, and *H. underwoodi* by the presence of strongly keeled dorsal scales (vs. smooth dorsal scales in *H. andicola*, *H. whitii*, and *H.*

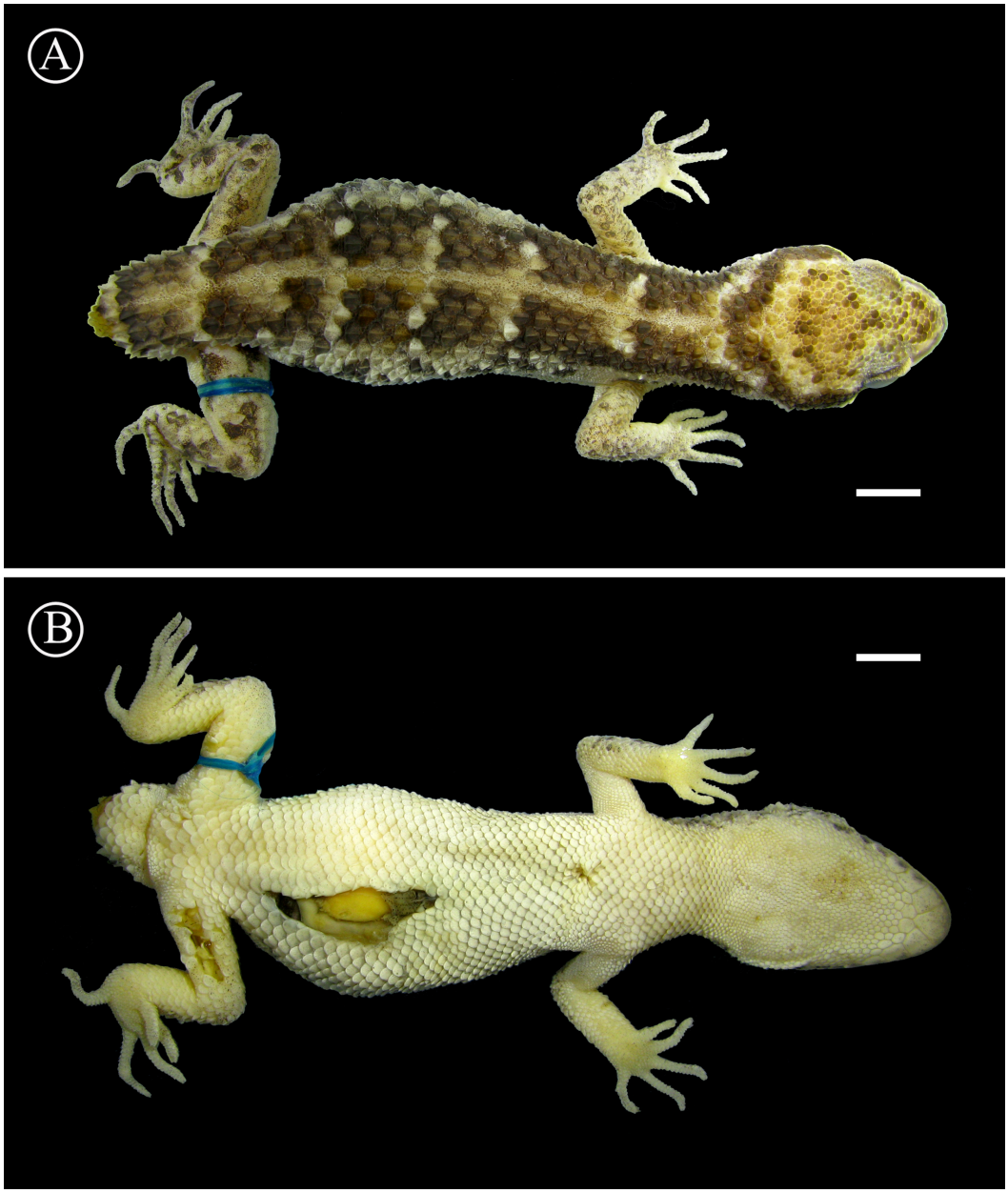

Figure 10 **Holotype of *Homonota septentrionalis*** Dorsal (A) and ventral (B) views of the holotype of *Homonota septentrionalis* (MNHNP 12238). Scale bar = 5 mm.

underwoodi), transversal clear bands on a darker dorsum (vs. reticulated pattern), and from *H. underwoodi* also by a lower number of 4TL (16–20) and 3FL (11–15) (vs. 20–25 and 15–17 respectively in *H. underwoodi*). From *H. borellii* and *H. rupicola* by the oblique shape of the auditory meatus (vs. round in *H. borellii* and *H. rupicola*), transversal clear bands on a darker dorsum (vs. reticulated pattern), and also from *H. borelli* by the presence of strongly keeled dorsal scales (vs. moderately keeled), and from *H. rupicola* by a higher number of 4TL (16–20) (vs. 14–15). From *H. darwinii* by the presence of strongly keeled

dorsal scales (vs. smooth at least on the anterior part of the dorsum in *H. darwinii*), and by transversal clear bands on a darker dorsum (vs. reticulated pattern). From *H. rupicola* and *H. taragui* by the presence of enlarged keeled tubercles on the sides of the head behind the orbits (vs. homogeneous granular scales). From *H. uruguayensis* by a higher number of IL scales (6–7, vs. 4–5 in *H. uruguayensis*), by the coloration, and by the serrated edge of the auditory meatus (vs. smooth granular edge in *H. uruguayensis*). From *H. williamsii* by the presence of strongly keeled dorsal scales (vs. moderately keeled) and by transversal clear bands on a darker dorsum (vs. reticulated pattern). From *H. horrida* (the most similar species) by the high position of the auditory meatus relative to the mouth commissure (vs. lower position in *H. horrida*) (Fig. 11); less developed tubercles on the sides of the head, including a narrow area between the orbit and the auditory meatus covered with small granular scales with without or with few tubercles (vs. several big tubercles on the sides of the head even in the area between the orbit and the auditory meatus) (Fig. 11).

Description of the holotype: Adult female, SVL 60 mm, TrL 26 mm, tail broken near the base, FL 11.0 mm, TL 10.8 mm, AL 14.1 mm, HL 14.8 mm, HW 13.3 mm, HH 7.9 mm, END 4.6 mm, ESD 6.6 mm, EMD 5.1 mm, ID 5.5 mm, IND 2.5 mm; rostral wide with a median groove at the upper half; nares surrounded by rostral (slight contact), supranasal, two postnasals, and first SL (slight contact); SL 9/8; two elongated tubercular scales on the mouth commissure; upper region of the muzzle slightly convex covered by big homogeneous juxtaposed scales; upper surface of the head covered with big homogeneous juxtaposed scales intermixed with small granules; superciliary scales imbricated forming a serrated edge, associated to spiny-like scales on the posterior half of the orbit; lateral sides of the head heterogeneously covered with large keeled tubercles and small granular (sometimes elongated) scales; auditory meatus oblique and with serrated edge, and two big scales on the upper border; IL 6/6; mental triangular; postmentals big (less than twice the size of the following posterior scales) contacting the mental, the first IL, and a row of six posterior scales (the two centrals smaller); scales under the head reducing in size posteriorly; dorsolateral parts of the neck with granular juxtaposed scales mixed with tubercles; throat region covered by imbricated cycloid scales; dorsum covered with eight strongly keeled scales separated by one or two small granular scales, except on the vertebral area where keeled scales are separated by four granules; ventral scales cycloid and imbricated arranged in 20 longitudinal rows at midbody; suprascapular, axillary, and inguinal regions and cloacal opening surrounded by small imbricated granules; anterior and dorsal surfaces of limbs covered by large imbricated scales, keeled on the dorsal surface; posterior region of limbs covered by small juxtaposed granules; ventral surface of forelimbs with juxtaposed granules, and ventral surface of hind limbs with large imbricated scales; subdigital lamellae of hands starting from pollex were recorded as follows: 7/8 – 12/10 – 13/14 – 13/13 – 12/10 ; subdigital lamellae of feet starting from hallux were recorded as follow: 13/13 – 18/18 – 15/14 – 12/12 – 10/10 ; large imbricated scales around the tail (stump) with the eight uppermost strongly keeled.

Coloration in life: Dorsal surface of head Grayish Horn Color (268) with groups of Dusky Brown (285) scales, irregularly mixed with Hair Brown (277) scales; posterior surface of the head with a curved Hair Brown (277) line interrupted by five groups of

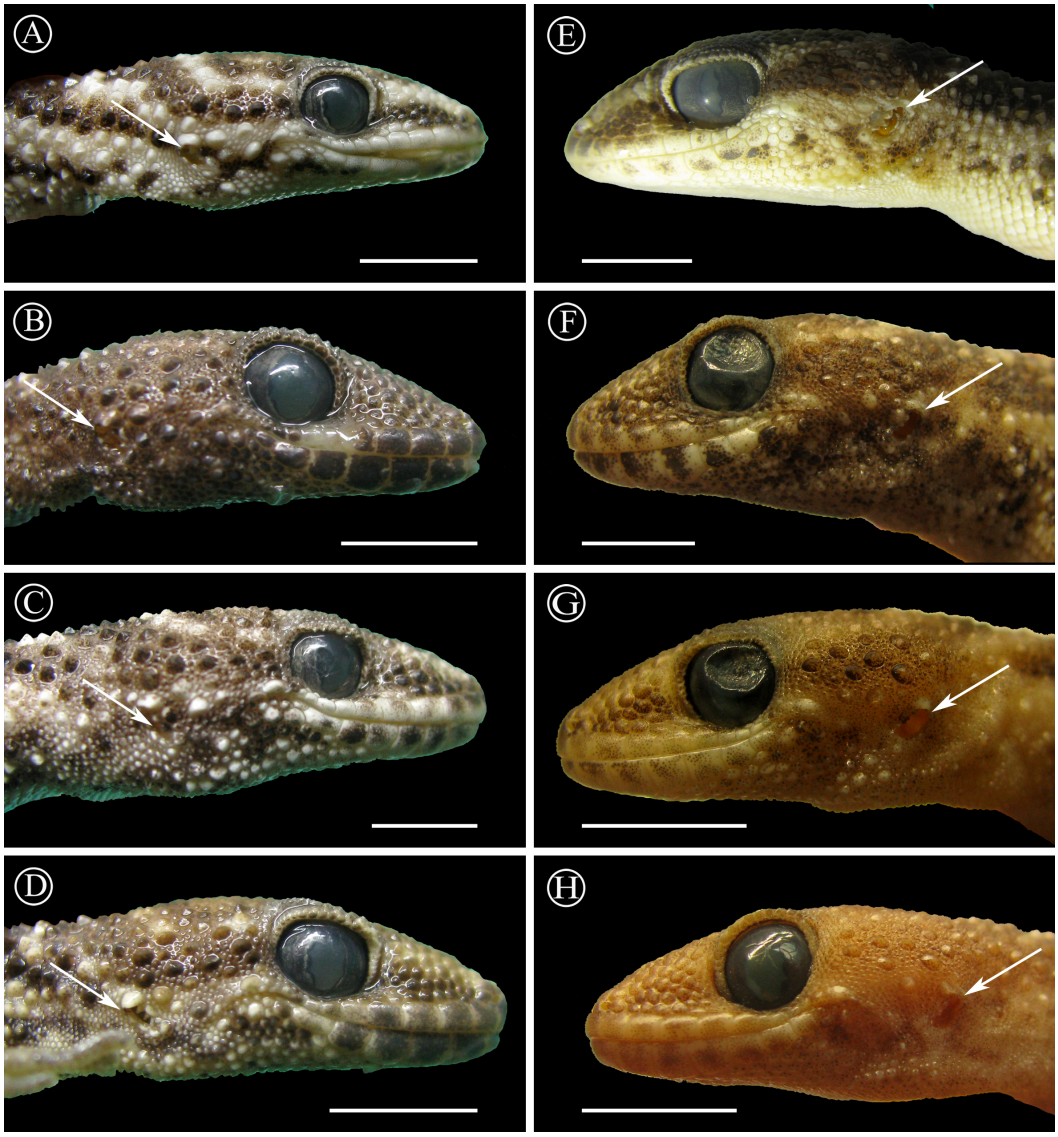

Figure 11 Position of ear opening. Lateral sides of the head of *Homonota horrida* (A–D) compared with *H. septentrionalis* (E–H) showing differences in the disposition of ear opening (EO), indicated with white arrows, and the tubercles between the EO and the commissure of the mouth. Vouchers: LJAMM-CNP 6520, 6532, 6533, 7670 from A to D respectively, and MNHNP 12238, 11855, 11406, 9131 from E to H respectively. Scale bars = 5 mm.

Dusky Brown (285) scales; upper lateral view of the head Grayish Horn Color (268), edged below by a thick Dusky Brown (285) stripe from the muzzle (interrupted by the orbit) to the temporal region; supralabial and infralabial regions Smoky White (261) with irregular Raw Umber (280) suffusions on the 1st and 2nd SL and 1st to 5th IL; region between mouth commissure and shoulder Smoky White (261) with irregular Dusky Brown (285) speckles, edged above (bordering the upper edge of the ear opening) by an irregular Cream Yellow (82) stripe; ventral surface of the head Smoky White (261); dorsal ground color Dusky Brown (285), with a Light Straw Yellow (95) vertebral stripe, and five transversal

Light Sulphur Yellow (93) lines; lateral parts of the body Cream Yellow (82) with irregular Dusky Brown (285) speckles; venter Smoky White (261); dorsal surface of limbs Cream Color (12) with irregular Dusky Brown (285) speckles on the forelimbs, and groups of Dusky Brown (285) scales (eventually forming short stripes) on the hind limbs; ventral surface of limbs Smoky White (261).

Coloration in preservative: Dorsal surface of head Drab (19) with groups of Vandyke Brown (282) scales; posterior surface of the head with a curved Vandyke Brown (282) line; upper lateral view of the head Smoke Gray (266), edged below by a thick Raw Umber (260) stripe from the muzzle (interrupted by the orbit) to the temporal region; supralabial and infralabial regions Cream White (52) with irregular Raw Umber (260) suffusions on the 1st and 2nd SL and 1st to 5th IL; region between mouth commissure and shoulder Cream White (52) with irregular Raw Umber (260) speckles; ventral surface of the head Cream White (52); dorsal ground color Raw Umber (260), with a Beige (254) vertebral stripe, and five transversal Cream White (52) lines; lateral parts of the body Cream White (52) with irregular Raw Umber (260) speckles; venter Cream White (52); dorsal surface of limbs Beige (254) with irregular Sepia (279) speckles on the forelimbs, and groups of Sepia (279) scales (eventually forming short stripes) on the hind limbs; ventral surface of limbs Cream White (52).

Variation: SVL 37–65 mm; TrL 15–28 mm (43.3–48.2% of SVL in females, 38.3–48.8% in males); Tail length 47–63 mm (ratio SVL:Tail - 1:1 in one female, 1:1.18–1:1.22 in two males, and 1:1.17 in a juvenile of unknown sex); FL 8–9 mm (8.8 $\pm$ 0.37) in males, 10–12 mm (11.2 $\pm$ 0.83) in females; TL 7.2–9.8 mm (8.7 $\pm$ 0.36) in males, 9.4–11.3 mm (10.5 $\pm$0.81) in females; AL 10.2–13.1 mm (11.7 $\pm$ 0.91) in males, 13.1–15.0 mm (14.1 $\pm$ 0.76) in females; HL 10.7–13.3 mm (11.8 $\pm$ 0.38) in males, 12.9–17.3 mm (14.6 $\pm$ 1.66) in females; HW 8.1–13.3 mm (71.6–89.8% of HL in females, 75.7–84.4% in males); HH 5.8–8.6 mm (49.7–61.3% of HL in females, 54.1–61.4% in males); END 3.7–5.8 mm (31.9–37.9% of HL in females, 29.3–39.1% in males); ESD 3.6–6.8 mm (39.3–46.7% of HL in females, 31.6–45.9% in males); EMD 3.6–5.6 mm (34.4–40.8% of HL in females, 33.0–38.6% in males); ID 3.7–5.5 mm (30.1–38.7% of HL in females, 33.0–38.3% in males); IND 1.4–2.5 mm (14.4–16.9% of HL in females, 12.3–18.8% in males); SL 6–9; one or two elongated tubercular scales on the mouth commissure; upper region of the muzzle slightly convex or flattened; auditory meatus with one or two big scales on the upper border; IL 6–7; 12–20 longitudinal rows of ventral scales at midbody.

The coloration variation follows the same pattern observed for the holotype. Smaller animals (MNHNP 11419, 11423) are clearer and the clear transversal bands are reduced to the paravertebral area; vertebral stripe reduced in MNHNP 11855; three paratypes (MNHNP 2821, 9037, 9131) have a darker pattern being reddish dorsal background color, and in two of them (MNHNP 2821, 9131) the transversal bands are almost faded; the original tail (MNHNP 9131, 11419, 11421, 11850, 11860, 11872, SMF 29277) has transversal dark and clear bands dorsally, and clear or reddish hue ventrally.

Distribution: *Homonota septentrionalis* is distributed in the northernmost range of the genus. The examined specimens come from the Dry Chaco, at the westernmost part of the Paraguayan Chaco and southeast of Bolivia (Fig. 12).

Habitat: The environment inhabited by *H. septentrionalis* is a xerophytic (precipitation varies between 300 and 400 mm per year) and thorny dry forest, with null or scarce herbaceous stratum (Fig. 13). This species is a nocturnal ground dweller, being abundant in natural areas, and also present in anthropogenically modified areas.

DISCUSSION

The analysis of genetic barcodes of the mtDNA gene 16S provided the first evidence for the existence of an undescribed species of *Homonota* in Paraguay, which was posteriorly tested with additional data. The uncorrected genetic distance of the 16S fragment between *H. horrida* and *H. septentrionalis* is rather low (1.8–2.5%) compared to distances between species of other genera of geckos such as *Diplodactylus* (4–12%; *Pepper, Doughty & Keogh, 2006*), *Phyllopezus* (6–15%; *Gamble et al., 2012*), and *Lepidoblepharis* (12–23%; *Batista et al., 2015*). Using the species delimitation program ABGD, we estimated the intraspecific variation since this program explores the pairwise differences in barcode datasets, providing limits for intraspecific divergence (*Puillandre et al., 2012*). The expected intraspecific variation for *Homonota* Species A and Species B, matches with the variation in uncorrected pairwise distance (Table 1), with a clear difference between the two taxa. The tree-based PTP analysis provides speciation models based on number of substitution in a phylogenetic hypothesis, for which the branch length of a tree represents the number of substitutions (*Zhang et al., 2013*). This algorithm also suggested two putative species, one from Argentina (Species A) and the other from Paraguay (Species B).

The topology of the species tree (Fig. 2) shows *Phyllodactylus* as the sister genus of *Homonota*, congruent with *Gamble et al. (2008b)*; *Gamble et al. (2011)* and *Morando et al. (2014)*. The arrangement among groups of *Homonota* inferred the *fasciata* group as the most basal clade, a hypothesis contrary to that proposed by *Morando et al. (2014)* where the *whitii* group was the most basal clade within *Homonota*. The majority of the topological arrangements among the concatenated trees are identical, with the exception of the position of *H. taragui* which was closely related to *H. rupicola* using mitochondrial genes, and related to *H. borellii* using nuclear genes (Appendix S9); a conflict that was already reported by *Morando et al. (2014)*. In our phylogeny *H. horrida* and *H. septentrionalis* were inferred to as sister taxa with high statistical support ($PP = 1$, Fig. 2). Given the taxonomic modifications proposed here, we suggest referring to the group that contains *H. underwoodi*, *H. horrida*, and *H. septentrionalis* as the *H. horrida* species group.

The holotype of *Homonota fasciata* was sent to Paris by Auguste Plée who was a botanist who collected several samples of plants and animals in the Antilles, and some of his collections are valid records for Martinique (i.e., type locality of *H. fasciata*) such as *Monstera adansonii* (Alismatales: Araceae), *Auxis thazard* (Actinopterygii: Scombridae), *Eleutherodactylus martinicensis* (Amphibia: Eleutherodactylidae), *Mabuya mabouya* (Reptilia: Scincidae), *Megalomys desmarestii* (Mammalia: Cricetidae), whereas some others were recorded but currently extinct as *Leptodactylus fallax* (Amphibia: Leptodactylidae) and *Leiocephalus herminieri* (Reptilia: Leiocephalidae) (*Madison, 1977*; *Collette & Aadland, 1996*; *Borroto-Páez & García, 2012*; *Hedges & Conn, 2012*; *Breuil, 2015*). Thus, although

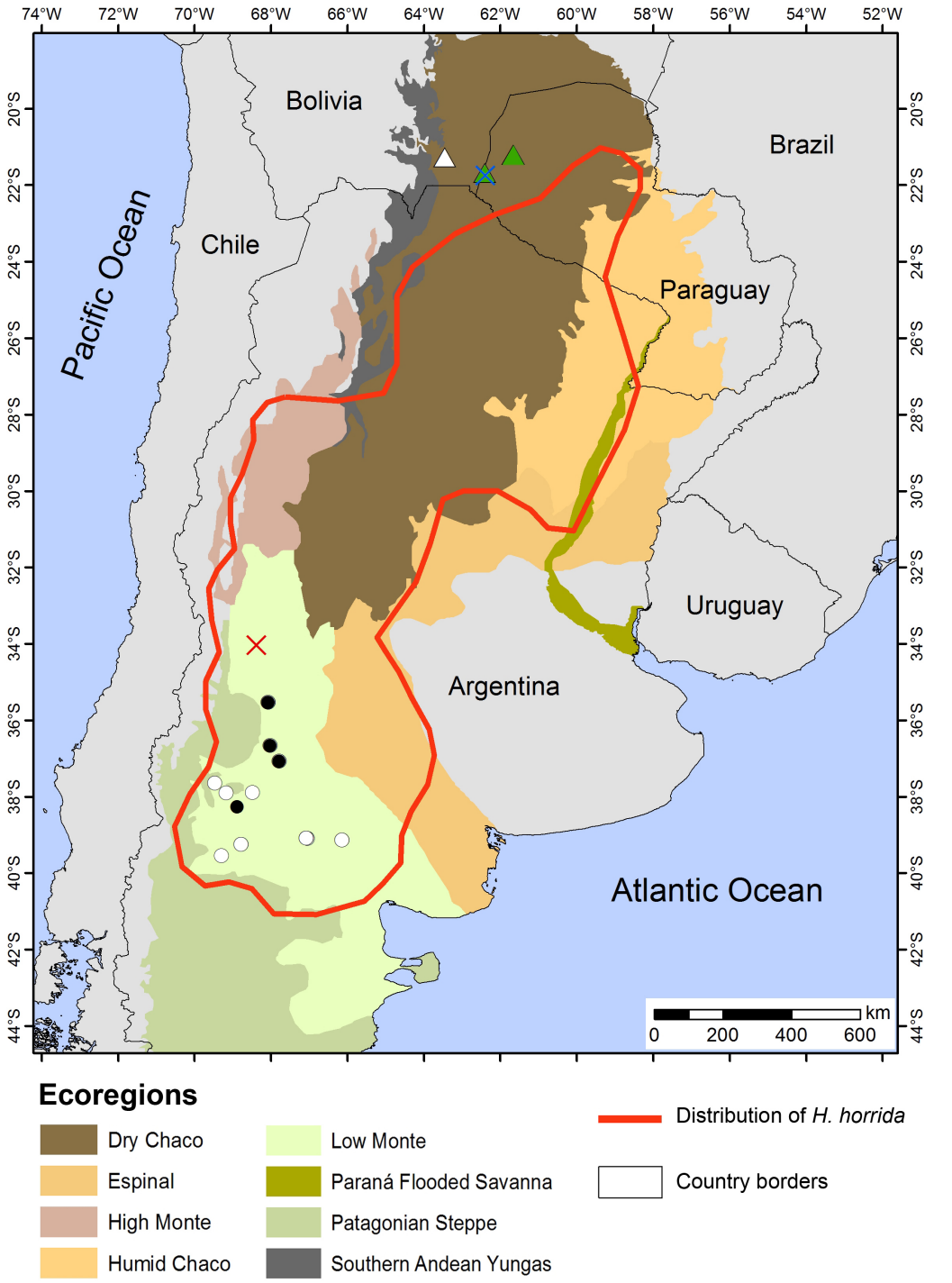

Figure 12 Distribution Map. Locality records of *Homonota septentrionalis* (triangles) highlighting localities of specimens used for genetic analyses (green triangles), and the distribution of *Homonota horrida* (red line) according to *Morando et al. (2014)* with localities of specimens used for morphological analyses (white circles) and genetic analyses (black circles). Crosses represent type localities: blue for *H. septentrionalis*, and red for *H. horrida*.

Figure 13 Habitat of *Homonota septentrionalis*. Environmental characteristics of the type locality of *H. septentrionalis*.

some locality records provided by Plée are trustable, the name *H. fasciata* based on specimen MNHN 6756, remains has to be considered as a *species inquirenda*. More historical analyses could shine some light on the real origin of this specimen.

Abdala & Lavilla (1993) stated that differences between *Homonota horrida* and the type of *H. fasciata* were due to variation, which is true for some meristic characters. Nevertheless, the small size of postmental scales and serrated edge of auditory meatus are common morphological traits of *H. horrida*. These authors suggested that some specimens of *H. horrida* could have big postmentals and smooth auditory meatus (referring to specimens FML 35 and FML 114) which is rare for the species. Another common trait for *H. horrida* is the presence of a tubercular scale on the upper edge of the auditory meatus, which is absent in the type of *H. fasciata*. Further genetic and morphological analyses of Argentinean populations of *H. horrida* are required for a better understanding of variation within the species.

Homonota septentrionalis is a large species of *Homonota*, with a marked sexual dimorphism in measurable characters according to the DA analysis (Fig. 3), where SVL and TrL are the variables that contribute more to the differentiation (Appendix S10). This differs from what is known for *Homonota darwinii* where *Ibargüengoytía & Casalinas (2007)* found no sexual dimorphism, although *Fitch (1981)* reported differences in SVL between males and females in Gekkonidae with females usually larger than males. More analyses

are needed in order to explore the extent of this pattern in other species of the genus.

Genetic analyses were key for the recognition of the new species, since the morphological differences between *H. septentrionalis* and *H. horrida* are subtle and they could be considered cryptic species. High degree of genetic differentiation and low degree of morphological distinction is a common phenomenon for lizards, leading to situations in which authors designate candidate species without formal descriptions (*Gamble et al., 2012*; *Werneck et al., 2012*), or cases in which authors base the entire diagnosis upon genetic clustering (*Leaché & Fujita, 2010*).

Currently, *Homonota septentrionalis* is known from the type locality (Fig. 11), in plain areas and xerophytic environments. Given the similarity in external morphology between *H. septentrionalis* and *H. horrida* it is difficult to elaborate a cresonymy list of the previous records for these species. Records published by *Mendoza, Rivas & Muñoz (2015)* as *H. fasciata* from Bolivia, probably are *H. septentrionalis*, but further morphological and genetic analyses are required for a better understanding of the distribution pattern of *H. septentrionalis*.

Based on these results, the actual diversity of the genus *Homonota* is as follows: *borellii* group: *H. borellii*, *H. uruguayensis*, *H. rupicola*, and *H. taragui*; *horrida* group: *H. horrida*, *H. underwoodi*, and *H. septentrionalis* sp. nov; *whitii* group: *H. whitii*, *H. darwinii*, *H. andicola*, and *H. williamsii*; *Incertae sedis*: *H. fasciata*.

Currently, the conservation status of *Homonota septentrionalis* is totally unknown. *Homonota fasciata* was categorized as Least Concern (LC) by *Motte et al. (2009)* given its big range, but since we actually do not know the range of *H. septentrionalis*, the conservation status might be different. This species is related to the Dry Chaco, which for a long time was a sanctuary for wildlife because of the lack of anthropogenic impacts; but unfortunately in the last decade the deforestation is severely threatening many areas of the Dry Chaco (*Eva et al., 2004*; *Caballero et al., 2014*). An assessment of the status of this new taxon is required.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Pier Cacciali conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Mariana Morando conceived and designed the experiments, performed the experiments, analyzed the data, reviewed drafts of the paper.
- Cintia D. Medina performed the experiments, analyzed the data, prepared figures and/or tables, reviewed drafts of the paper.
- Gunther Köhler conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Martha Motte performed the experiments, reviewed drafts of the paper.
- Luciano J. Avila conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Animal Ethics

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Data Availability

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Raw data are available as Appendices in Supplemental Information 1. Raw data for MF535517 are available as Supplemental Information 2.

New Species Registration

The following information was supplied regarding the registration of a newly described species:

Genus name: urn:lsid:zoobank.org:act:22AF067B-1B91-4736-AE2E-779B97BF1F23
Publication: urn:lsid:zoobank.org:pub:04A88748-40CA-4243-BF28-96B67A646E35
Publication: urn:lsid:zoobank.org:pub:7233E738-D8B3-424D-B1FC-7CA903BED5A0
Species name: urn:lsid:zoobank.org:act:8AE7D2A8-0D62-4AF2-8CB9-3D4346F63B52.

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Taxonomic analysis of Paraguayan samples of *Homonota fasciata* Duméril & Bibron (1836) with the revalidation of *Homonota horrida* Burmeister (1861) (Reptilia: Squamata: Phyllodactylidae) and the description of a new species

Cacciali P, Morando M, Medina CD, Köhler G, Motte M, Avila LJ - PeerJ 5:e3523; DOI 10.7717/peerj.3523

Supplementary Information

Appendix S1

GenBank accession numbers (16S)

GenBank accession numbers (GBAN) of specimens used for genetic analyses with 16S mitochondrial gene for each voucher (institutional acronyms follow Sabaj Pérez, 2014). Specimen indicated with [1] is currently BYU 47941.

Country	Specimen	GBAN
Argentina	LJAMM-CNP 5047[1]	MF278828
	LJAMM-CNP 10495	MF278829
	LJAMM-CNP 10576	MF278830
Paraguay	MNHNP 11873	MF278831
	MNHNP 12238	MF278832
	SMF 101984	MF278833

Appendix S2

Localities of specimens used for genetic analyses

Codes for countries are: ARG (Argentina), BRA (Brazil), CHI (Chile), PAR (Paraguay), and PER (Peru). Locality information is provided from the major political division of the country to the most specific location data. References to routes are RP (Provincial Route) and RN (National Route). Elevation is given in meters above sea level. Coordinates given with only one decimal are approximately, in which case elevation is not provided. Species treated as "*Homonota* sp. A" and "*Homonota* sp. B" were formerly referred as *H. fasciata*. Specimens with superindexes ([1–3]) are currently in BYU: 1- BYU 47960, 2- BYU 47941, 3- 47931.

Species	Voucher	Country	Specific locality	Lat/Long	Elevation
H. andicola	LJAMM-CNP 12490	ARG	Mendoza, Las Heras, RP 39, 3 km S from limit San Juan-Mendoza	-32.098º, -69.371º	2231
	LJAMM-CNP 12493	ARG	Mendoza, Las Heras, RP 39, 3 km S from limit San Juan-Mendoza	-32.098º, -69.371º	2231
	LJAMM-CNP 12494	ARG	Mendoza, Las Heras, RP 39, 3 km S from limit San Juan-Mendoza	-32.098º, -69.371º	2231
	LJAMM-CNP 12495	ARG	Mendoza, Las Heras, RP 39, 3 km S from limit San Juan-Mendoza	-32.098º, -69.371º	2231
H. borellii	LJAMM-CNP 5842[1]	ARG	La Rioja, Anillaco	-28.814º, -66.933º	1362
	LJAMM-CNP 12116	ARG	Santiago del Estero, Ojo de Agua, Sierra de Ambargasta	-29.249º, -63.920º	314
	LJAMM-CNP 12125	ARG	Santiago del Estero, Ojo de Agua, Sierra de Ambargasta	-29.249º, -63.920º	314
H. darwinii	LJAMM-CNP 9266	ARG	Santa Cruz, RP 45, E of Estancia El Cerrito	-46.263º, -71.378º	640
	LJAMM-CNP 9813	ARG	Santa Cruz, 4 km NE of Puerto Deseado	-47.715º, -65.839º	16
	LJAMM-CNP 10638	ARG	Santa Cruz, RP 77, ~500 m from detour to Estancia La María	-48.498º, -68.864º	214
	LJAMM-CNP 11424	ARG	Santa Cruz, RN 3, 5 km N of Estancia El Rancho	-46.781º, -67.354º	266
	LJAMM-CNP 11432	ARG	Santa Cruz, NR 3, 12 km S of detour to Cerro Vanguardia	-48.512º, -67.731º	85
Homonota sp. A	LJAMM-CNP 5047[2]	ARG	Mendoza, RP 190, 2 km S (on road) from Punta del Agua	-35.538º, -68.080º	795
	LJAMM-CNP 10495	ARG	Mendoza, RP 190, 2 km S (on road) from Punta del Agua	-35.538º, -68.080º	795
	LJAMM-CNP 10505	ARG	La Pampa, RP 27, 4 km N of intersection with RP 14	-36.668º, -68.022º	767

Species	Voucher	Country	Specific locality	Lat/Long	Elevation
	LJAMM-CNP 10528	ARG	La Pampa, RP 16, 23 km W of intersection with RN 151	-37.075º, -67.785º	538
	LJAMM-CNP 10576	ARG	Mendoza, Secondary road 88 m from RP 190, 2.3 km S (on road) from Punta del Agua	-35.541º, -68.079º	786
	LJAMM-CNP 10577	ARG	Mendoza, Secondary road 88 m from RP 190, 2.3 km S (on road) from Punta del Agua	-35.541º, -68.079º	786
	LJAMM-CNP 12415	ARG	Neuquén, Secondary road, 80 m from RP 7, 13 km NW of Añelo	-38.264º, -68.891º	477
Homonota sp. B	MNHNP 11406	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655º	253
	MNHNP 11409	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655º	253
	MNHNP 11873	PAR	Boquerón, Cruce San Miguel, in front to Parque Nacional Teniente Enciso	-21.203º, -61.662º	254
	MNHNP 12238	PAR	Boquerón, Fortín Mayor Infante Rivarola	-21.679º, -62.401º	277
	SMF 101984	PAR	Boquerón, Fortín Mayor Infante Rivarola	-21.679º, -62.401º	277
H. rupicola	RUPI-1	PAR	Cordillera, Cerro Pedregal	-25.515º, -57.042º	288
	RUPI-2	PAR	Cordillera, Cerro Pedregal	-25.515º, -57.042º	288
H. taragui	LJAMM-CNP 14419	ARG	Corrientes, Paraje Tres Cerros, Cerro Capará	-29.112º, -56.919º	147
	LJAMM-CNP 14420	ARG	Corrientes, Paraje Tres Cerros, Cerro Capará	-29.112º, -56.919º	147
H. underwoodi	LJAMM-CNP 10923	ARG	San Juan, Baños del Salado, 5 km N of Baños de la Laja	-31.313º, -68.446º	641
	LJAMM-CNP 10931	ARG	San Juan, 5.5 km E of Caucete	-31.662º, -68.209º	580
H. uruguayensis	UFRGS 1568	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
	UFRGS 2139	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
	UFRGS 2140	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
	UFRGS 2579	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
	UFRGS 2580	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
	UFRGS 3929	BRA	Rio Grande do Sul, Rosário do Sul	-30.2º, -54.9º	
H. whitii	LJAMM-CNP 12110	ARG	Catamarca, W of Animan	-29.358º, -63.949º	410
	LJAMM-CNP 12111	ARG	Catamarca, W of Animan		

Species	Voucher	Country	Specific locality	Lat/Long	Elevation
	LJAMM-CNP 14387	ARG	Córdoba, Tulumba, RP 18, 9.5 km N of intersection with RP 16	-30.332º, -64.207º	907
	LJAMM-CNP 14388	ARG	Córdoba, Tulumba, RP 18, 9.5 km N of intersection with RP 16	-30.332º, -64.207º	907
	LJAMM-CNP 4467[3]	ARG	Buenos Aires, Tornquist, Parque Provincial Ernesto Tornquist	-38.055º, -62.014º	822
H. williamsii	LJAMM-CNP 6517	ARG	Buenos Aires, Tornquist, Parque Provincial Ernesto Tornquist	-38.136º, -61.986º	589
	LJAMM-CNP 6518	ARG	Buenos Aires, Tornquist, Parque Provincial Ernesto Tornquist	-38.136º, -61.986º	589
Garthia	IBE-G1(1)	CHI	Unknown locality		
gaudichaudi	IBE-G1(2)	CHI	Unknown locality		
Gymnodactylus	LG 911	BRA	Alagoas, Xingo		
geckoides	LG 1050	BRA	Sergipe, Barra dos Coqueiros	-10.9º, -37.0º	
Phyllodactylus kofordi	TG 266	PER	Chongoyape, Lambayeque	-6.7º, -79.9º	
Phyllopezus pollicaris	CHUNB 57388	BRA	Ceara, Tianguá	-3.7º, -40.9º	
Phyllopezus przewalskii	LJAMM-CNP 12089	ARG	Chaco, Fuerte Esperanza	-25.160º, -61.844º	169

Appendix S3

GenBank accession numbers (multi-genes)

GenBank accession numbers for each gene, and numbers of new sequences highlighted in bold. MD indicates missing data. Specimens with superindexes ([1–3]) are currently in BYU: 1- BYU 47960, 2- BYU 47941, 3- 47931.

Species	Voucher	ANL-19b	ANL-30b	RBMX	NKTR	SINCAIP	MXRA5	ACA4	DMXL1	PRLR	12S	Cytb
H. andicola	LJAMM-CNP 12490	KJ484255	KJ484235	KJ484346	KJ484394	KJ484369	KJ484348	KJ484324	KJ484298	KJ484274	MD	MD
	LJAMM-CNP 12493	MD	MD	MD	MD	MD	MD	MD	MD	MD	KJ484211	KJ484188
	LJAMM-CNP 12494	MD	MD	MD	MD	MD	MD	KJ484325	MD	MD	MD	MD
	LJAMM-CNP 12495	KJ484256	KJ484236	KJ484347	KJ484395	MD	KJ484349	MD	KJ484299	KJ484275	KJ484212	KJ484189
H. borellii	LJAMM-CNP 5842[1]	MD	MD	MD	MD	KJ484370	MD	MD	MD	MD	MD	MD
	LJAMM-CNP 12116	KJ484257	KJ484237	KJ484411	KJ484396	MD	KJ484350	KJ484326	KJ484300	KJ484276	KJ484213	KJ484205
	LJAMM-CNP 12125	KJ484258	KJ484238	KJ484412	KJ484397	KJ484393	KJ484351	KJ484327	KJ484301	KJ484277	KJ484214	KJ484206
H. darwinii	LJAMM-CNP 9266	KJ484260	KJ484240	KJ484414	KJ484409	KJ484372	KJ484352	KJ484329	KJ484303	MD	MD	KJ484191
	LJAMM-CNP 9813	MD	MD	MD	MD	MD	MD	MD	MD	KJ484278	MD	MD
	LJAMM-CNP 10638	MD	MD	MD	MD	MD	MD	MD	MD	KJ484295	MD	MD
	LJAMM-CNP 11424	MD	KJ484239	KJ484413	KJ484408	KJ484371	KJ484367	KJ484328	KJ484302	MD	KJ484215	KJ484190
	LJAMM-CNP 11432	KJ484259	MD	MD	MD	MD	MD	MD	MD	MD	KJ484216	MD
Homonota sp. A	LJAMM-CNP 5047[2]	KJ484262	KJ484242	KJ484416	MD	KJ484374	KJ484354	MD	KJ484305	KJ484280	KJ484218	KJ484192
	LJAMM-CNP 10505	MD	MD	MD	MD	KJ484373	MD	KJ484330	MD	MD	MD	MD
	LJAMM-CNP 10528	MD	MD	MD	KJ484399	MD	MD	KJ484331	MD	MD	MD	MD
	LJAMM-CNP 10577	KJ484261	KJ484241	KJ484415	KJ484398	MD	KJ484353	MD	KJ484304	MD	KJ484217	KJ484208
	LJAMM-CNP 12415	MD	MD	MD	MD	MD	MD	MD	MD	KJ484279	MD	MD

Species	Voucher	ANL-19b	ANL-30b	RBMX	NKTR	SINCAIP	MXRA5	ACA4	DMXL1	PRLR	12S	Cytb
Homonota sp. B	MNHNP 11406	**MF278837**	**MF278839**	**MF278851**	**MF278847**	**MF278853**	**MF278845**	**MF278841**	MD	**MF278849**	**MF278835**	**MF278843**
	MNHNP 11409	**MF278838**	**MF278840**	**MF278852**	**MF278848**	**MF278854**	**MF278846**	**MF278842**	**MF535517**	**MF278850**	**MF278836**	**MF278844**
H. rupicola	RUPI-1	KJ484263	KJ484243	KJ484426	MD	KJ484375	MD	MD	KJ484306	KJ484281	KJ484219	KJ484193
	RUPI-2	KJ484264	KJ484244	KJ484427	KJ484410	KJ484376	MD	MD	KJ484320	KJ484282	KJ484220	KJ484194
H. taragui	LJAMM-CNP 14419	KJ484265	KJ484245	KJ484428	MD	KJ484377	MD	KJ484332	KJ484321	KJ484283	KJ484221	KJ484195
	LJAMM-CNP 14420	KJ484266	KJ484246	KJ484429	KJ484400	KJ484378	MD	KJ484333	KJ484322	KJ484284	KJ484222	KJ484196
H. underwoodi	LJAMM-CNP 10923	KJ484269	KJ484249	MD	KJ484402	KJ484381	KJ484356	KJ484335	KJ484308	KJ484286	MD	KJ484197
	LJAMM-CNP 10931	KJ484270	KJ484250	MD	KJ484403	KJ484382	KJ484357	MD	KJ484309	KJ484297	MD	KJ484198
H. uruguayensis	UFRGS 1568	KJ484267	KJ484247	KJ484430	KJ484401	KJ484381	KJ484358	KJ484334	KJ484307	KJ484285	KJ484223	MD
	UFRGS 2139	MD	MD	MD	MD	KJ484382	MD	MD	MD	KJ484296	MD	MD
	UFRGS 2140	MD	MD	MD	MD	MD	MD	MD	MD	MD	KJ484224	MD
	UFRGS 2579	KJ484268	KJ484248	KJ484359	MD	MD	MD	MD	MD	MD	MD	MD
	UFRGS 2580	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	KJ484202
	UFRGS 3929	MD	MD	MD	MD	MD	MD	MD	KJ484323	MD	MD	MD
H. whitii	LJAMM-CNP 12110	MD	KJ484251	KJ484360	MD	KJ484383	KJ484359	KJ484336	MD	MD	MD	MD
	LJAMM-CNP 12111	KJ484271	MD	MD	MD	MD	KJ484360	KJ484337	MD	MD	MD	MD
	LJAMM-CNP 14387	MD	MD	MD	KJ484404	MD	MD	MD	KJ484310	MD	KJ484225	KJ484203
	LJAMM-CNP 14388	MD	KJ484252	KJ484432	KJ484405	KJ484384	MD	MD	KJ484311	MD	KJ484226	KJ484204
H. williamsii	LJAMM-CNP 4467[3]	KJ484272	KJ484253	KJ484417	MD	KJ484385	KJ484360	KJ484338	KJ484312	KJ484287	KJ484227	KJ484205
	LJAMM-CNP 6517	KJ484273	KJ484254	KJ484418	KJ484407	KJ484386	KJ484361	KJ484339	KJ484313	KJ484288	KJ484228	KJ484206
	LJAMM-CNP 6518	MD	MD	MD	KJ484406	MD	MD	MD	MD	MD	MD	MD
Garthia gaudichaudi	IBE-G1(1)	MD	MD	KJ484419	MD	KJ484387	KJ484362	MD	KJ484314	KJ484289	KJ484229	MD
	IBE-G1(2)	MD	MD	KJ484420	MD	MD	MD	KJ484340	MD	MD	KJ484230	MD
Gymnodactylus geckoides	LG 911	MD	MD	KJ484421	MD	KJ484389	KJ484364	KJ484342	KJ484316	KJ484291	KJ484232	KJ484210
	LG 1050	MD	MD	KJ484431	MD	KJ484388	KJ484363	KJ484341	KJ484315	KJ484290	KJ484231	KJ484209

Species	Voucher	ANL-19b	ANL-30b	RBMX	NKTR	SINCAIP	MXRA5	ACA4	DMXL1	PRLR	12S	Cytb
Phyllodactylus kofordi	TG 266	MD	MD	KJ484422	MD	KJ484390	KJ484368	KJ484343	KJ484317	KJ484292	KJ484233	KJ484207
Phyllopezus pollicaris	CHUNB 57388	MD	MD	KJ484424	MD	KJ484392	KJ484366	KJ484345	KJ484319	KJ484294	KJ484234	KJ484204
Phyllopezus przewalskii	LJAMM-CNP 12089	MD	MD	KJ484423	MD	KJ484391	KJ484365	MD	KJ484318	KJ484293	MD	KJ484203

Appendix S4

Primers used for genetic analyses

Primers' sequences for each gene, used for analyses. For every primer we indicate the author, and the full reference is provided below the table. We maintain the original name of the primers, providing an indication for forward (F) and reverse (R) primers in square brackets when not specified in the original name.

Gene	Primer	Sequence (5' -> 3')	Author
16S	L2510 [F]	GCCTGTTTAACAAAAACAT	Miya & Nishida 1996
	H3056 [R]	CGGTCTG AACTCAGATCACGT	Miya & Nishida 1996
12S	tPhe [F]	AGCACRGCACTGA	Wiens et al. 1999
	12e [R]	RCGCTTACCWTGTTAC	Wiens et al. 1999
Cytb	CB3	GGCAAATAGGAARTATCATTC	Palumbi 1996
	CB3R	CATATTAAACCCGAATGATAYTT	Palumbi 1996
ANL-19b	Homo19b_F	CCTAAGAAAAGAGAAGGCAATTCA	Morando et al. 2014
	Homo19b_R	TGCATGCTACTCAGATTCCTG	Morando et al. 2014
ANL-30b	Homo30b_F	CAATCCAGTCGAAGGAAGGA	Morando et al. 2014
	Homo30b_R	AAACTTGGTTGGGTGCAGAG	Morando et al. 2014
RBMX	HNRNP1F	CCACGAGATTATGCCTACCG	Gamble et al. 2011
	HNRNP1R	CATCATAKCGACTGCTTCCA	Gamble et al. 2011
	RBMX-F1	TCCTCTTACAGTGAYCGTGATG	Gamble et al. 2011

	RBMX-R1	TCCCGTAATCATCATAGCGACT	Gamble et al. 2011
NKTR	NTKRf1	AGTAAATGGGAYTCKGARTCAAA	Townsend et al. 2011
	NKTRr3	KCGTGCYGTCTTYCTWACTTCA	Townsend et al. 2011
SINCAIP	SNCAIP_f10	CGCCAGYTGYTGGGRAARGAWAT	Townsend et al. 2008
	SNCAIP_r13	GGWGAYTTGAGDGCACTCTTRGGRCT	Townsend et al. 2008
MXRA5	MXRA5_F1	YATTTTGGCAAARGTCCGTGGGAARA	Portik et al. 2012.
	MXRA5_F2	KGCTGAGCCTKCCTGGGTGA	Portik et al. 2012.
	MXRA5_R1	WTGTGCTGCATATGCTGTWATCTCWGGT	Portik et al. 2012.
	MXRA5_R2	YCTMCGGCCYTCTGCAACATTK	Portik et al. 2012.
ACA4	ACA4 [F]	GAGCGTGGCTAYTCCTTTGT	Waltari & Edwards 2002
	ACA4 [R]	GTGGCCATTTCATTCTCAAA	Waltari & Edwards 2002
DMXL1	DMXL1(F2)	GTCTAGGGAGGATGGTTCACATA	Werneck et al. 2012
	DMXL1(R2)	GAATGAAGCAAGTGACSAGAAAGA	Werneck et al. 2012
PRLR	PRLR_f1	GACARYGARGACCAGCAACTRATGCC	Townsend et al. 2008
	PRLR_r3	GACYTTGTGRACTTCYACRTAATCCAT	Townsend et al. 2008

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Appendix S5

Examined specimens for morphology

Specimens used for morphological comparison. Specimens marked with "[H]" and "[P]" indicate holotype and paralectotypes of *Gymnodactylus horridus* respectively. Codes for countries are: ARG (Argentina), BOL (Bolivia), and PAR (Paraguay). Locality information is provided from the major political division of the country to the most specific location data. References to routes are RP (Provincial Route) and RN (National Route). Coordinates given with only one decimal are approximately, in which case elevation is not provided.

Specimen	Country	Specific locality	Lat/Long	Elevation
IZH-R 1 [H]	ARG	Mendoza, Canyons of the Sierra del Challao	-32.9º, -68.9º	
IZH-R 2 [P]	ARG	Mendoza, Canyons of the Sierra del Challao	-32.9º, -68.9º	
IZH-R 3 [P]	ARG	Mendoza, Canyons of the Sierra del Challao	-32.9º, -68.9º	
LJAMM-CNP 6520	ARG	Río Negro, Villa Regina, fosa del tiro federal	-39.096º, -67.051º	214
LJAMM-CNP 6530	ARG	Río Negro, Villa Regina, bardas	-39.092º, -67.076º	261
LJAMM-CNP 6532	ARG	Río Negro, Villa Regina, bardas	-39.092º, -67.076º	261
LJAMM-CNP 6533	ARG	Río Negro, Villa Regina, bardas	-39.092º, -67.076º	261
LJAMM-CNP 6535	ARG	Río Negro, Villa Regina, bardas	-39.092º, -67.076º	261
LJAMM-CNP 6967	ARG	Neuquén, Villa El Chocón, N margin of Limay river	-39.254º, -68.781º	469
LJAMM-CNP 6968	ARG	Neuquén, Villa El Chocón	-39.254º, -68.781º	469

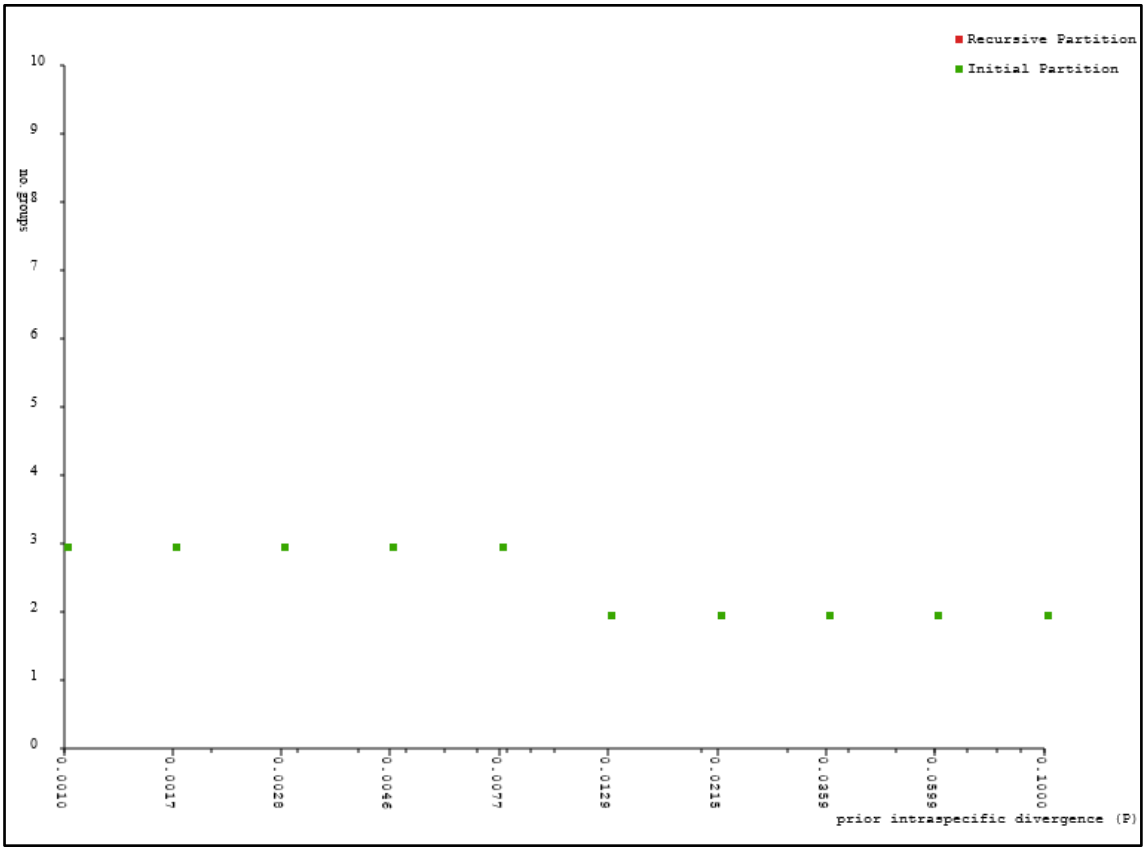
Specimen	Country	Specific locality	Lat/Long	Elevation
LJAMM-CNP 7670	ARG	Río Negro, Avellaneda, Chimpay	-39.145º, -66.145	157
LJAMM-CNP 7674	ARG	Río Negro, Avellaneda, Chimpay	-39.145º, -66.145	157
LJAMM-CNP 7804	ARG	Neuquén, RP 5, 10 km N from RP 7	-37.906º, -69.174º	555
LJAMM-CNP 8713	ARG	Neuquén, RP 7, 41 km NW Punta Carranza	-37.656º, -69.472º	662
LJAMM-CNP 10493	ARG	Mendoza, RP 190, 1 km S Punta de Agua	-35.541º, -68.079º	789
LJAMM-CNP 10496	ARG	Mendoza, RP 190, 1 km S Punta de Agua	-35.541º, -68.079º	789
LJAMM-CNP 10523	ARG	La Pampa, RP 1, 23.6 km W intersection with RN 151	-37.075º, -67.785º	542
LJAMM-CNP 10526	ARG	La Pampa, RP 1, 23.6 km W intersection with RN 151	-37.075º, -67.785º	542
LJAMM-CNP 10576	ARG	Mendoza, RP 190, 1 km S Punta de Agua	-35.541º, -68.079º	789
LJAMM-CNP 10577	ARG	Mendoza, RP 190, 1 km S Punta de Agua	-35.541º, -68.079º	789
LJAMM-CNP 10578	ARG	La Pampa, RP 27, 37.7 km S intersection with RP 14	-36.668º, -68.022º	766
LJAMM-CNP 10579	ARG	La Pampa, RP 27, 37.7 km S junction with RP 14	-36.668º, -68.022º	766
LJAMM-CNP 10584	ARG	La Pampa, RP 1, 23.6 km W intersection with RN 151	-37.075º, -67.785º	542
LJAMM-CNP 13948	ARG	Neuquén, RN 237, 6 km SW Picun Leufu	-39.555º, -69.301º	431
LJAMM-CNP 14551	ARG	Neuquén, Mina La Casualidad	-37.904º, -68.488º	462
MNHNP 2821	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253

Specimen	Country	Specific locality	Lat/Long	Elevation
MNHNP 9037	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 9038	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 9131	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11406	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11410	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11419	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11421	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11423	PAR	Boquerón, Parque Nacional Teniente Enciso	-21.209º, -61.655	253
MNHNP 11850	PAR	Boquerón, Cruce San Miguel, in front of Parque Nacional Teniente Enciso	-21.203º, -61.662º	254
MNHNP 11855	PAR	Boquerón, Cruce San Miguel, in front of Parque Nacional Teniente Enciso	-21.203º, -61.662º	254
MNHNP 11860	PAR	Boquerón, Cruce San Miguel, in front of Parque Nacional Teniente Enciso	-21.203º, -61.662º	254
MNHNP 11872	PAR	Boquerón, Cruce San Miguel, in front of Parque Nacional Teniente Enciso	-21.203º, -61.662º	254
MNHNP 12238	PAR	Boquerón, Fortín Mayor Infante Rivarola	-21.679º, -62.401º	277
SMF 29277	BOL	Tarija, Villamontes	-21.266º, -63.451º	398
SMF 101984	PAR	Boquerón, Fortín Mayor Infante Rivarola	-21.679º, -62.401º	277

Appendix S6

Results of ABGD analysis

Number of groups according to the prior intraspecific divergence. Color of "Initial Partitions" were modified from original for a better contrast. Under the graphic, is presented the list of partitions with suggested grouping and each maximal intraspecific divergence.



Partition 1 : found 3 groups (prior maximal distance P= 0.001000)

Partition 2 : found 3 groups (prior maximal distance P= 0.001668)

Partition 3 : found 3 groups (prior maximal distance P= 0.002783)

Partition 4 : found 3 groups (prior maximal distance P= 0.004642)

Partition 5 : found 3 groups (prior maximal distance P= 0.007743)

Partition 6 : found 2 groups (prior maximal distance P= 0.012915)

Partition 7 : found 2 groups (prior maximal distance P= 0.021544)

Partition 8 : found 2 groups (prior maximal distance P= 0.035938)

Partition 9 : found 2 groups (prior maximal distance P= 0.059948)

Partition 10 : found 2 groups (prior maximal distance P= 0.100000)

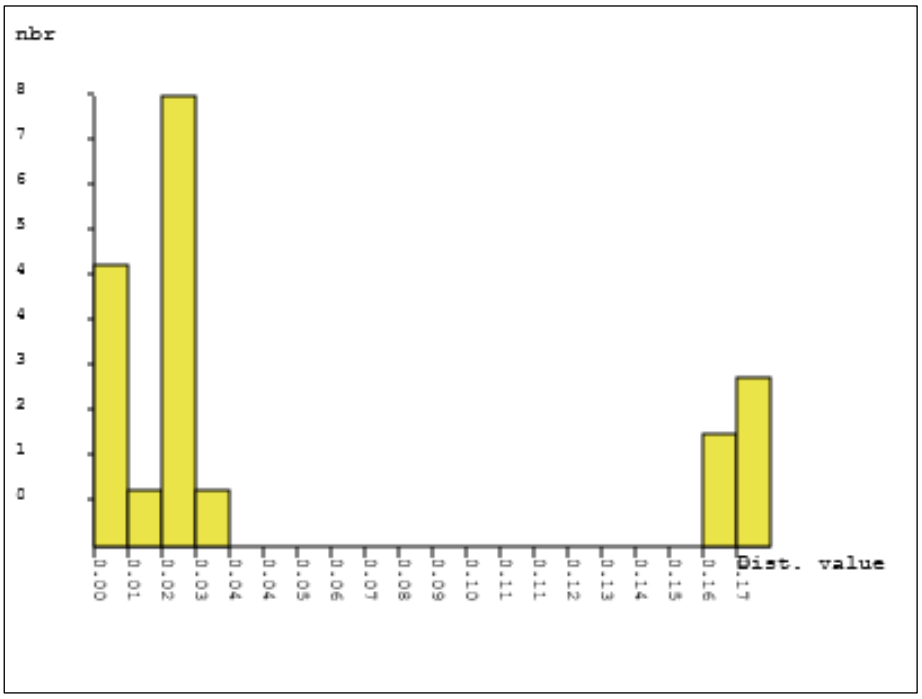
Groups detected when Initial Partition with prior maximal distance P=7.74e^{-03}

Group[1] n: 3 ;id: LJAMM-CNP_10495 LJAMM-CNP_10576 LJAMM-CNP_5047

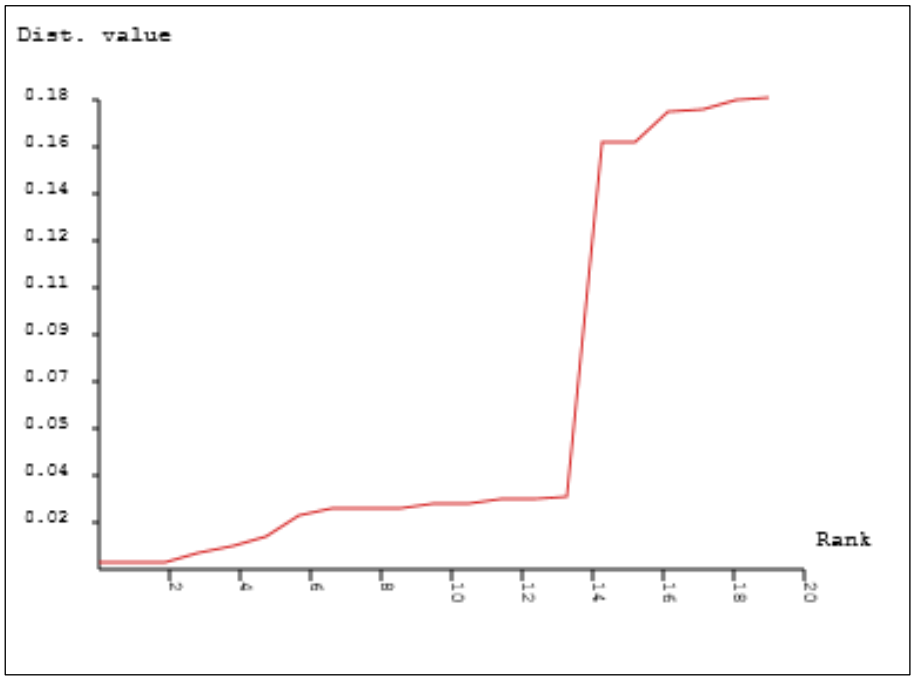
Group[2] n: 3 ;id: MNHNP_11873 MNHNP_12238 SMF_101984

Group[3] n: 1 ;id: Phyllopezus_SMF-100495

Histogram of distances



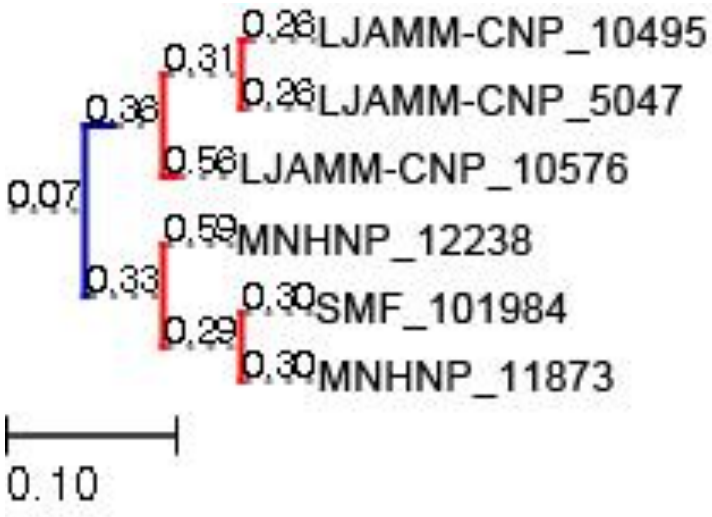
Ranked distances



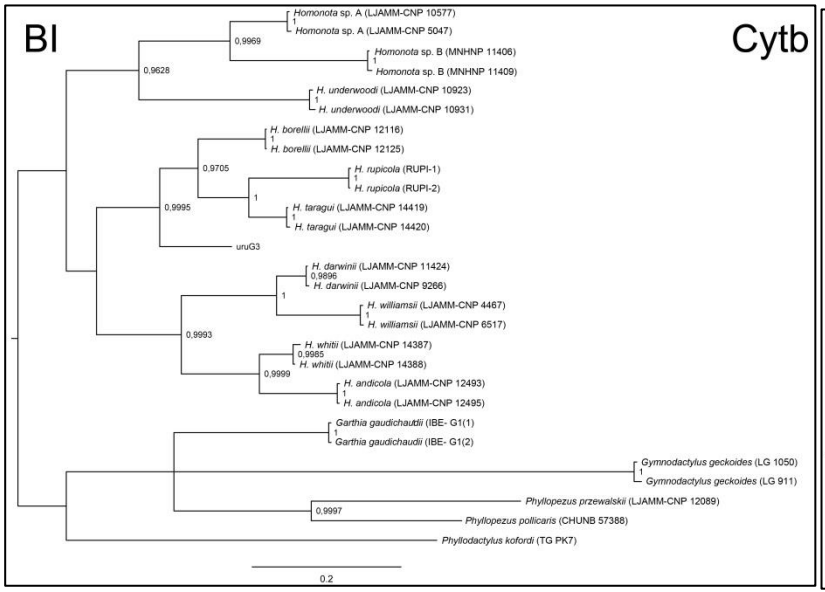
Appendix S7

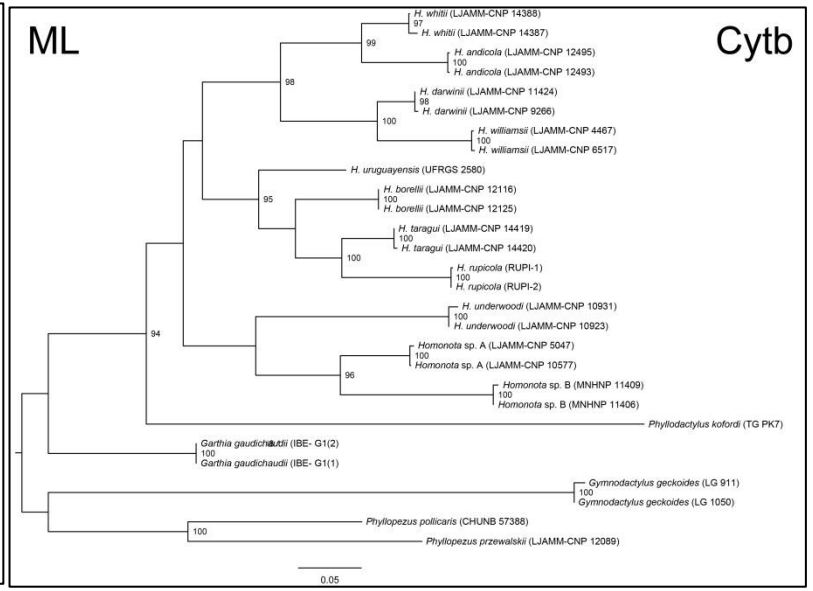
Results of PTP analysis

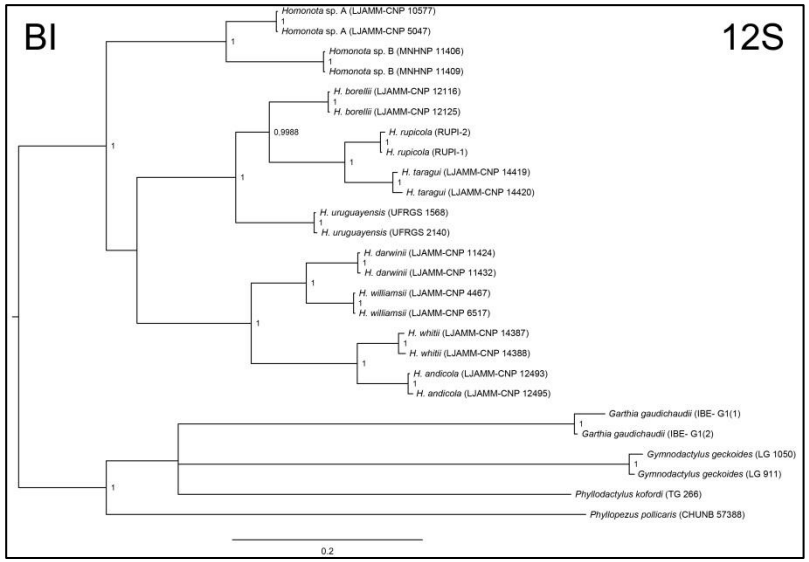
ML solution produced with PTP. Numbers on nodes indicate support values. The red clades represent putative species. In this case, one species is composed of Argentinean samples (LJAMM-CNP 5047, 10495, 10576), and the other of Paraguayan specimens (MNHNP 11873, 12238, SMF 101984).

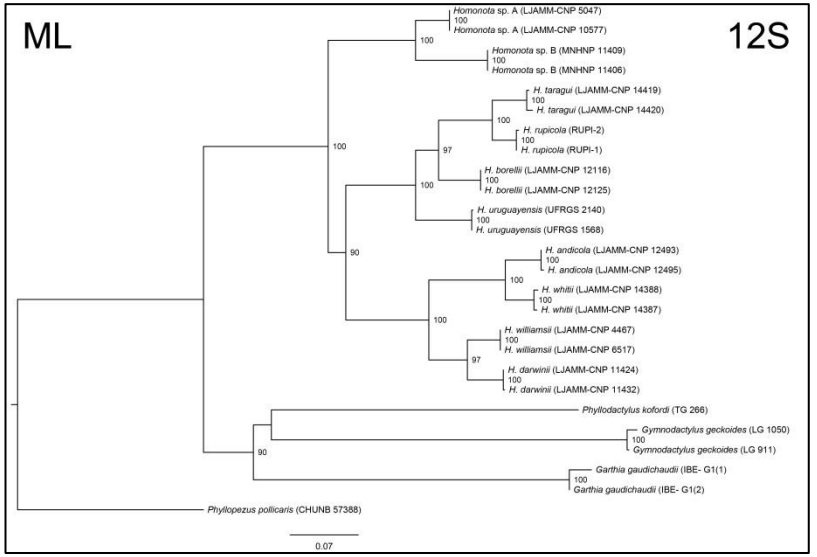


Appendix S8 - Individual gene trees (Explanations at the end)

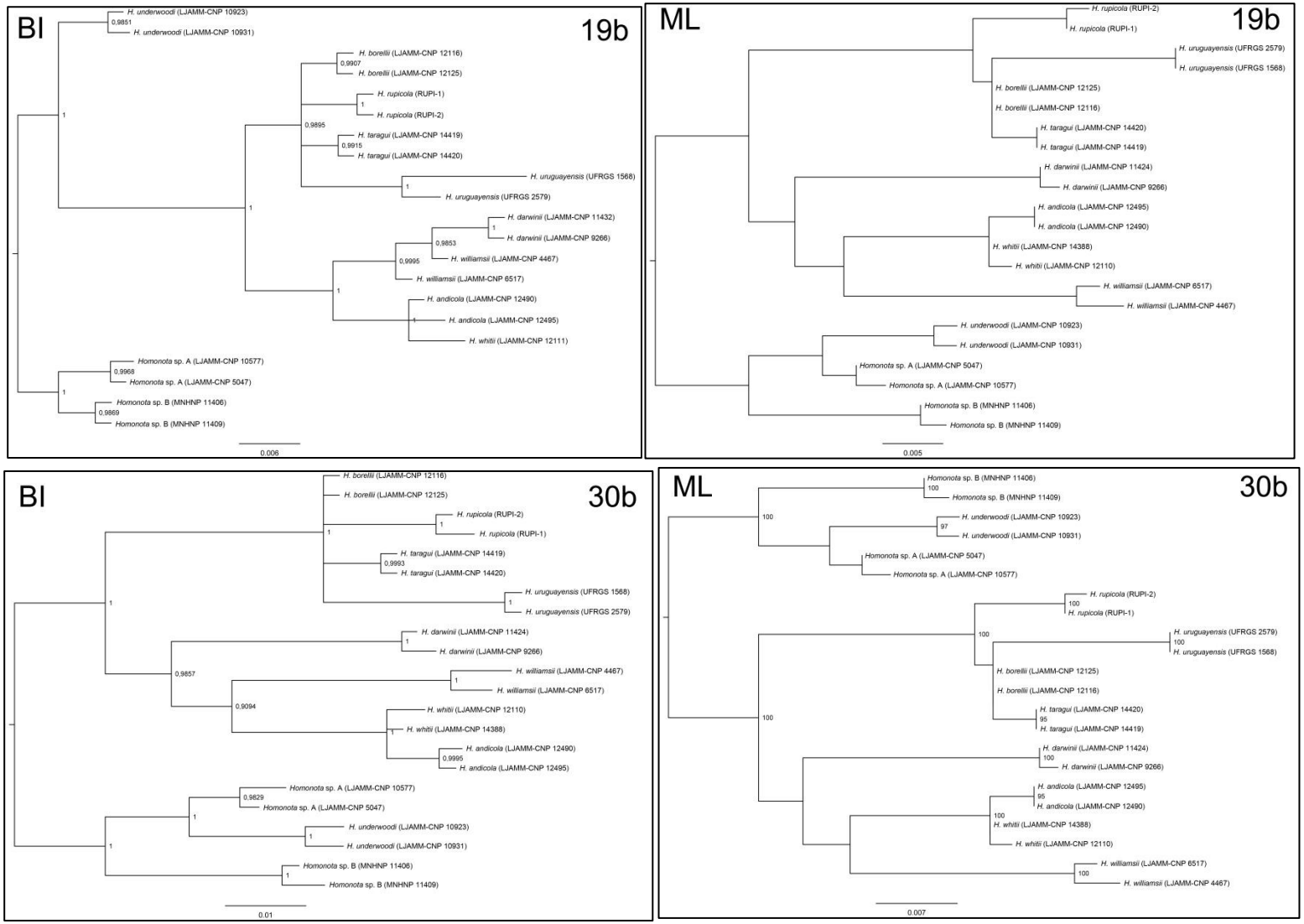




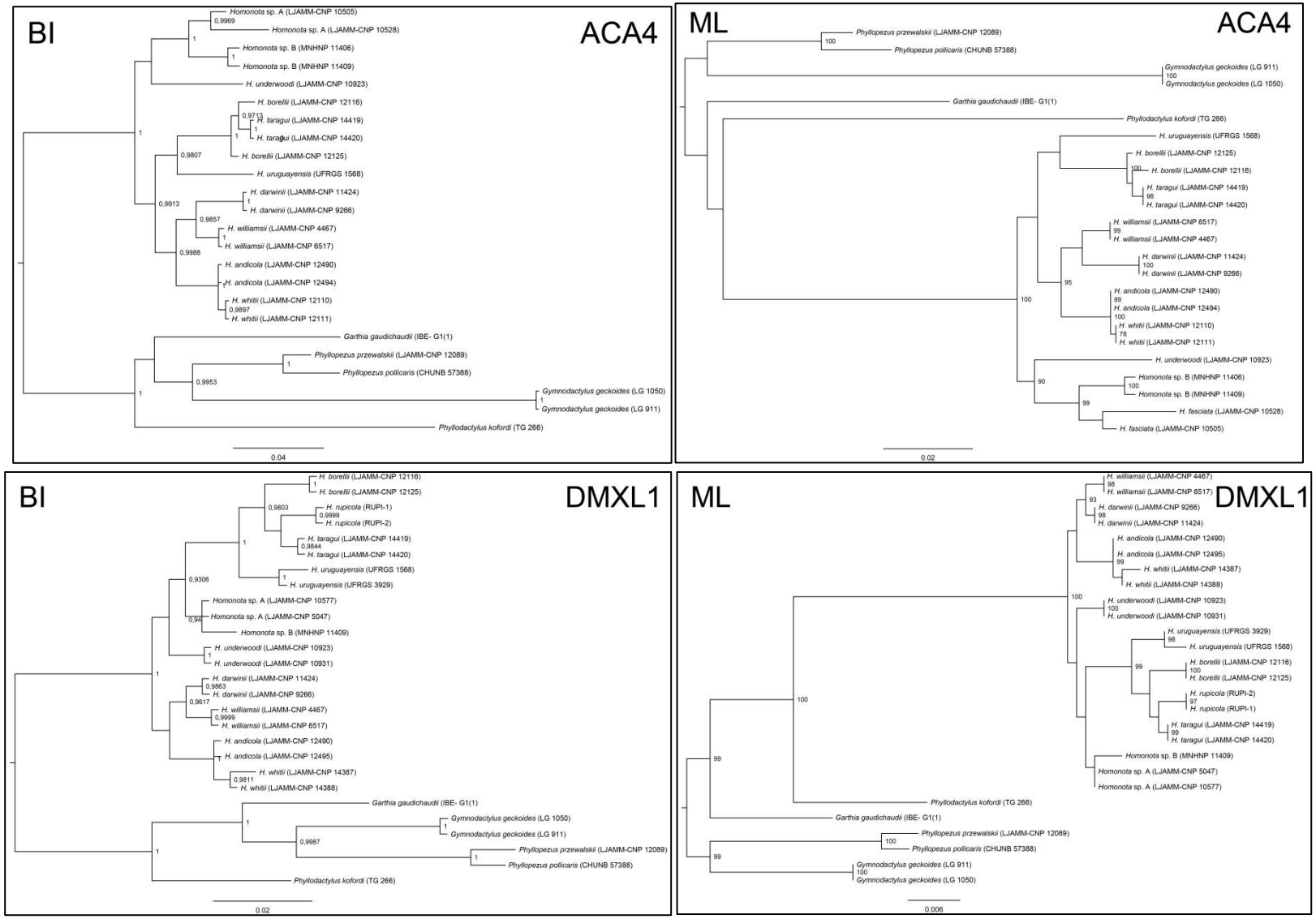




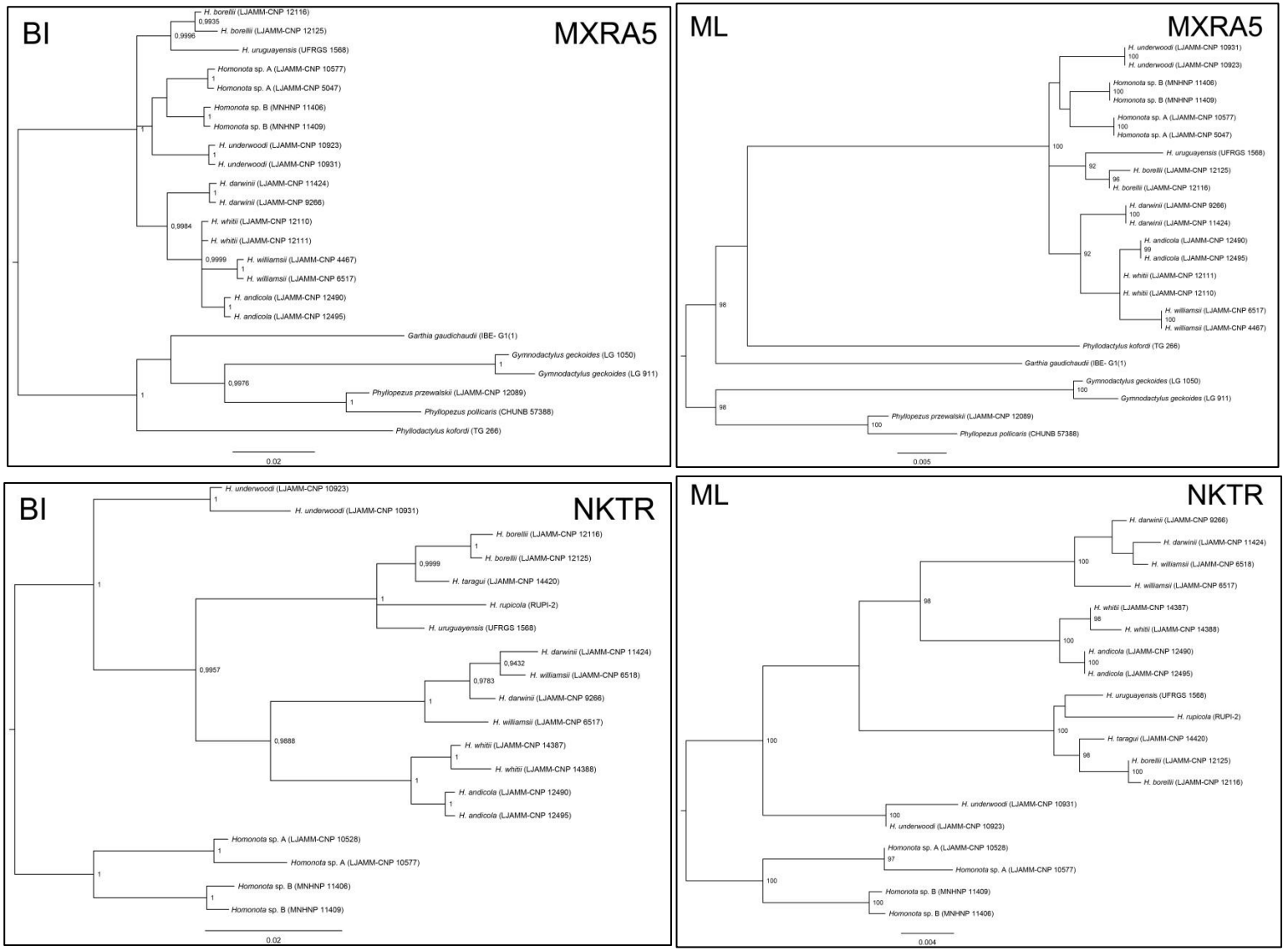
Appendix S8 (continuation)



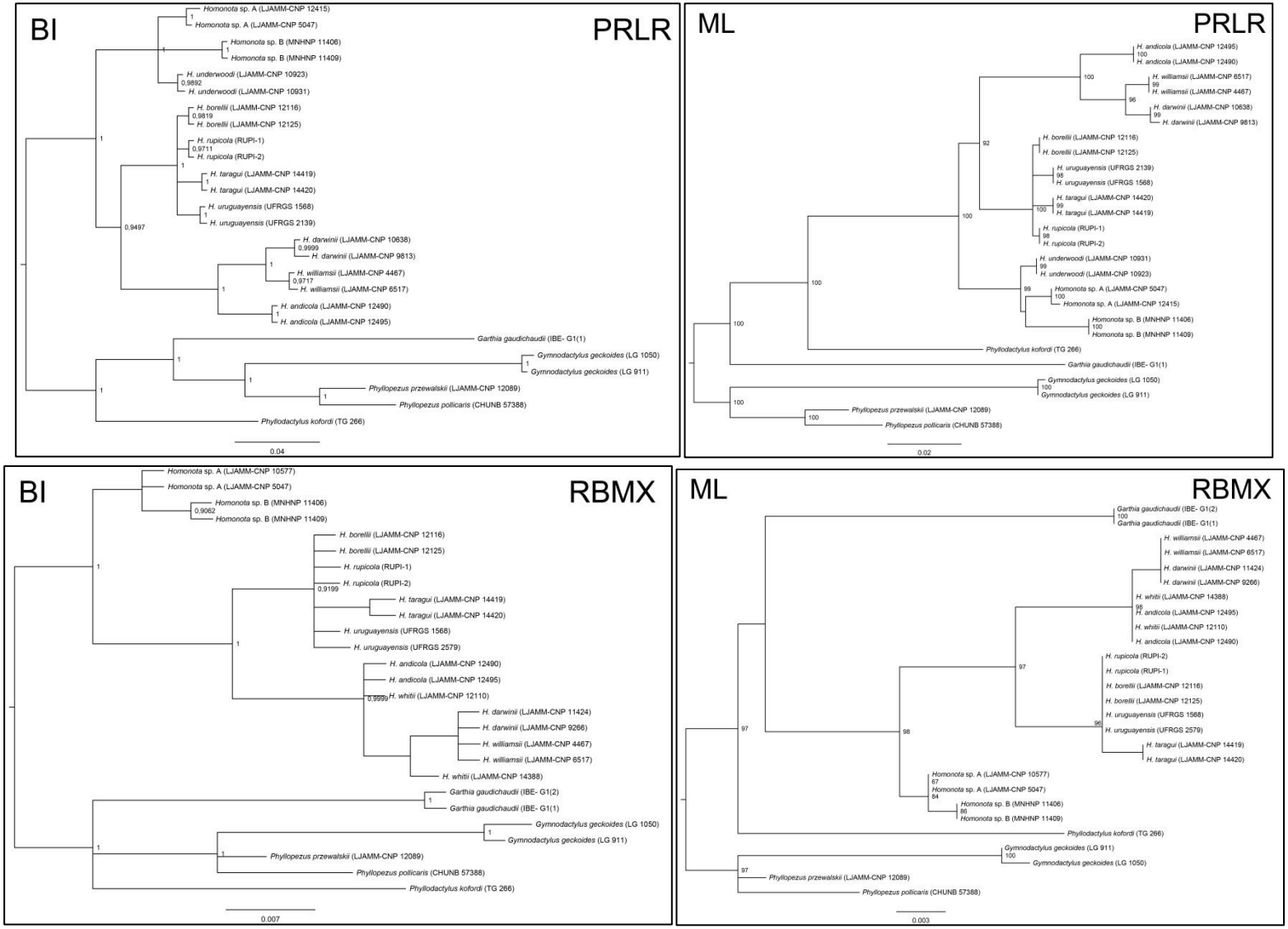
Appendix S8 (continuation)



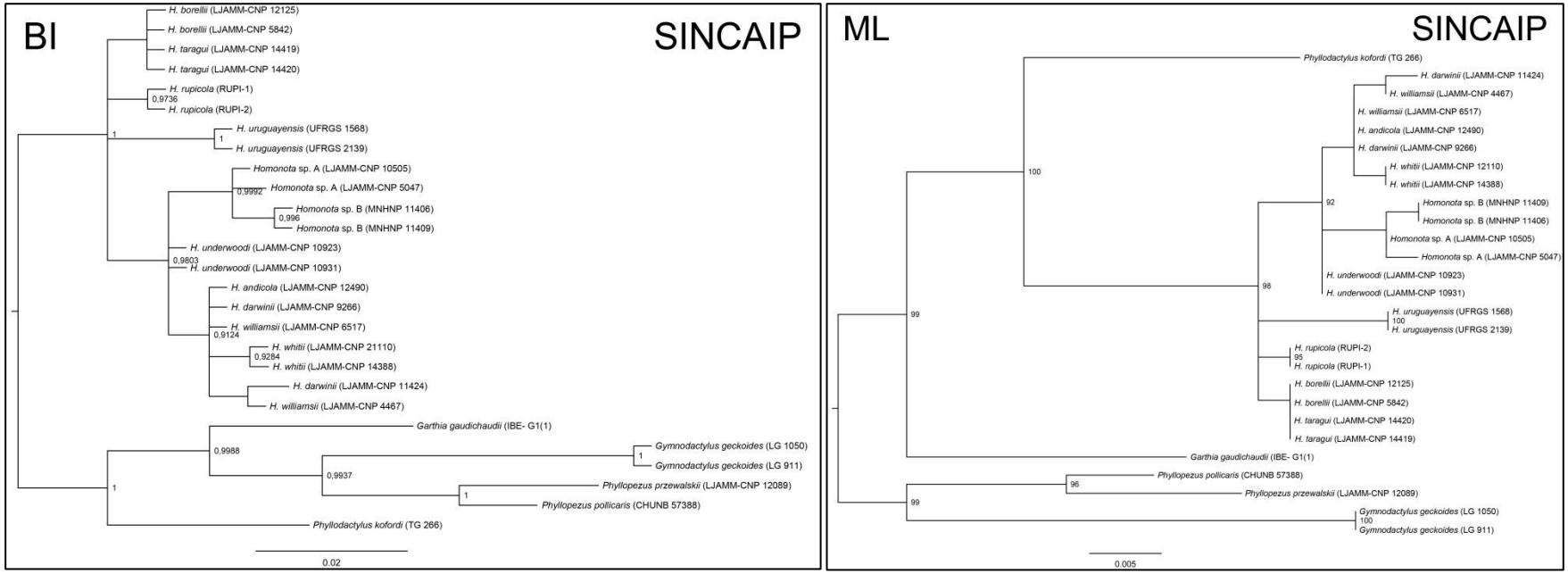
Appendix S8 (continuation)



Appendix S8 (continuation)

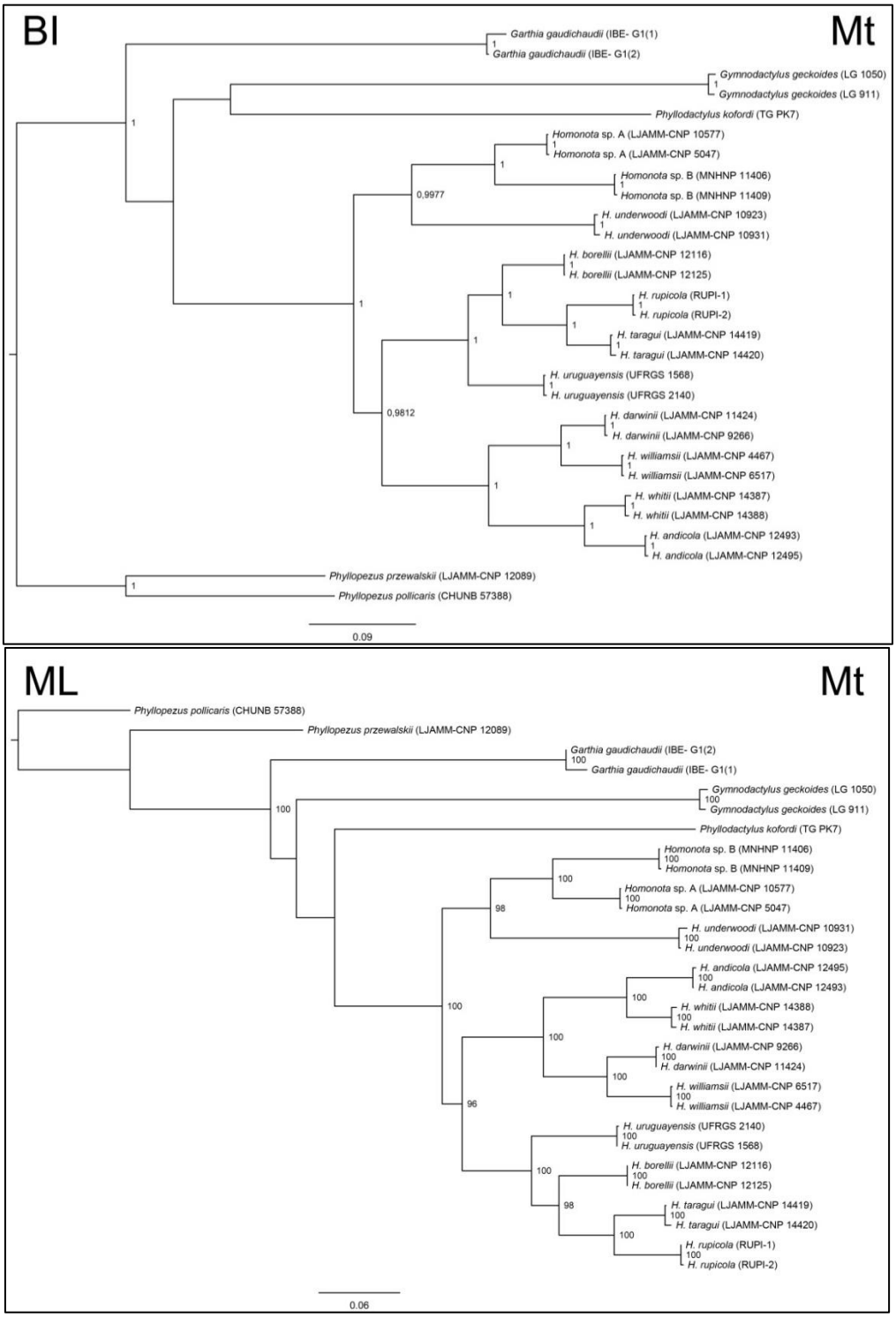


Appendix S8 (continuation)



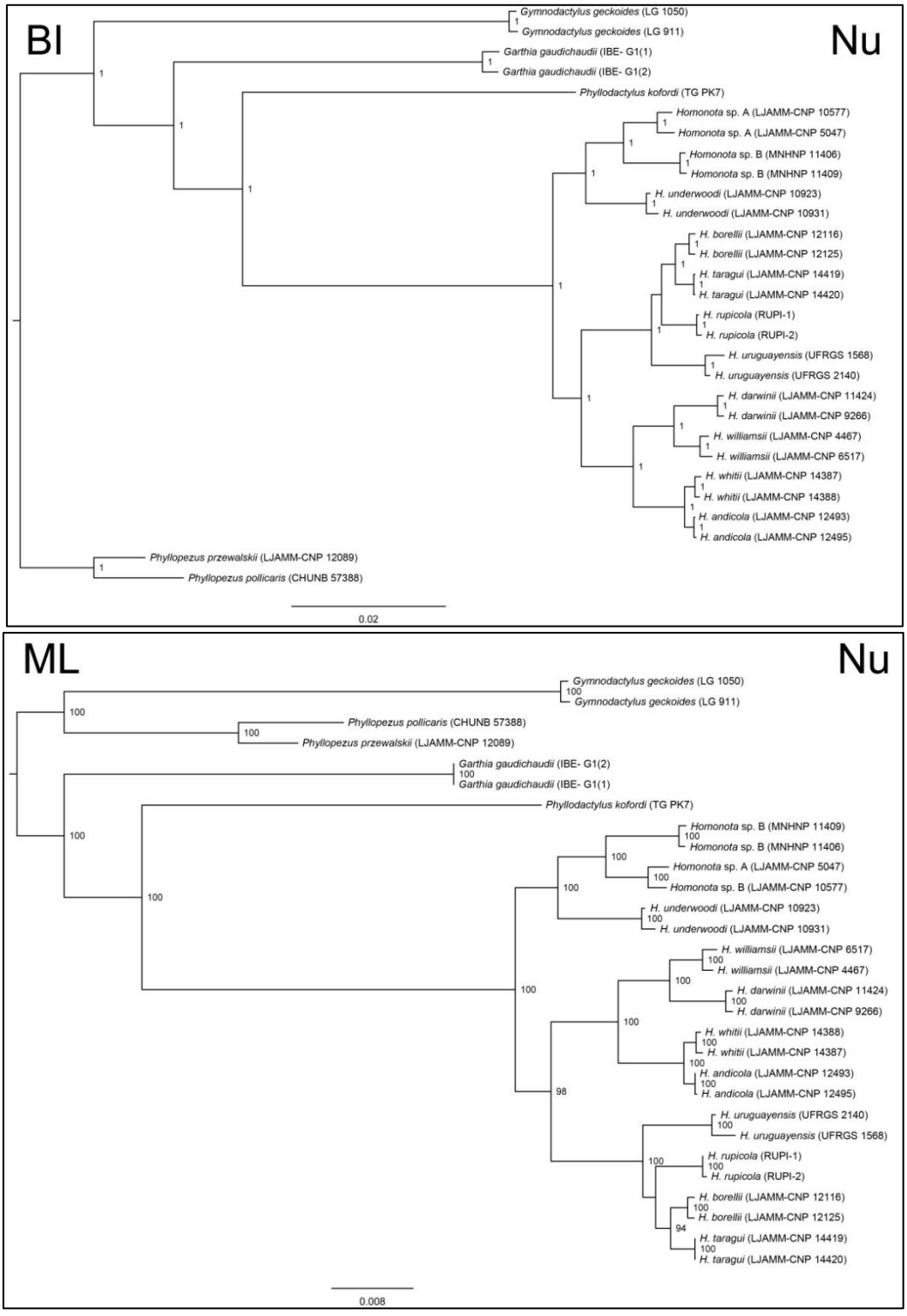
Individual gene trees generated by Bayesian analysis (BI) and Maximum Likelihood (ML). Acronyms for genes are cytb (cytochrome *b*), 12S (small ribosomal subunit), ACA4 (alpha-cardiac actin intron 4), DMXL1 (dmX-like protein 1), MXRA5 (encoding matrix remodeling associated intron 5), NKTR (natural killer-tumor recognition sequence), PRLR (prolactin receptor), RBMX (intron 8 and flanking exon regions of RNA binding motif protein), SINCAIP (synuclein alpha interacting protein), 19b and 30b (anonymous nuclear loci). The two first genes are mitochondrial, and the remaining nine are nuclear. Only values of ≥0.90 (for BI) and ≥90 (for ML) are shown.

Appendix S9 – Concatenated trees



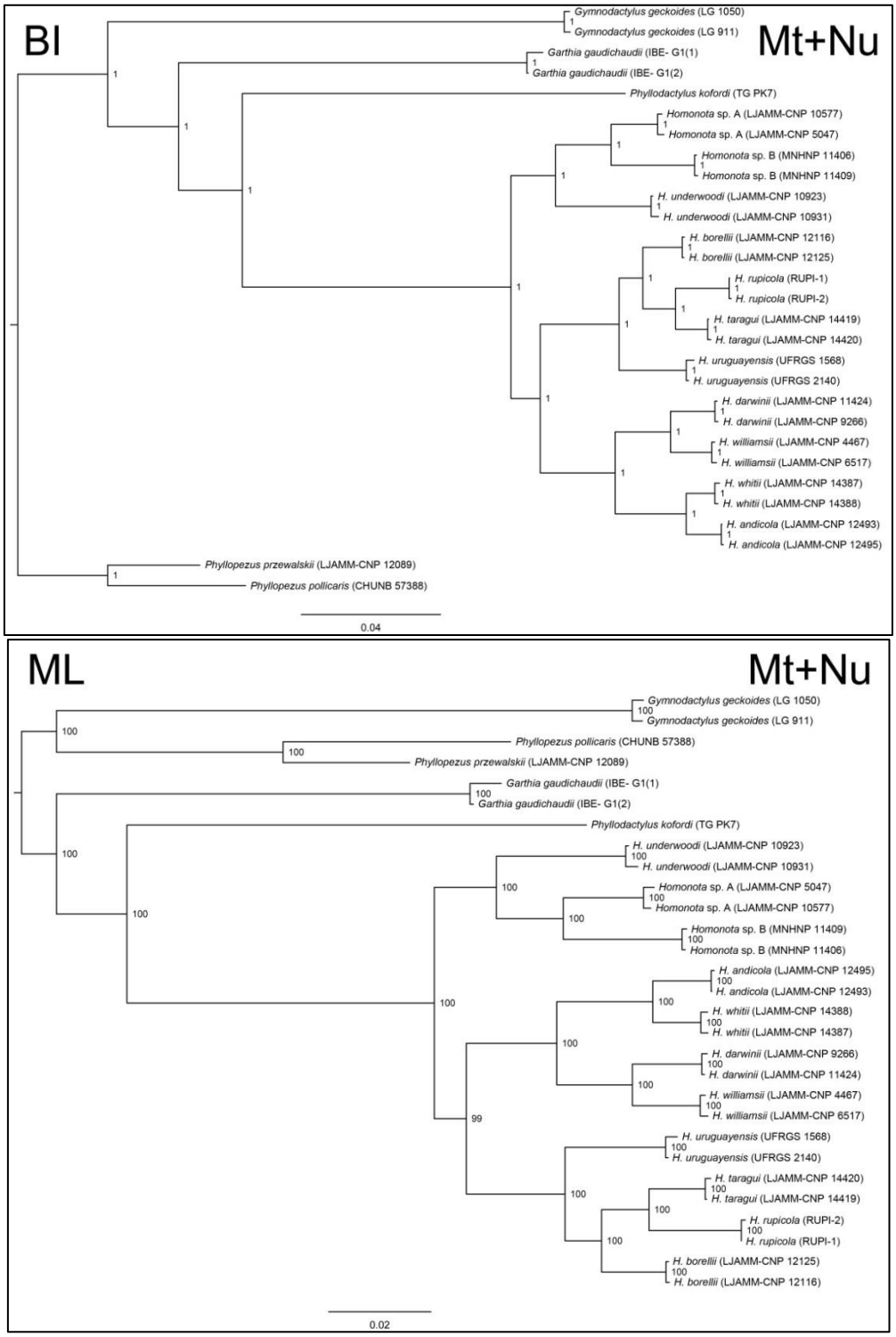
Bayesian (above) and Maximum Likelihood (below) trees inferred from concatenated mitochondrial genes. See Materials and Methods for indication of mitochondrial genes. Only values of ≥0.90 (for BI) and ≥90 (for ML) are shown.

Appendix S9 (continuation)



Bayesian (above) and Maximum Likelihood (below) trees inferred from concatenated nuclear genes. See Materials and Methods for indication of nuclear genes. Only values of ≥0.90 (for BI) and ≥90 (for ML) are shown.

Appendix S9 (continuation)



Bayesian (above) and Maximum Likelihood (below) trees inferred from concatenated mitochondrial and nuclear genes. Only values of ≥0.90 (for BI) and ≥90 (for ML) are shown.

Appendix S10

Most contributing variables for DA

A- Continuous variables

Contribution values for the three principal axes of the discriminant function analysis of continuous variables. Most highly contributing variables in bold. Abbreviations: AL (arm length), EMD (eye–meatus distance), END (eye–nostril distance), ESD (eye–snout distance), FL (foot length), HH (head height), HL (head length), HW (head width), ID (interorbital distance), IND (internostril distance), SVL (snout–vent length), TL (tibial length), TrL (trunk length). See "Morphological Approach" in the Materials and Methods section for details about measurements.

Variables	Axis 1	Axis 2	Axis 3
SVL	**-0.25403**	**2.77550**	**0.40997**
TrL	**-0.45427**	**1.26620**	0.25683
FL	-0.09004	0.50099	-0.07282
TL	0.06197	0.43801	0.05459
AL	0.03385	0.50497	**0.39286**
HL	-0.21662	0.47604	**0.37498**
HW	-0.21465	0.32961	0.33726
HH	-0.23825	0.11503	0.23913
END	-0.08492	0.14669	0.02613
ESD	-0.11675	0.25690	0.20150
EMD	0.02475	0.24455	0.21194
ID	-0.01922	0.17126	0.20578
IND	-0.06752	0.06858	0.05570

B- Discrete variables

Contribution values for the three principal axes of the discriminant function analysis of discrete variables. Most highly contributing variables in bold. Abbreviations: 3FL (third finger lamellae), 4TL (fourth toe lamellae), DT (dorsal tubercles), LVS (longitudinal rows of ventral scales), TVS (transversal rows of ventral scales). See "Morphological Approach" in the Materials and Methods section for details about counts.

Variables	Axis 1	Axis 2	Axis 3
DT	**1.08450**	0.23782	1.22050
TVS	0.67569	**-1.77600**	-0.65160
LVS	-0.34039	-0.87093	**1.46070**
4TL	0.59106	0.39061	0.33669
3FL	0.84406	-0.14672	0.42342

Appendix S11

Matrix of metric data

Raw metric data (in mm) used for analyses. See Materials and Methods section for explanation of the characters. Missing data indicated with "?". Argentinean populations belong to LJAMM-CNP collection, whereas Paraguayan specimens are housed in the MNHNP and SMF.

Specimen	SVL	TrL	FL	TL	AL	HL	HW	HH	END	ESD	EMD	ID	IND	Sex
LJAMM-CNP 6520	57	24	10	9.2	12.2	12.8	8.6	6.7	4.3	5.3	4.8	3.8	1.7	F
LJAMM-CNP 6530	63	29	11	12.5	15.4	14.6	10.4	7.6	4.7	6.3	5.3	4.8	1.7	F
LJAMM-CNP 6532	55	23	9	9.6	?	13.5	10.5	6.9	4.6	5.8	5.2	5.2	1.8	M
LJAMM-CNP 6533	59	25	?	11.2	13.4	13.9	11.1	7.1	4.8	6.1	4.9	4.9	2.3	F
LJAMM-CNP 6535	52	20	11	9.6	?	?	?	?	?	?	?	?	?	M
LJAMM-CNP 6967	41	18	7	8.3	11.9	10.5	10.4	5.8	3.5	4.1	4.4	4.5	1.7	M
LJAMM-CNP 6968	45	18	9	9.0	13.7	?	8.9	?	?	?	?	4.6	1.6	M
LJAMM-CNP 7670	55	22	11	11.3	?	10.5	8.6	5.8	4.2	4.9	4.6	4.5	1.8	F
LJAMM-CNP 7674	50	23	9	9.9	11.9	11.8	8.3	6.3	4.1	4.8	4.7	4.5	1.6	F
LJAMM-CNP 7804	52	20	11	10.2	12.1	9.8	10.1	6.1	2.9	3.6	4.7	5.3	2.3	F
LJAMM-CNP 8713	56	25	8	10.5	?	?	10.6	?	?	?	?	4.8	2.2	M

Specimen	SVL	TrL	FL	TL	AL	HL	HW	HH	END	ESD	EMD	ID	IND	Sex
LJAMM-CNP 10493	53	21	10	10.4	14.4	11.7	8.7	?	3.7	4.6	4.5	4.5	2.0	M
LJAMM-CNP 10496	51	23	9	9.4	12.9	12.5	9.9	5.5	4.0	4.8	5.1	4.4	1.8	F
LJAMM-CNP 10523	57	22	?	10.3	14.5	14.5	12.4	6.7	4.8	6.0	5.9	5.8	1.8	F
LJAMM-CNP 10526	64	27	12	12.0	16.8	14.3	10.8	6.3	4.9	6.1	5.5	5.2	1.9	F
LJAMM-CNP 10576	51	18	9	10.1	12.7	13.2	10.8	6.1	4.5	5.8	5.3	4.6	1.8	M
LJAMM-CNP 10577	42	16	8	8.3	11.8	10.6	8.5	4.9	3.7	4.2	4.2	4.1	1.2	F
LJAMM-CNP 10578	47	17	9	9.5	14.2	14.1	9.2	6.7	4.8	6.0	5.5	4.4	2.2	F
LJAMM-CNP 10579	53	20	?	10.4	13.1	13.4	9.9	6.4	4.1	5.3	5.3	4.1	2.0	F
LJAMM-CNP 10584	59	23	9	11.4	14.7	16.1	10.4	7.8	5.0	6.6	6.5	5.1	2.3	M
LJAMM-CNP 13948	44	19	9	8.9	12.8	10.7	8.2	5.5	3.2	4.3	4.4	4.1	1.7	M
LJAMM-CNP 14551	51	24	9	9.9	12.8	12.0	10.0	?	3.9	5.0	4.7	4.8	1.5	M
MNHNP 2821	61	28	12	9.9	14.6	14.4	11	7.7	4.5	5.9	5.3	4.8	2.4	F
MNHNP 9037	65	28	12	11.3	15	17.3	12.4	8.6	5.8	6.8	6.0	5.2	2.5	F
MNHNP 9038	51	23	9	9.8	13.1	13.1	10.2	7.1	4.3	5.1	4.9	4.4	2	M
MNHNP 9131	41	20	9	8.4	11.8	11.4	9.5	6.4	3.9	3.6	4.4	4.2	1.4	M
MNHNP 11406	47	18	7	8.3	11.5	11.4	8.8	6.3	3.7	5	3.6	4.1	1.8	?

Specimen	SVL	TrL	FL	TL	AL	HL	HW	HH	END	ESD	EMD	ID	IND	Sex
MNHNP 11410	39	15	8	7.2	10.2	10.7	8.1	5.8	3.7	4.4	3.6	3.7	1.7	M
MNHNP 11419	40	17	8	7.6	9.4	10.5	8.1	6.6	3.4	4.3	3.6	3.5	1.5	?
MNHNP 11421	45	17	9	8.1	11	10.9	9.2	6.7	3.7	5	3.9	3.8	1.7	M
MNHNP 11423	45	19	9	9.1	11.6	11.6	9	6.4	3.4	4.5	4.0	4	1.6	M
MNHNP 11850	53	25	9	9.8	12.2	13.3	10.6	7.9	4.7	5.9	4.5	5.1	2.5	M
MNHNP 11855	58	28	11	11.1	13.6	13.7	11.2	8.4	4.6	6.4	5.6	5.3	2.3	F
MNHNP 11860	51	23	10	9.4	13.1	12.9	9.7	6.8	4.9	5.8	4.6	4.6	1.9	F
MNHNP 11872	48	20	9	8.4	11.8	11.5	8.9	6.4	4.5	4.9	3.8	3.8	1.9	M
MNHNP 12238	60	26	11	10.8	14.1	14.8	13.3	7.9	4.6	6.6	5.1	5.5	2.5	F
SMF 29277	37	16	6	7.1	9.9	9.8	7.8	6.4	2.8	4.2	3.6	3.6	1.9	?
SMF 101984	53	24	9	9.8	13.2	13.4	10.4	6.7	4.1	5.2	4.6	4.6	1.9	?

Appendix S12

Matrix of meristic data

Raw meristic data used for analyses. See Materials and Methods section for explanation of the characters. Missing data indicated with "?". Argentinean populations belong to LJAMM-CNP collection, whereas Paraguayan specimens are housed in the MNHNP and SMF.

Specimen	DT	TVS	LVS	SL	IF	4TL	3FL	Sex
LJAMM-CNP 6520	30	43	19	9	7	19	16	F
LJAMM-CNP 6530	28	45	15	9	7	20	14	F
LJAMM-CNP 6532	33	48	16	7	6	18	13	M
LJAMM-CNP 6533	31	36	15	8	7	19	14	F
LJAMM-CNP 6535	34	44	18	8	7	21	17	M
LJAMM-CNP 6967	34	42	17	9	7	16	15	M
LJAMM-CNP 6968	30	42	18	8	8	17	15	M
LJAMM-CNP 7670	35	47	14	8	6	20	14	F
LJAMM-CNP 7674	35	40	13	8	6	20	14	F
LJAMM-CNP 7804	34	43	17	8	7	20	16	F
LJAMM-CNP 8713	32	48	17	8	6	20	16	M

Specimen	DT	TVS	LVS	SL	IF	4TL	3FL	Sex
LJAMM-CNP 10493	32	39	14	8	6	17	14	M
LJAMM-CNP 10496	33	39	16	8	6	18	15	F
LJAMM-CNP 10523	32	41	15	8	6	19	14	F
LJAMM-CNP 10526	34	39	13	8	6	21	16	F
LJAMM-CNP 10576	33	46	20	8	7	22	17	M
LJAMM-CNP 10577	34	42	16	7	7	20	16	F
LJAMM-CNP 10578	36	42	19	9	7	21	15	F
LJAMM-CNP 10579	37	42	15	8	7	?	16	F
LJAMM-CNP 10584	33	43	16	9	6	20	15	M
LJAMM-CNP 13948	29	42	14	8	6	18	14	M
LJAMM-CNP 14551	36	45	16	9	8	21	14	M
MNHNP 2821	29	42	17	8	6	20	14	F
MNHNP 9037	34	42	16	7	6	19	15	F
MNHNP 9038	31	40	12	7	6	17	12	M
MNHNP 9131	29	40	17	8	6	17	13	M
MNHNP 11406	32	?	?	8	6	19	13	?

Specimen	DT	TVS	LVS	SL	IF	4TL	3FL	Sex
MNHNP 11410	28	?	?	9	6	?	13	M
MNHNP 11419	29	43	15	8	6	18	11	?
MNHNP 11421	28	41	15	9	7	17	13	M
MNHNP 11423	32	41	19	8	6	19	14	M
MNHNP 11850	31	39	18	8	6	18	12	M
MNHNP 11855	28	43	17	8	6	16	12	F
MNHNP 11860	31	37	19	6	7	18	12	F
MNHNP 11872	32	47	16	8	7	18	13	M
MNHNP 12238	32	42	19	9	6	17	13	F
SMF 29277	34	42	15	8	6	17	11	?
SMF 101984	33	39	17	7	7	20	13	?

APPENDIX V

Declaration on the contributions of authors
to the publication: Description of a new species of *Homonota* (Reptilia, Squamata, Phyllodactylidae) from the central region of northern Paraguay.
Status: Accepted (2018).
Name of the journal: Zoosystematics and Evolution.
Authors involved: Pier Cacciali (PC), Mariana Morando (MM), Luciano J. Avila (LJA), Gunther Köhler (GK).

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 25%
Coauthor MM: 25%
Coauthor LJA: 25%
Coauthor GK: 25%

(2) to the implementation of the respective studies and experiments

PhD candidate: 70% – molecular analyses, morphological analyses, revision of museum vouchers.
Coauthor MM: 5% – molecular analyses.
Coauthor LJA: 5% – morphological analysis.
Coauthor GK: 20% – field work (collecting and documenting specimens).

(3) to the creation of the data collection and figures

PhD candidate: 80% – created database, sequenced DNA barcodes, provided photographs, created figures, created maps.
Coauthor MM: 5% – sequenced DNA barcodes.
Coauthor LJA: 5% – created databases.
Coauthor GK: 10% – provided photographs.

(4) to the analysis and interpretation of the data

PhD candidate: 65% – analysis and interpretation of molecular, morphological, and biogeographical data.
Coauthor MM: 10% – contributed to data analysis and interpretation.
Coauthor LJA: 10% – contributed to data analysis and interpretation.
Coauthor GK: 15% – contributed to data analysis and interpretation.

(5) to writing the manuscript

PhD candidate: 75%
Coauthor MM: 5%
Coauthor LJA: 5%
Coauthor GK: 15%

Date/place: _____

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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PENSOFT museum für naturkunde

Description of a new species of *Homonota* (Reptilia, Squamata, Phyllodactylidae) from the central region of northern Paraguay

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Abstract

Homonota is a gecko distributed in central and southern South America with 12 species allocated in three groups. In this work, we performed molecular and morphological analyses of samples of *Homonota* from the central region of northern Paraguay, comparing the data with those of related species of the group: *H. horrida* and *H. septentrionalis*. We found strong molecular evidence (based on 16S, Cyt-b, and PRLR gene sequences) to distinguish this lineage as a new species. Morphological statistical analysis showed that females of the three species are different in metric characters (SVL and TL as the most contributing variables), whereas males are less differentiated. No robust differences were found in meristic characters. The most remarkable trait for the diagnosis of the new species is the presence of well-developed keeled tubercles on the sides of the neck, and lack of a white band (crescent-shaped) in the occipital area, which is present in *H. horrida* and *H. septentrionalis*. Nevertheless, in our sample, we found three specimens (one juvenile and two young adults) that exhibit the white occipital band. Thus, this character seems only reliable in adults of the new species. The new species is parapatric to *H. septentrionalis*, both inhabiting the Dry Chaco of Paraguay.

Introduction

Homonota is a gecko, inhabiting mainly xeric and rocky areas in central and southern South America (Cei 1993, Avila et al. 2012), with *Homonota darwinii* reaching the most austral distribution of the genus at 54° latitude south (Morando et al. 2014). Most of the species in the genus are nocturnal, although *H. uruguayensis* can be either diurnal or nocturnal (Carreira et al. 2005). *Homonota horrida* (Burmeister 1861), distributed in Argentina and Paraguay, was the second described species of the genus, after the controversial *H. fasciata* (Duméril and Bibron 1836). This latter species was described from "Martinique", a Caribbean island located out of the distribution of the southern cone Neotropical genus. Both species were considered synonyms by Abdala and Lavilla (1993), which was followed by posterior researchers until recently when Cacciali et al. (2017) found that the type specimen of *H. fasciata* is distinct from the types of *H. horrida*, and recognized them as different taxa. Currently, both species are considered valid, although *H. fasciata* remains a *species inquirenda* because of the lack of information on its distribution and uncertainty in its diagnostic characters (Cacciali et al. 2017). The most recently described species of the genus was *H. septentrionalis* Cacciali, Morando, Medina, Köhler, Motte & Avila, 2017, which is present in the western part of the Dry Chaco (western Paraguay and southern Bolivia). Three groups are currently recognized: *whitii* group composed of *H. whitii* Boulenger, 1885, *H. darwinii* Boulenger, 1885, *H. andicola* Cei, 1978, and *H. williamsii* Avila, Pérez, Minoli & Morando, 2012; *borelli* group with *H. borellii*

(Peracca, 1897), *H. uruguayensis* (Vaz-Ferreira & Sierra de Soriano, 1961), *H. rupicola* Cacciali, Ávila & Bauer, 2007, and *H. taragui* Cájade, Etchepare, Falcione, Barrasso & Álvarez, 2013; and the *horrida* group (indicated as *fasciata* group by Morando et al. 2014) which contains *H. horrida* (Burmeister, 1861), *H. underwoodi* Kluge, 1964, and *H. septentrionalis* Cacciali, Morando, Medina, Köhler, Motte & Avila, 2017. Cacciali et al. (2017) suggested that more revisions are needed to understand the true taxonomic status of *H. fasciata* because currently it is not possible to know to which group it belongs and it is considered *incertae sedis*.

Four species of *Homonota* are recorded in Paraguay: *H. borellii*, *H. rupicola*, *H. horrida*, and *H. septentrionalis*. The most commonly known species was *Homonota horrida* recorded for the "Chaco" (Kluge 1964, Talbot 1978, 1979). Even after the synonymy of *H. horrida* with *H. fasciata* (Abdala and Lavilla 1993) the name *H. horrida* was still used in Paraguayan reports (Aquino et al. 1996, Ziegler et al. 2002). Many specimens of *H. septentrionalis* were referred to as *H. horrida* (those from the westernmost part of the Paraguayan Chaco) according to Cacciali et al. (2017). *Homonota rupicola* is an endemic species found in a rocky hill, east of the Paraguay River; and *H. borellii* was recorded from a few specimens from "Defensores del Chaco" and "Médanos del Chaco" National Parks (Cacciali et al. 2016). Thus, most of the species of *Homonota* from Paraguay are present in the Chaco, which is part of the "Dry Diagonal" formed by Caatinga, Cerrado, and Chaco, characterized by dry seasonal woodlands (Prado and Gibbs 1993). In Paraguay the Chaco is divided in two ecoregions: Humid Chaco and Dry Chaco, and most of the *Homonota* samples are located in the latter (Cacciali et al. 2016).

After the description of *H. septentrionalis*, the same authors continued to study and analyze the taxonomy of Paraguayan samples of *Homonota* from the Chaco, within the framework of a barcoding initiative of the herpetofauna from Paraguay. We performed genetic and morphological analyses among different populations of *Homonota* from the central region of northern Paraguay. Based on genetic and morphological differences, and applying a species delimitation algorithm, we found enough differences to consider these new samples as a different taxonomic unit from those previously recorded for Paraguay. We present here a detailed analysis along with the description of this new species.

Methods

We extracted DNA from three samples of *Homonota* from the central area of northern Paraguay (Occidental Region), which were compared with available sequences of the remaining members of the genus (except *H. fasciata*) to assess its taxonomic relationships in the gene tree. We sequenced fragments of mitochondrial genes rRNA 16S and Cytochrome b (Cytb) and the nuclear gene prolactine receptor (PRLR). Samples used and GenBank accession numbers are specified in Table 1. Samples of 16S were available only for the *horrida* group. To root the tree we included two outgroups (*Garthia gaudichaudii* and *Phyllopezus przewalskii* (Table 1) based on Morando et al. (2014).

Tissue samples were first washed for 15 h with 50 ml Phosphate-buffered saline (PBS) (diluted of 1:9 PBS: H_2O). The DNA extraction was carried out with the DNeasey kit of Qiagen. We used 25 µl of reaction mix for every sample for the PCR (except for PRLR where we used 20 µl). Reagents and concentrations for the PCR mix for the amplification of every gene, are provided in Suppl. material 1: Appendix S1. Primers (produced by Eurofins MWG Operon) used for amplification and sequencing, along with PCR conditions for each gene are detailed in Suppl. material 1: Appendix S2.

We used SeqTrace 0.9.0 (Stucky 2012) for examination of chromatograms and to generate the consensus sequences. We used MAFFT 7 (Katoh and Standley 2013) to automatically align the sequences through its webserver. For alignment of sequences of 16S, we included the Q-INS-i search strategy for corrections with the secondary structure of that gene (Katoh and Toh 2008). We used MSA Viewer (Yachdav et al. 2016) to visualize the alignments and export them to fasta format. We estimated the best substitution model for each gene (separately) with PartitionFinder2 (Lanfear et al. 2016) using the PhyML 3.0 algorithm (Guindon et al. 2010). We used the corrected Akaike Information Criterion (AICc) (Burnham and Anderson 2002) to select the best substitution model, but under the premise that it is not correct to use models that include both +G and +I (Sullivan et al. 1999, Mayrose et al. 2005). Then we chose the subsequent model in the best partition schemes when both were suggested by the AICc.

We performed two phylogenetic analyses, first using a Maximum Likelihood (ML) approach, and then a Bayesian inference (BI) to compare the trees topologies. These analyses were made for each gene individually and for a concatenated dataset of the three genes together. For the ML analysis we used IQ-Tree (Nguyen et al. 2015) through its webserver (Trifinopoulos et al. 2016) using 10,000 non parametric bootstrap replicates plus 10,000 replicates of Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT) (Anisimova et al. 2011) and 10,000 ultrafast bootstrap (UFBoot) approximation replicates (Minh et al. 2013). We converted the alignment to nexus format in the online server Alter (Glez-Peña et al. 2010) available at http://sing.ei.uvigo.es/ALTER/, to be used in MrBayes v3.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) for a BI. For this, we ran the analysis in independent duplicates, each with 1,000,000 generations for MCMC with a sampling frequency of 500 generations. We visualized the trees and exported them using FigTree v1.4.3 (available at http://tree.bio.ed.ac.uk/software/figtree/). We considered convergence when the standard deviation of split frequencies was 0.015 or less and when the Potential Scale Reduction Factor approached 1.0 (Gelman and Rubin 1992).

Table 1. Specimens used for genetic analyses and GenBank accession numbers for every gene. Asterisks (*) indicate tissue samples without voucher. Numbers in bold are samples generated for this work.

Species	Voucher	16S	Cytb	PRLR	GenSeq Nomenclature
Homonota andicola	LJAMM-CNP 12493	MD	KJ484188	KJ484274	genseq-3
	LJAMM-CNP 12495	MD	KJ484189	KJ484275	genseq-3
Homonota borellii	LJAMM-CNP 12116	MD	KJ484205	KJ484276	genseq-4
	LJAMM-CNP 12119	MD	KM677796	MD	genseq-4
	LJAMM-CNP 12125	MD	KJ484206	KJ484277	genseq-4
Homonota darwinii	LJAMM-CNP 9266	MD	KJ484191	MD	genseq-3
	LJAMM-CNP 9813	MD	MD	KJ484278	genseq-3
	LJAMM-CNP 11424	MD	KJ484190	MD	genseq-3
Homonota horrida	BYU 47941	MF278828	KJ484192	**MG950402**	genseq-3
	LJAMM-CNP 10493	MD	KM677795	MD	genseq-3
	LJAMM-CNP 10495	MF278829	MD	**MG950403**	genseq-3
	LJAMM-CNP 10576	MF278830	MD	**MG950404**	genseq-3
	LJAMM-CNP 10577	MD	KJ484208	MD	genseq-3
Homonota rupicola	MNHNP-1*	MD	KJ484193	KJ484281	genseq-3
	MNHNP-2*	MD	KJ484194	KJ484282	genseq-3
Homonota septentrionalis	MNHNP 11406	MD	MF278843	MF278849	genseq-2
	MNHNP 11409	MD	MF278844	MF278850	genseq-2
	MNHNP 11873	MF278831	MD	**MG950405**	genseq-3
	MNHNP 12238	MF278832	MD	MD	genseq-1
	SMF 101984	MF278833	MD	**MG950406**	genseq-2
Homonota taragui	LJAMM-CNP 14419	MD	KJ484195	KJ484283	genseq-3
	LJAMM-CNP 14420	MD	KJ484196	KJ484284	genseq-3
Homonota underwoodi	LJAMM-CNP 10923	MD	KJ484197	KJ484286	genseq-4
	LJAMM-CNP 10931	MD	KJ484198	KJ484297	genseq-4
Homonota uruguayensis	UFRGS 2139	MD	MD	KJ484296	genseq-4
	UFRGS 5769	MD	KM677689	MD	genseq-4
	UFRGS 5770	MD	KM677690	MD	genseq-4
	UFRGS 5771	MD	KM677691	MD	genseq-4
Homonota whitii	LJAMM-CNP 14387	MD	KJ484199	MD	genseq-4
	LJAMM-CNP 14388	MD	KJ484200	MD	genseq-4
Homonota williamsii	LJAMM-CNP 4467	MD	KJ484201	KJ484287	genseq-3
	LJAMM-CNP 6517	MD	KJ484202	KJ484288	genseq-2
Homonota sp. n.	SMF 101436	MD	**MG950409**	**MG950407**	genseq-2
	SMF 101438	**MG947388**	**MG950410**	**MG950408**	genseq-2
	SMF 101439	MD	**MG950411**	MD	genseq-2
Outgroups					
Garthia gaudichaudii	E61214	MD	FJ985045	MD	
	IBE_G1(1)	MD	MD	KJ484289	
Phyllopezus przewalskii	LG1093	JN935567	JQ826890	JQ825640	
	LJAMM-CNP 12089	MD	KJ484203	MF278849	

When frequencies did not converge we continued adding 500,000 generations until convergence was achieved.

We assessed the degree of intraspecific divergence within the alignment (removing the outgroups) with the species delimitation test ABGD (Puillandre et al. 2012) through its webserver (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html), using 10 steps of prior minimum and maximum simple genetic distance from 0.001 to 0.1 (default), and 0.5 of relative gap width, since higher (default) values tend to exceedingly split clades (Kekkonen et al. 2015, Yang et al. 2016). For this analysis we used only Cytb which was the mitochondrial gene better represented in our samples, and available for all the species within the genus. The last step using genetic data was the assessment of a species tree based on the clustering proposed by the species delimitation test. To do this we used *BEAST (Drummond et al. 2012) in BEAST 2.4.7 (Ogilvie et al. 2017) under 1,000,000 generations for the mcmc model, visualizing the posterior probability in DensiTree 2.2.6 (Bouckaert et al. 2014).

Additionally, we generated morphological data for 13 specimens (7 males and 6 females) of the new species and

taxa with similar pattern (related taxa of the *horrida* group) looking for potential diagnostic characters. Thus, we used for comparison *H. horrida* (7 males and 5 females) and *H. septentrionalis* (10 males and 12 females), using standard variables (continuous data expressed in mm) already used by Avila et al. (2012) and Cacciali et al. (2017):

SVL snout–vent length, from tip of snout to vent.
TrL trunk length, distance from axilla to groin from posterior edge of forelimb insertion to anterior edge of hind limb insertion.
FL foot length, from the tip of the claw of the 4th straightened toe to the back of the heel.
TL tibial length, measured between the level of the knee and the level of the heel, as shown by Köhler (2014).
AL arm length, from tip of claws of the 3rd finger to elbow.
HL head length, distance between anterior edge of auditory meatus and snout tip.
HW head width, taken at the level of the temporal region, corresponding to the widest part of the head.
HH head height, maximum height of head, at level of parietal area.
END eye–nostril distance, from the anterior edge of the eye to the posterior edge of the nostril.
ESD eye–snout distance, from the anterior edge of the eye to the tip of the snout.
EMD eye–meatus distance, from the posterior edge of the eye to the anterior border of the ear opening.
ID interorbital distance, shortest distance between orbits.
IND internostril distance, shortest distance between nares.
DT number of keeled dorsal tubercles from occipital area to cloaca level.
TVS number of transversal rows of ventral scales, counted longitudinally at midline from the chest (shoulder level) to inguinal level.
LVS number of longitudinal rows of ventral scales, counted transversally at midbody.
SL number of supralabial scales.
IL number of infralabial scales.
4TL number of lamellae under the fourth toe.
3FL number of lamellae under the third finger.

Measurements were taken with digital calipers (precision 0.01), but only the first decimal considered to limit discrepancies. For the morphological analyses only specimens of ~40 mm or larger were included. When paired structures exist, data are presented in left/right orientation, and only the left side was used for statistical analyses. In the color descriptions, the capitalized colors and the color codes (in parentheses) are those of Smithe (1981) for live animals and Köhler (2012) for preserved specimens.

We compared the morphological variation among species through a discriminant function analysis (DFA), testing the normality of the variables with a Shapiro-Wilk (*W*) test (Shapiro et al. 1968, Zar 1999), and for the DFA we only used variables that were normally distributed. We used PAST 3.14 (Hammer et al. 2001) to perform these tests. Meristic (discrete) and metric (continuous) data were analyzed separately. Examined specimens are detailed in Appendix 1. We present a table of localities of the specimens examined in Suppl. material 1: Appendix S3.

Results

The final alignments of 16S, Cytb, and PRLR consisted of 539, 793, and 457 bp, respectively. Alignments and trees are available at TreeBASE (ID: 22305). The best substitution model for 16S was GTR+G, for Cytb TVM+I(1stpos)|TIM+G(2ndpos)|SYM+G(3rdpos), and for PRLRK81(1stpos)|GTR+G(2nd+3rdpos). The complete table with scores is provided in Suppl. material 1: Appendix S4. The topology of the ML (Suppl. material 1: Fig. S1) and BI (Suppl. material 1: Fig. S2) trees using 16S coincide in recognizing three clusters, but the ML tree shows the new species as a sister clade to *H. septentrionalis*, whereas BI shows a trichotomy including *H. horrida*, *H. septentrionalis*, and the new species. Trees of ML and BI based on Cytb have the same topology (Suppl. material 1: Figs S3–S4), with strong support values. In these trees *H. andicola* and *H. whitii* are sister clades, as are *H. darwinii* and *H. williamsii*, and they are sister to the remaining *Homonota* species. The *borellii* group shows *H. uruguayensis* as sister to *H. borellii* + *H. rupicola* + *H. taragui*. Finally, within the *horrida* group, *H. underwoodi* appears as the sister to the remaining *Homonota* with banded coloration pattern. In this part of the tree *H. horrida* is rendered as sister to the clade *H. septentrionalis* plus the new taxon. The topologies of the ML and BI trees using PRLR are also the same (Suppl. material 1: Figs S5–S6). Species in the *whitii* group are clustered together, and the *borellii* group also shows monophyly but with a unresolved polytomy. In the *horrida* group *H. underwoodi* is also suggested as sister to the remaining species, with the new species and *H. septentrionalis* showing the most recent divergence. The trees using the concatenated dataset (with both ML and BI) show similar branch arrangement previously observed in trees of individual genes (Suppl. material 1: Figs S7–S8). Only two samples (UFRGS 2139 of *H. uruguayensis* and LJAMM-CNP 9813 of *H. darwinii*) are not allocated within their respective taxa, probably because some genes are lacking for some species.

The analysis of intraspecific genetic divergence with ABGD results in 12 groups (Suppl. material 1: Appendix S5), which represent nearly all described species (except *H. fasciata*) and the new species, providing evidence for its recognition as a distinct taxon. This is highly congruent with the clusters shown by the gene trees. The species tree shows consensus in the clusters of the three groups of the genus *Homonota*, with slight differences in the branch arrangements. The lower value (density of green lines in Figure 1) shows a trichotomy where the three groups are nested together, and with similar proba-

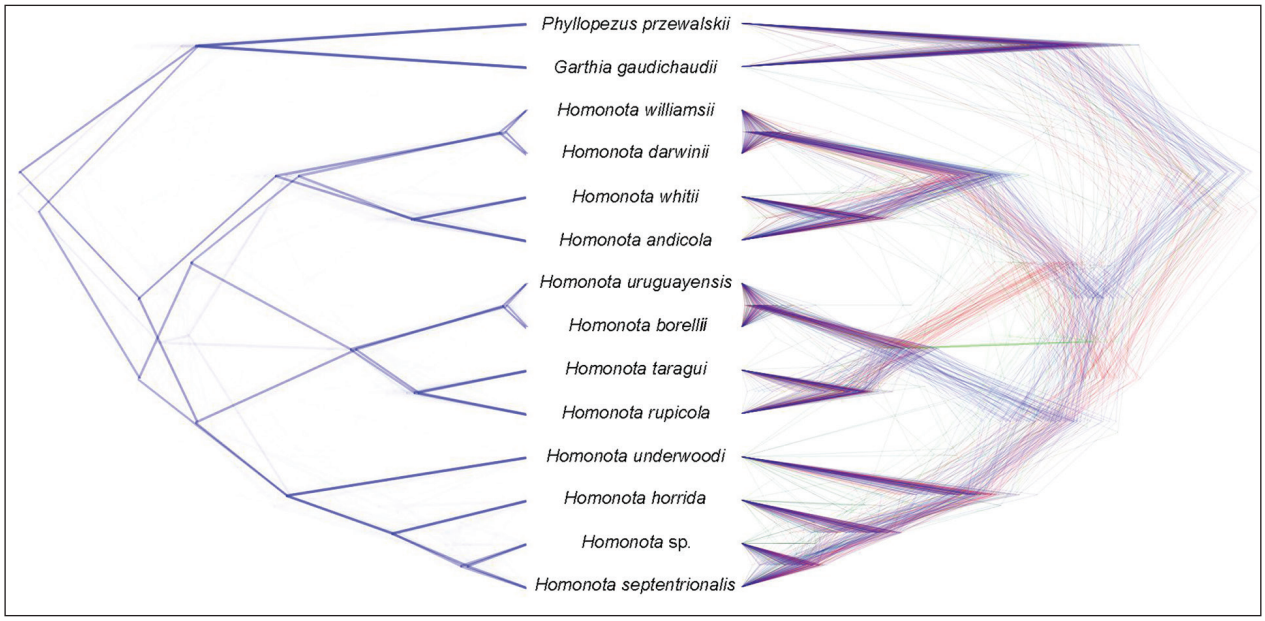

Figure 1. Species tree (left) and density of trees (right) for the species of the genus *Homonota*, based on the genes 16S, Cytb, and PRLR. The intensity in the color of the species tree is proportional to the probability.

Table 2. Normality Shapiro-Wilk (*W*) values for metric (above) and meristic (below) characters showing the *p* value. Values shaded in gray did not reach normality. See Methods section for reference to the acronyms.

	Continuous												
	SVL	TrL	FL	TL	AL	HL	HW	HH	END	ESD	EMD	ID	IND
W	0.978	0.979	0.957	0.987	0.982	0.979	0.976	0.983	0.979	0.967	0.969	0.975	0.952
p	0.503	0.506	0.087	0.849	0.696	0.575	0.401	0.758	0.555	0.199	0.284	0.400	0.050
	Discrete												
	DT	TVS	LVS	SL	IL	4TL	3FL						
W	0.962	0.971	0.965	0.779	0.788	0.913	0.948						
p	0.109	0.291	0.164	$3.05E^{-7}$	$4.65E^{-7}$	0.008	0.023						

bilities a species tree that clusters the *whitii* group as sister to the *borellii* group (density of red lines) and another where the *whitii* group is sister to the *borellii* group + *horrida* group (density of blue lines). Same as observed in the gene trees, *H. underwoodi* is presented as the sister clade to the remaining members of the group, and *H. horrida* sister to the new species of *Homonota* and *H. septentrionalis* with a rather deep divergence between these two taxa.

All continuous morphological variables had normal distributions (Table 2). The DFA for metric data showed that females of the three species are more differentiated than males (Fig. 2). The most contributing variables were SVL and TL for Axis 1, and SVL and TrL for Axis 2 and 3 (Table 3). Given the high eigenvalue of axes 1 and 2 (3.79 and 2.34 respectively, Fig. 2) suggests that the groups are significantly differentiated. For the meristic data, only DT, TVS, and LVS reached normality (Table 2), and DFA with these variables showed a high degree of overlapping without group discrimination and low eigenvalues (Fig. 3), and weak discrimination values (Table 3). Raw metric and meristic data are presented in Suppl. material 1: Tables S1 and S2.

Table 3. Most contributing continuous (Cont.) and discrete (Disc.) variables (highlighted in bold) for Axis 1–3 of the DFA.

	Variables	Axis 1	Axis 2	Axis 3
Cont.	SVL	**0,417**	**1,202**	**–3,447**
	TrL	–0,187	**0,690**	**–1,798**
	FL	0,132	0,401	–0,479
	TL	**0,228**	0,193	–0,417
	AL	0,201	0,213	–0,553
	HL	–0,017	0,357	–0,605
	HW	–0,128	0,218	–0,525
	HH	–0,199	0,187	–0,258
	END	–0,021	0,099	–0,193
	ESD	–0,052	0,111	–0,392
	EMD	0,144	0,134	–0,221
	ID	–0,028	–0,013	–0,302
	IND	0,002	0,127	–0,050
Disc.	DT	**1.424**	–1.232	**–1.338**
	TVS	**2.166**	**1.825**	0.921
	LVS	<0.001	**1.482**	**–1.255**

There is a strong molecular congruence in the recognition of 12 taxa within the genus *Homonota* (three of them with a banded coloration pattern), which added

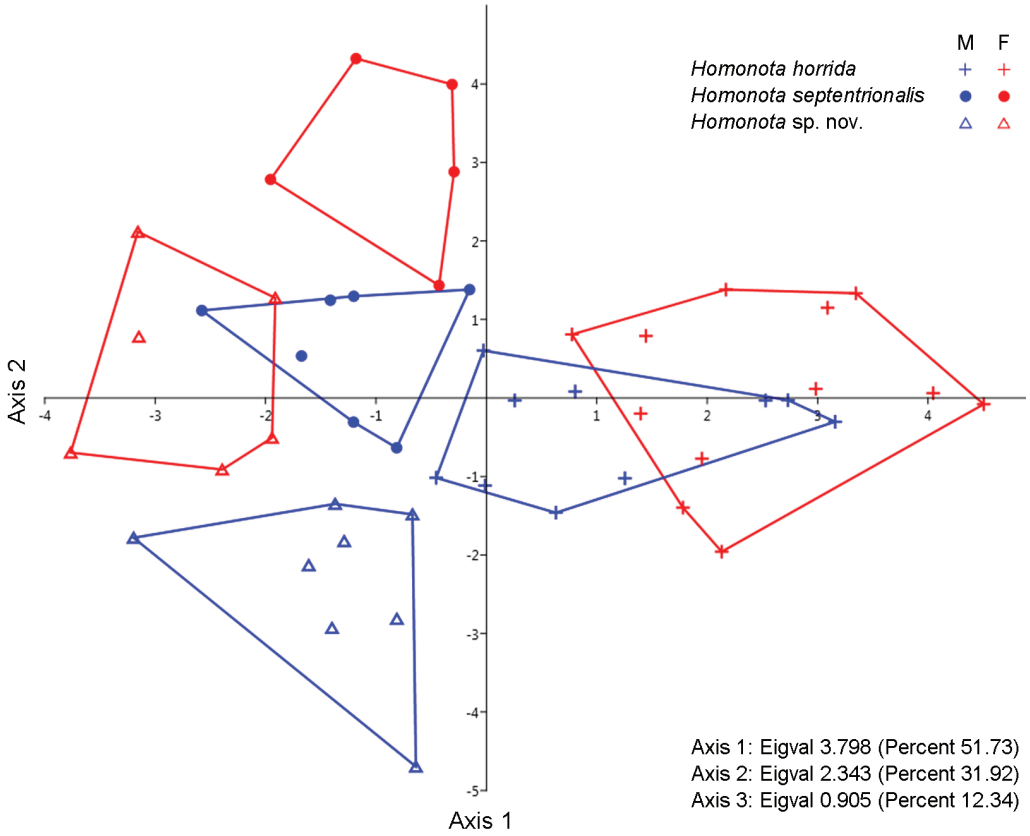

Figure 2. Discriminant Function Analysis scatter plot of individual scores for the three most informative axes for continuous variables of *Homonota horrida*, *H. septentrionalis*, and *Homonota* sp. n. Eigval: Eigenvalues. M: males. F: females.

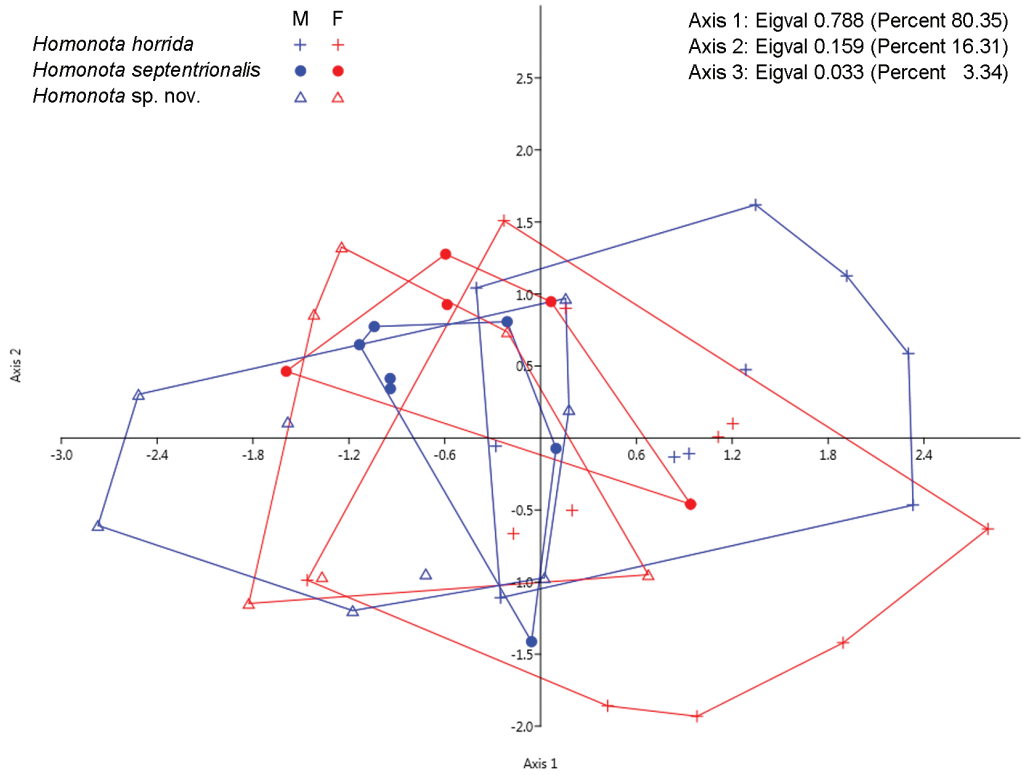

Figure 3. Discriminant Function Analysis scatter plot of individual scores for the three most informative axes for discrete variables of *Homonota horrida*, *H. septentrionalis*, and *Homonota* sp. n. Eigval: Eigenvalues. M: males. F: females.

to the significant differences among the three species with banded pattern based on the DFA, and the additional morphological distinctions discussed below are used to identify the new taxon described here.

Homonota marthae sp. n.

http://zoobank.org/FAB96653-46FF-4291-A23B-0FDB390AC54D

Holotype. SMF 101441 (field number GK-3783) (Fig. 4), adult female, collected on February 17th 2012 by Gunther Köhler in Dry Chaco, near the main house of Estancia Amistad (22.406°S, 60.728°W, elevation ca. 190 masl), Boquerón Department, Paraguay (Fig. 5).

Paratypes. Paraguay: Boquerón Department: Comunidad Ayoreo Jesudi (MNHNP 10744); Comunidad Ayoreo Tunucojai (MNHNP 10534); Estancia Amistad (SMF 101437); Estancia Jabalí (MNHNP 7832); Filadelfia (MNHNP 2795, 2798, 2810, 11790, 11791, 11793, SMF 101436, 101438–40, 101442); 31.5 km S Filadelfia (MNHNP 9726).

Diagnosis. A species of *Homonota* assigned to the *horrida* group given its relationship (based on molecular evidence) with *H. horrida*, and by the color pattern composed of a vertebral and five to seven transversal clear lines appearing as a banded *Homonota* smilar to *H. horrida* and *H. septentrionalis*. *Homonota marthae* has a robust body, and prominently keeled tubercles disposed in four to eight longitudinal rows on the dorsum.

Homonota marthae can be differentiated from all species in the genus, except *H. fasciata*, *H. horrida*, *H. darwinii*, and *H. septentrionalis* by the color pattern of transversal bands on the dorsum (reticulated pattern in the remaining species). *Homonota marthae* is further differ-

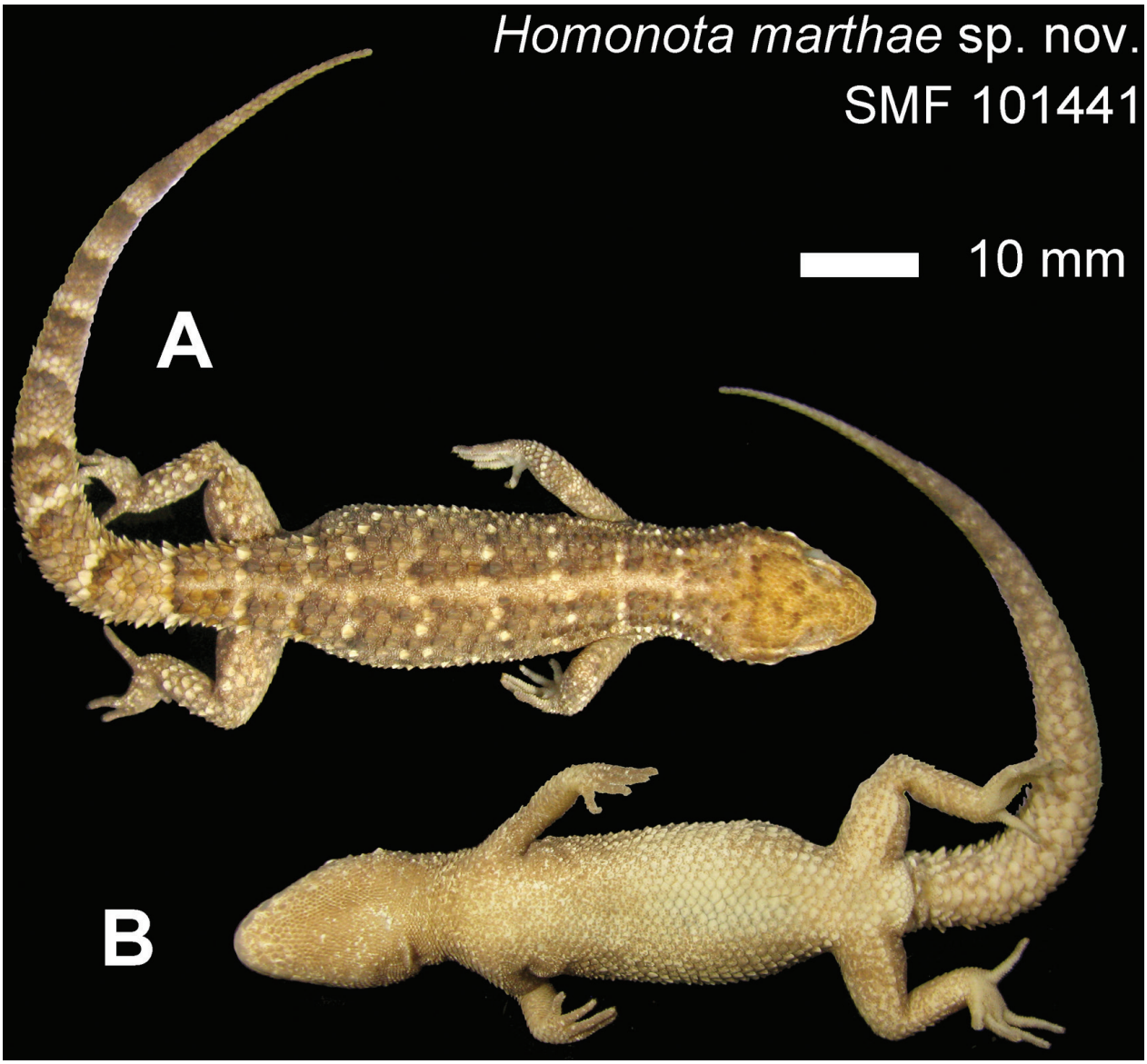

Figure 4. Dorsal (**A**) and ventral (**B**) views of the holotype of *Homonota marthae*.

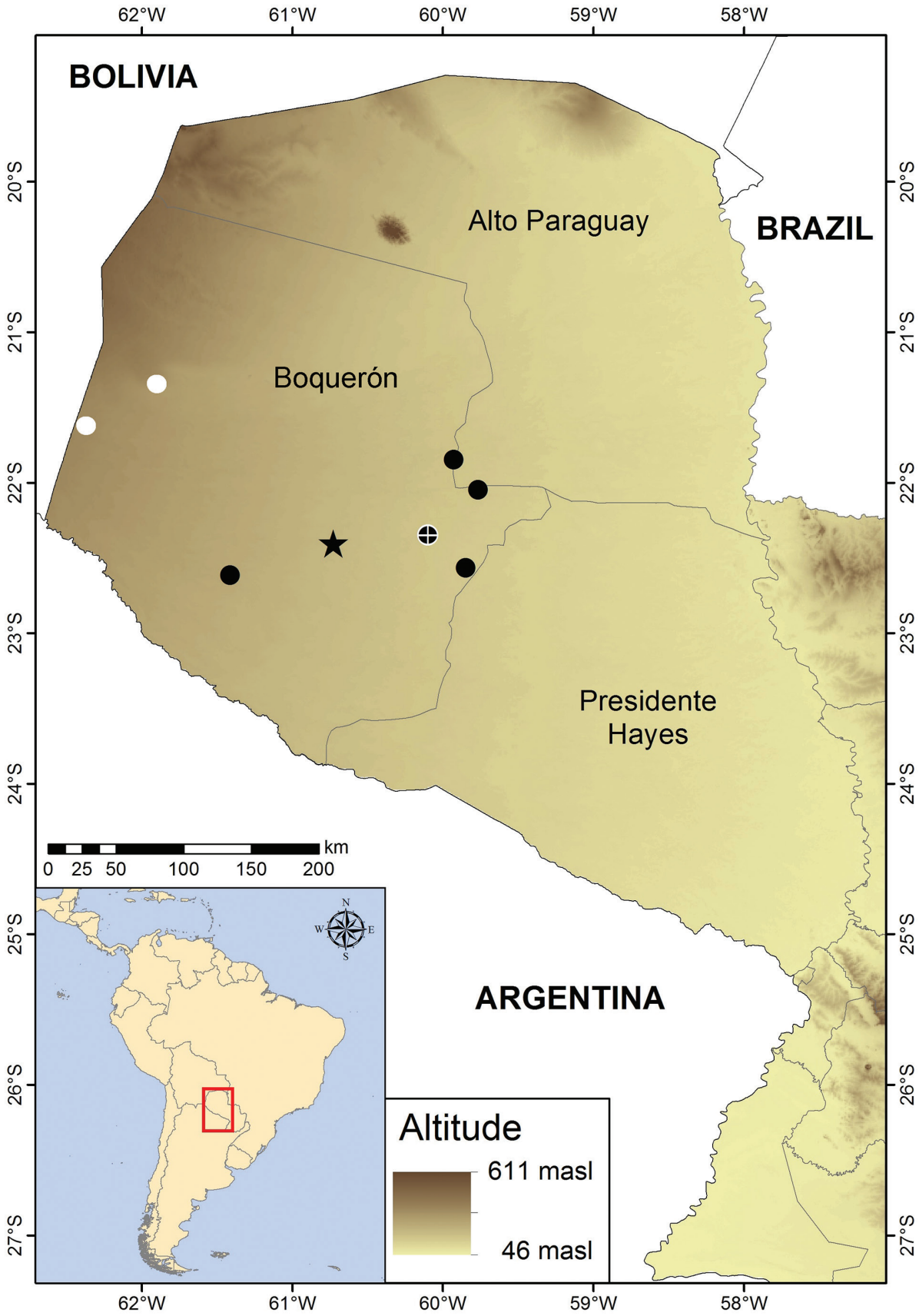

Figure 5. Occidental Region of Paraguay, indicating the political division, showing the known records for *Homonota septentrionalis* (white circles) and the analyzed records of *Homonota marthae* (black circles), and its type locality (star). Circle with a white cross, indicates origin of the genetic samples. High resolution elevation base map (30 seconds resolution) taken from Consortium for Spatial Information (CGIAR-CSI) available on http://www.diva-gis.org/gdata (Jarvis et al. 2008).

entiated from *H. andicola*, *H. whitii*, *H. darwinii*, and *H. underwoodi* by the keeled scales along the whole dorsum (vs. smooth dorsal scales in *H. andicola*, *H. whitii*, and *H. underwoodi*, and keeled scales restricted to the posterior part of the dorsum in *H. darwinii*). It differs from *H. fasciata* by having a serrated edge of the auditory meatus (vs. smooth anterior margin in *H. fasciata*); presence of one or two enlarged tubercles on the upper edge of the auditory meatus (vs. no enlarged tubercles in *H. fasciata*); and a smaller size of the postmental scales (vs. postmentals of the size of the first infralabials in *H. fasciata*). *Homonota marthae* differs from *H. horrida* by the higher position of the ear opening in relation to the level of the mouth (vs. lower positioned in *H. horrida*); from *H. septentrionalis* by more developed keeled tubercles on the sides of the neck (Fig. 6) (vs. less developed tubercles in *H. septentrionalis*). Finally, adults of *H. marthae* differ from these both species by the lack of a white band (usually crescent-shaped) on the occipital area (vs. white occipital crescent-shaped band present in *H. horrida* and *H. septentrionalis*) (Fig. 7). An artificial key for identification of the species of the genus is presented at the end of the work.

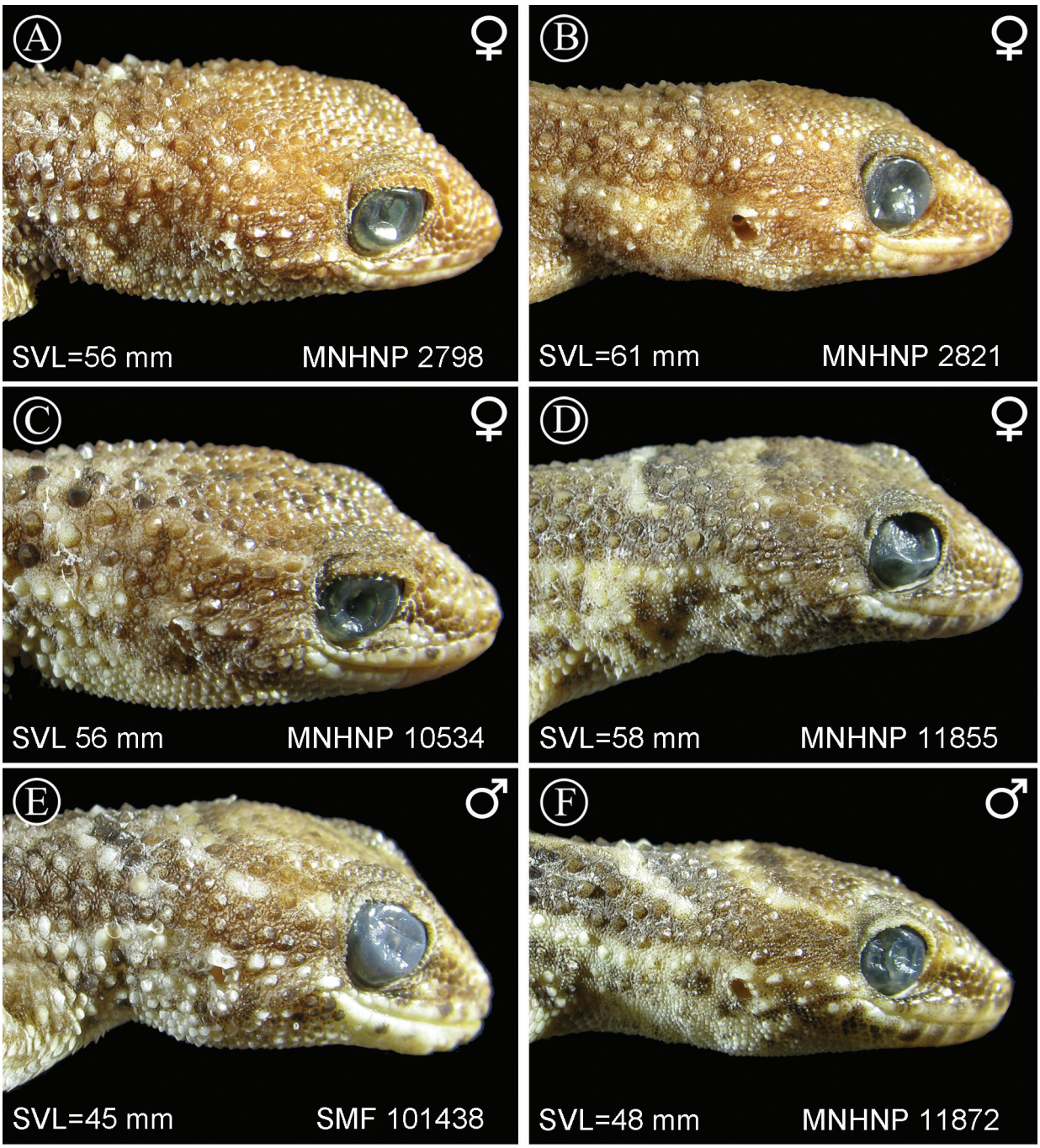

Figure 6. Plate showing the difference in scalation among individuals of similar sizes and same gender, of *Homonota marthae* (**A**, **C**, **E**) and *H. septentrionalis* (**B**, **D**, **F**). Note the more developed keeled tubercles on the sides of the neck in the former species.

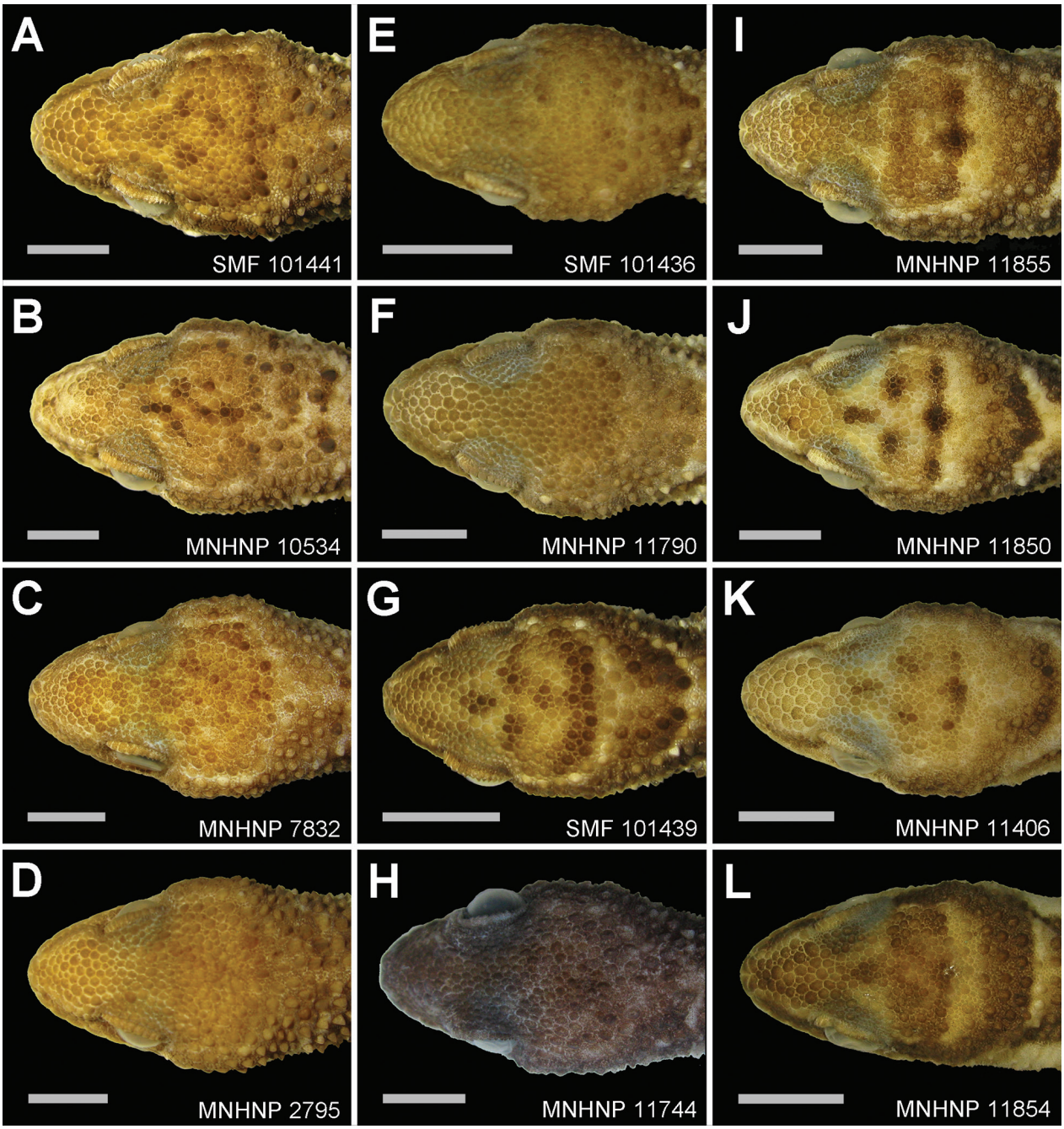

Figure 7. Variation in color patterns of *Homonota marthae* (**A**–**H**). The lack of the white occipital crescent-shaped line (present in *H. septentrionalis*, **I**–**L**) is evident in most of the specimens. Scale bars: 5 mm.

Description of the holotype. Adult female, SVL 56 mm (4.1 times the HL), TrL 26 mm, tail length 70 mm, FL 11.0 mm, TL 9.6 mm, AL 13.3 mm, HL 13.6 mm, HW 10.8 mm, HH 8.3 mm, END 4.2 mm, ESD 5.9 mm, EMD 4.7 mm, ID 4.6 mm, IND 2.0 mm; rostral wider (2.7 mm) than high (1.5 mm) with a median groove covering the upper two thirds of the scale; nares surrounded by rostral, supranasal, and postnasal; SL 8/8; one elongated tubercular scale on the mouth commissure; muzzle slightly convex, covered by large homogeneous juxtaposed scales; head covered with big homogeneous juxtaposed scales on the dorsal area, intermixed with small granules; supraciliary scales imbricated, associated to spiny-like scales on the posterior half of the orbit; scales on lateral surface of the head heterogeneously covered with strongly conical tubercles intermixed with small granules; auditory meatus oblique and with serrated edge, and one large elongated scale on the upper border; IL 6/6, the last less than half the size of the others; mental bell-shaped; two postmentals less than twice the size of the following posterior scales, contacting the mental, the first IL, and four posterior scales; scales under the head gradually reducing in size posteriorly; dorsal and lateral parts of the neck with granular juxtaposed scales mixed with tubercles;

ventral side of the head covered by imbricate cycloid scales; body dorsally covered with 14–16 rows of strongly keeled scales, separated by one to two small granules in the pleural areas, and three to four granules in the vertebral area; ventral scales cycloid and imbricate arranged in 16 longitudinal rows at midbody; suprascapular, axillary, inguinal regions, and cloacal opening surrounded by small imbricate granules; anterior and dorsal surfaces of limbs covered by large imbricate scales, keeled on the dorsal surface; posterior region of limbs covered by small juxtaposed granules; ventral surface of forelimbs with juxtaposed granules, and ventral surface of hind limbs with large imbricate scales; subdigital lamellae of hands starting from pollex were recorded as follows: 8/8 – 10/12 – 15/13 – 16/16 – 11/11; subdigital lamellae of feet starting from hallux were recorded as follow: 15/13 – 19/17 – 15/16 – 12/12 – 9/9; tail with large imbricated and mucronate scales, 10–12 per caudal whorl.

Coloration of the holotype (in preservative). After five years in preservative, the coloration was recorded as follows: Head Mikado Brown (42) with Warm Sepia (40) speckling on the dorsal surface; Warm Sepia (40) on the sides, with a Light Buff (2) line from nares to orbit, and continuing behind the orbit above the temporal region; supralabials and infralabials Medium Neutral Gray (298) with suffusions of Smoky White (261); and Fawn Color (258) ventrally. Dorsal background color of the body Beige (254) with Vandyke Brown (282) splotches, and poorly defined Chamois (84) transversal lines; Drab (19) laterally, with Dusky Brown (285) and Pale Buff (1) splotches; and Ground Cinnamon (270) ventrally, with Smoky White (261) suffusions. Tail with Grayish Horn Color (268), Sepia (286), and Cream White (52) transversal bands dorsally; Drab (19) laterally; and Smoky White (261) ventrally. Limbs dorsally covered with a reticulation of Drab (19), Chamois (84), and Dusky Brown (285), ventrally grading to Fawn Color (258) in forelimbs, and Ground Cinnamon (270) with suffusions of Smoky White (261) in hind limbs.

Coloration in life. Coloration in life of a young male (SMF 101438) was recorded as follows: Dorsum Mars Brown (223A) with a Tawny Olive (223D) vertebral stripe and transverse lines; dorsum of head Tawny Olive (223D) with a Verona Brown (223B) nuchal band that contains a central Tawny Olive (223D) line; iris Clay Color (123B) with a suffusion of Verona Brown (223B) centrally; dorsal surface of limbs Beige (219D) with Sepia (219) spots; ventral surfaces of head, body and limbs dirty white; dorsal surface of (regenerated) tail light Drab (119C) with scattered Sepia (119) spots; ventral surface of tail Light Drab (119C) with a suffusion of Sepia (119) medially.

Coloration in life of a juvenile female (SMF 101436) was recorded as follows: Dorsal ground color Raw Umber (123) with Raw Umber (223) transverse lines, edged with Pale Horn Color (92) posteriorly. Postocular stripe Ground Cinnamon (239); iris Yellow Ocher (123C) with a suffusion of Dark Drab (119B); dorsal surface of tail Cinnamon Drab (219C) with Sepia (119) bands, borders posteriorly by Chamois (123C); ventral surface of head, body and limbs dirty white, palmar and plantar surfaces Light Drab (119C); anterior portion of ventral tail Beige (219D), with Sepia (119) band on distal portion.

Color variation. One juvenile (SMF 101439, 36 mm SVL) and two young adults (MNHNP 11793, 45 mm SVL; SMF 101438, 45 mm SVL) out of the 17 examined specimens of *Homonota marthae* have a trace of white crescent-shaped band on the occipital area (more visible in the SMF 101439, Fig. 7G), typical of *H. horrida* and *H. septentrionalis*. Nevertheless, many juveniles (such as SMF 101436) show the same coloration as adults (Fig. 7). The specimen MNHNP 7832 has a narrow occipital white band, joined to the postocular lines (Fig. 7C). Some specimens have a darkish coloration (MNHNP 2810, 10744, 11791, 11793) dorsally, and ventrally most of the specimens have a clearer color than the holotype, except for MNHNP 2798, 2810, and 10744. In some specimens (MNHNP 2795, 2798, 2810, 10744) the dorsal color is diffused and the transversal bands are little visible.

Morphological variation. SVL 36–59 mm; TrL 16–27 mm (43.8–48.2% of SVL in females, 40.7–46.7% in males); FL 9–11 mm ($\bar{x}$ 10±0.36) in females, 7–11 mm ($\bar{x}$ 8.7±0.52) in males; TL 8.7–10.1 mm ($\bar{x}$ 9.5±0.2) in females, 8–10.2 mm ($\bar{x}$ 9.1±0.31) in males; AL 9.3–13.7 mm ($\bar{x}$ 12.8±0.28) in females, 11.2–14 mm ($\bar{x}$ 12.4±0.38) in males; HL 9.3–13.8 mm ($\bar{x}$ 13.2±0.19) in females, 11.1–13.5 mm ($\bar{x}$ 12.2±0.31) in males; HW 7.1–11.2 mm (79.4–88% of HL in females, 78.9–85.9% in males); HH 5.5–8.3 mm (52.6–61% of HL in females, 52.6–60.5% in males); END 2.8–5.1 mm (30.8–35.1% of HL in females, 31.4–38.9% in males); ESD 3.9–6.1 mm (40.3–45% of HL in females, 40.5–46.5% in males); EMD 3.1–5 mm (31.3–34.7% of HL in females, 33.8–37% in males); ID 3.8–5.8 mm (33.8–40% of HL in females, 37.1–44.7% in males); IND 1.4–2.1 mm (12.2–16% of HL in females, 11.8–14% in males); SL 5–8; one or two elongated tubercular scales on the mouth commissure; auditory meatus with one large scale on the upper border; IL 5–7; 14–20 longitudinal rows of ventral scales at mid-body; 34–49 transversal rows of ventral scales.

Etymology. This species is named in honor of our indefatigable colleague Martha Motte, who is not only dedicated to safekeeping the herpetological collection of the "Museo Nacional de Historia Natural del Paraguay", but also does a great job in providing selfless support to scientists that are striving to improve the knowledge of the Paraguayan herpetofauna.

Habitat and distribution. *Homonota marthae* is known from the central area of the Paraguayan Dry Chaco in the Department of Boquerón (Fig. 5). The environment is a xeric forest with abundance of thorny vegetation and

almost absence of a herbaceous stratum. Nevertheless, a more detailed analysis of museum collections is advisable for a better knowledge of the distribution of this species.

This species is a dry forest inhabitant, but it is also frequently found in human dwellings. Talbot (1978) recorded the use of logs of Drunken tree (*Chorisia speciosa*: Malvaceae) as shelter by *Homonota* in the Dry Chaco, since the wood of this tree keeps high water levels. Additionally, Cacciali et al. (2007a) demonstrated the use of subterranean caves (usually armadillo burrows) by *Homonota* in several areas of the Paraguayan Chaco.

Discussion

The diversity of species groups within the genus *Homonota* was explored in the last decade, and resulted in the description of *H. williamsii* (Avila et al. 2012) of the *whitii* group, and *H. rupicola* (Cacciali et al. 2007b) and *H. taragui* (Cajade et al. 2013) of the *borellii* group. However, the taxonomy of the *horrida* group (referred to as *fasciata* in Morando et al. 2014) was untouched for many years, and was comprised of two species (*H. horrida* and *H. underwoodi*). Recently, with the description of *H. septentrionalis* by Cacciali et al. (2017), and adding *H. marthae* described herein, the diversity of the *horrida* group currently includes four species. Morando et al. (2014) and Cacciali et al. (2017) presented species trees where the *whitii* group is sister to *horrida* and *borellii* groups. Our deep cluster arrangement is not completely resolved probably due to the use of fewer genes. Nevertheless, there is a strong consensus in the topology of the *horrida* group, where *H. underwoodi* appears as the sister of the remaining taxa (Fig. 1).

No obvious external synapomorphy is known to diagnose the *horrida* group. Three of the four species (*H. horrida*, *H. septentrionalis*, and *H. marthae*) have a pattern characterized by transversal body bands and the presence of a vertebral line. This coloration is different from the remaining species of the genus. The fourth species of the *horrida* group, *H. underwoodi*, has homogeneous body scalation and a completely different pattern, and therefore *H. horrida* and *H. underwoodi* were considered not to be in the same group (Kluge 1964).

The most obvious external difference between *H. marthae* and its presumed closest relative, *H. septentrionalis*, is the lack of a white occipital band in the former taxon, although we found some specimens (mainly juveniles or hatchlings) of *H. marthae* that do have the occipital band. Given that this white occipital band is also present in *H. horrida*, it could be a plesiomorphic character, and therefore the lack of it could be interpreted as the derived state.

Both species seem to inhabit in parapatry the Dry Chaco in Paraguay, and although a major revision of the whole distribution of the group is needed in order to know their actual ranges, *H. septentrionalis* is distributed in the north-westernmost part of the Dry Chaco, whereas *H. marthae* occurs in the central and easternmost areas of the Dry Chaco. Due to the lack of evident geographic barriers between these two species and considering their relatively low morphological variation (especially in males), they remained recognized as a single taxonomic unit until now. Parapatric speciation or breaks to gene flow without evident geographic barriers were observed and discussed by Irwin (2002), and also documented for other geckos in South America's Dry Diagonal, where Werneck et al. (2012) documented a high diversity in sympatric clades of *Phyllopezus* in Caatinga and Cerrado.

The degree of genetic differentiation between these two species is evident, and larger than the degree of morphological differences. Small morphological differentiation or even complete crypsis is common for many organisms, especially when they use the same ecological niche. Specifically for geckos, a recent study showed that it is difficult to find morphological diagnostic characters that match those observed by genetic evidence, as it is the case of the genera *Garthia* and *Homonota*, which are very similar morphologically (Daza et al. 2017). This is in agreement with previous studies that found that molecular genetic tools provided additional evidence for the interpretation of gecko's systematics in the Neotropics (Gamble et al. 2011, Gamble et al. 2012, Morando et al. 2014). The evolutionary processes that led to the molecular differentiation between *H. septentrionalis* and *H. marthae* remain unknown.

Finally, *Homonota marthae* is a common species that resists human perturbation and can be found in rural environments, and although its actual distribution limits are not yet known, and more revisions are needed to target this issue, probably the records of "*Homonota fasciata*" from Defensores del Chaco National Park referred by Cacciali et al. (2016) belong to *H. marthae*, and one of the records of *H. marthae* in Comunidad Ayoreo Tunucojai lays at ~70 km W from Yaguareté Porã Natural Reserve. Thus, we consider that *H. marthae* is not under extinction risk.

Key for identification of the species of the genus *Homonota*

Information to generate the key was based on Cacciali et al. (2007b), Avila et al. (2012), and Cájade et al. (2013). Given that the holotype of *H. fasciata* is completely bleeched, we consider the information on its coloration from the original color description (Duméril and Bibron 1836).

1 Coloration based on irregular or reticulated pattern 2
– Coloration pattern composed of transversal bands 10
2 Dorsal scales homogeneously smooth 3
– Dorsal scales smooth and granular mixed with series of enlarged keeled scales 5

3 Ventral surface of the body immaculate due to lack of chromatophores ..... *H. underwoodi*
– Ventral surface of the body pigmented with chromatophores 4
4 43–49 scales around midbody ..... *H. andicola*
– 55–59 scales around midbody ..... *H. whitii*
5 Series of keeled scales restricted to the posterior half of the dorsum ..... *H. darwinii*
– Series of keeled scales uniformly extended along the whole dorsum 6
6 Dorsal surface of thighs with keeled scales 7
– Dorsal surface of thighs with smooth scales 8
7 Dorsal surface of arms with keeled scales; temporal region with enlarged keeled scales ..... *H. uruguayensis*
– Dorsal surface of arms with smooth cycloid scales; temporal region homogeneously covered by granular scales ..... *H. taragui*
8 146–161 dorsal scales from occipital area to the level of the cloaca; oblique ear opening ..... *H. williamsii*
– 94–139 dorsal scales from occipital area to the level of the cloaca; round ear opening 9
9 45–50 scales around midbody; dorsal surface of the tail with a pattern of thin speckling ..... *H. borellii*
– 54–63 scales around midbody; dorsal surface of the tail with a pattern of black blotches ..... *H. rupicola*
10 Edge of ear opening smooth, without enlarged tubercular scales around; postmentals about five times larger than the scales behind it ..... *H. fasciata*
– Edge of ear opening serrated, with one or two tubercular scales above; postmentals twice larger than the scales behind it 11
11 Tubercles on the dorsal and lateral sides of the neck poorly developed; occipital area with a wide whitish crescent-shaped mark ..... *H. septentrionalis*
– Tubercles on the dorsal and lateral sides of the neck well developed; occipital coloration variable 12
12 Ear opening above the mouth level; occipital area with homogeneous coloration or with a faint reticulation in adults ..... *H. marthae*
– Ear opening at the level of the mouth; occipital area with a wide whitish crescent-shaped mark ..... *H. horrida*

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Appendix 1

Examined specimens

Homonota horrida

ARGENTINA: La Pampa: Ruta Provincial 1, 23.6 km W from intersection with Ruta Nacional 151 (LJAMM-CNP 10523, 10584); Ruta Provincial 27, 37.7 km S from intersection with Ruta Provincial 14 (LJAMM-CNP 10578–9). Mendoza: 1 km S Punta de Agua (LJAMM-CNP 10493, 10496, 10576–7). Neuquén: 41 km NW Punta Carranza (LJAMM-CNP 8713); 6 km SW Picun Leufu (LJAMM-CNP 13948); Ruta Provinicial 5, 10 km N from Ruta Provincial 7 (LJAMM-CNP 7804); Mina La Casualidad (LJAMM-CNP 14551); Villa El Chocón (LJAMM-CNP 6967–8). Río Negro: Avellaneda (LJAMM-CNP 7670, 7674); Villa Regina (LJAMM-CNP 6520, 6530, 6532–3, 6535).

Homonota septentrionalis

PARAGUAY: Boquerón: Cruce San Miguel (MNHNP 11850, 11855, 11860, 11872); Fortín Mayor Infante Rivarola (MNHNP 12238, SMF 101984); Parque Nacional Teniente Enciso (MNHNP 2821, 9037–8, 9131, 11410, 11421, 11423).

Acronyms

LJAMM-CNP: Colección de herpetología del Centro Nacional Patagónico.

MNHNP: Museo Nacional de Historia Natural del Paraguay.

SMF: Senckenberg Forschungsinstitut und Naturmuseum Frankfurt.

Supplementary material 1

Supplementary information

Authors: Pier Cacciali, Mariana Morando, Luciano J. Avila, Gunther Köhler

Data type: Adobe PDF file

Explanation note: Description of a new species of Homonota (Reptilia, Squamata, Phyllodactylidae) from the central region of northern Paraguay

Link: https://doi.org/10.3897/zse.21754.NN.suppl1

Description of a new species of *Homonota* (Reptilia, Squamata, Phyllodactylidae) from the central region of northern Paraguay

Pier Cacciali, Mariana Morando, Luciano J. Avila, Gunther Köhler

SUPPLEMENTARY INFORMATION

Appendix S1

PCR mix for amplification

Following we present the list of reagents and amounts for preparation of the master-mix for sequence amplification.

Amounts for 25µl of PCR reaction mix for 16S

Regeant	Concentration	Volume (µl)
Water		14
dNTPs	2.5 mM	4
Reaction Buffer Y	10×	2.5
$MgCl_2$	25 mM	1
Primer 1	10 µM	1
Primer 2	10 µM	1
TaqPolymerase	5 U/µl	0.5
DNA	25 ng/ µl	1

Amounts for 25µl of PCR reaction mix for Cytb

Regeant	Concentration	Volume (µl)
Water		11.8
dNTPs	2.5 mM	3.5
Reaction Buffer Y	10×	3.5
$MgCl_2$	25 mM	1.5
Primer 1	10 µM	1.5
Primer 2	10 µM	1.5
TaqPolymerase	5 U/µl	0.2
DNA	25 ng/ µl	1.5

Amounts for 20µl of PCR reaction mix for PRLR

Regeant	Concentration	Volume (µl)
Water		8
dNTPs	2.5 mM	3.2
Reaction Buffer Y	10×	2
$MgCl_2$	25 mM	1.4
Primer 1	10 µM	1.5
Primer 2	10 µM	1.5
TaqPolymerase	5 U/µl	0.4
DNA	25 ng/ µl	2

Appendix S2

Primers and PCR conditions

Following we detail the primers used for gene amplifications and the conditions for the thermocycler.

16S	L2510 (F) H3056 (R)	CGCCTGTTTAACAAAAACAT CGGTCTGAACTCAGATCACGT	Miya & Nishida 1996
Cytb	IguaF2 IguaR2	CCACCGTTGTTATTCAACTAC GGTTTACAAGACCAATGCTTT	Corl et al. 2010
PRLR	PRLR_f1 PRLR_r3	GACARYGARGACCAGCAACTRATGCC GACYTTGTGRACTTCYACRTAATCCAT	Townsend et al. 2008

Primers	PCR conditions					Reference
	Den	Den	Ann	Ext	Ext	
L2510 (F) H3056 (R)	94.0º 02:00	94.0º 00:35	48.5º 00:35	72.0º 01:00	72.0º 10:00	Lotzkat et al. 2013
		×40				
PRLR_f1 PRLR_r3	95.0º 01:30	95.0º 00:35	63.0º ↓ (*) 00:35	72.0º 02:00	72.0º 10:00	Noonan & Yoder 2009
		×10				
		95.0º 00:35	58.0º 00:35	72.0º 01:00		
		×10				
		94.0º 00:35	52.0º 00:35	72.0º 01:00		
		×15				
IguaF2 IguaR2	94.0º 04:00	94.0º 00:30	50.0º 00:35	72.0º 00:50	72.0º 07:00	Corl et al. 2010
		×25				

References

Corl et al. 2010. Evolution 64: 79–96.

Lotzkat et al. 2013. Zootaxa, 3626: 1–54.

Miya & Nishida 1996. Ichthyological Research, 43: 375–398.

Noonan & Yoder 2009 Molecular Ecology Resources, 9: 402–404.

Townsend et al. 2008. Molecular Phylogenetics and Evolution, 47: 129–42.

Appendix S3

Table of localities

List of localities included in the text, with geographic coordinates (DD format). Abbreviations: RP (Provincial Route), RN (National Route), PN (National Park).

Country	Division	Locality	Latitude	Longitude
Argentina	La Pampa	RP 1, 23.6 km W RN151	-37.075º	-67.785º
		RP 27, 37.7 km S RN 14	-36.668º	-68.022º
	Mendoza	1 km S Punta de Agua	-35.541º	-68.079º
	Neuquén	41 km NW Punta Carranza	-37.656º	-69.472º
		6 km SW Picun Leufu	-39.555º	-69.301º
		RP 5, 10 km N RP 7	-37.906º	-69.174º
		Mina La Casualidad	-37.904º	-68.488º
		Villa El Chocón	-39.261º	-68.779º
	Río Negro	Avellaneda	-39.145º	-66.145º
		Villa Regina	-39.101º	-67.091º
Paraguay	Boquerón	Comunidad Ayoreo Jesudi	-59.924º	-21.841º
		Comunidad Ayoreo Tunucojai	-59.772º	-22.050º
		Cruce San Miguel	-21.203º	-61.662º
		Estancia Amistad	-22.406º	-60.728º
		Estancia Jabalí	-61.424º	-22.611
		Filadelfia	-22.352º	-60.041º
		31.5 km S Filadelfia	-22.644º	-60.031
		Fortín Mayor Infante Rivarola	-21.679º	-62.401º
		PN Teniente Enciso	-21.209º	-61.655º

Appendix S4

Best substitution models

List of all the models considered by PartitionFinder2 and scores of AICc.

16S		Cytb						PRLR			
		1st Pos		2nd Pos		3rd Pos		1st Pos		2nd + 3rd Pos	
Model	AICc	Model	AICc	Model	AICc	Model	AICc	Model	AICc	Model	AICc
GTR+G	**2212.76**	TVM+I	**1469.37**	TIM+G	**6064.01**	SYM+G	**2538.22**	K81	**1184.44**	GTR+G	**1869.65**
GTR+I	2213.46	GTR+I	1471.43	TIM+I+G	6066.16	TVMEF+G	2538.25	K80	1184.97	TIM+G	1870.24
GTR	2213.55	TVM+I+G	1471.51	GTR+G	6068.27	GTR+G	2538.6	K81+I	1185.49	GTR+I	1871.76
GTR+I+G	2214.82	HKY+I	1471.99	GTR+I+G	6070.44	TVM+G	2539.41	K81+G	1185.8	GTR+I+G	1871.86
TVM+G	2215.11	HKY+I+G	1472.73	TRN+G	6072.19	TVMEF+I+G	2539.9	TIMEF	1185.84	SYM+G	1872.12
TVM	2215.65	GTR+I+G	1473.58	TRN+I+G	6073.92	SYM+I+G	2540.14	K80+I	1186.0	TVMEF+G	1872.19
TVM+I	2215.74	K81UF+I	1473.97	TIM	6075.59	GTR+I+G	2540.44	K80+G	1186.33	TIM+I+G	1872.35
TVM+I+G	2217.17	TRN+I	1474.05	K81UF+G	6077.49	TVM+I+G	2540.84	TRNEF	1186.34	TIM+I	1872.43
TIM+G	2218.12	K81UF+I+G	1474.6	TIM+I	6077.72	TIM+G	2540.86	K81UF	1186.98	TVM+G	1873.86
TIM+I	2218.75	TRN+I+G	1474.91	K81UF+I+G	6079.6	K81UF+G	2541.95	TIMEF+I	1187.06	SYM+I	1874.02
TRN+G	2219.28	TIM+I	1476.06	GTR	6079.74	TIMEF+G	2542.88	TIMEF+G	1187.31	TRN+G	1874.04
TRN+I	2219.85	TIM+I+G	1476.72	TVM+G	6081.2	TIM+I+G	2542.88	HKY	1187.49	TVMEF+I	1874.04
TIM	2219.89	TVM+G	1477.03	TRN	6081.69	K81+G	2543.2	TRNEF+I	1187.55	SYM+I+G	1874.28
TIM+I+G	2220.13	GTR+G	1479.17	GTR+I	6081.9	K81UF+I+G	2543.57	K81+I+G	1187.61	TVMEF+I+G	1874.34
K81UF+G	2220.32	HKY+G	1480.14	TVM+I+G	6083.37	TRN+G	2544.43	TRNEF+G	1187.81	TIMEF+G	1874.59
K81UF+I	2220.85	K81UF+G	1482.11	TRN+I	6083.78	TIMEF+I+G	2544.86	K80+I+G	1188.09	K81UF+G	1874.77
TRN+I+G	2221.26	TRN+G	1482.23	HKY+G	6084.6	K81+I+G	2544.95	TVMEF	1188.32	K81+G	1874.81
HKY+G	2221.28	TIM+G	1484.21	HKY+I+G	6086.71	HKY+G	2545.65	K81UF+I	1188.52	TVM+I	1875.84
HKY+I	2221.84	TRNEF+I+G	1506.38	K81UF	6091.5	TRN+I+G	2546.25	TIM	1188.67	TVM+I+G	1876.06
TRN	2221.88	SYM+I+G	1506.44	K81UF+I	6093.63	HKY+I+G	2546.96	K81UF+G	1188.79	TRN+I+G	1876.21
K81UF	2221.98	SYM+I	1506.96	TVM	6095.55	SYM+I	2547.66	HKY+I	1188.98	TRN+I	1876.23
K81UF+I+G	2222.33	TIMEF+I+G	1507.67	HKY	6097.28	TRNEF+G	2548.1	TRN	1189.15	GTR	1876.29
HKY+I+G	2223.26	TVMEF+I+G	1508.83	TVM+I	6097.7	TVMEF+I	2548.16	TIMEF+I+G	1189.22	TIMEF+I	1876.6
HKY	2223.89	TRNEF+I	1509.23	HKY+I	6099.38	K80+G	2548.52	HKY+G	1189.27	TIMEF+I+G	1876.65
SYM+G	2229.99	K80+I+G	1509.59	SYM+G	6517.49	GTR+I	2548.56	TVMEF+I	1189.49	TIM	1876.67
SYM	2230.24	TVMEF+I	1509.98	SYM+I+G	6519.62	TIM+I	2549.77	TRNEF+I+G	1189.68	K81+I	1876.73
SYM+I	2230.65	TIMEF+I	1510.45	TVMEF+G	6526.45	TRNEF+I+G	2549.91	SYM	1189.78	K81UF+I	1876.74
TVMEF+G	2231.1	K81+I+G	1510.92	TVMEF+I+G	6528.55	K80+I+G	2549.97	TVMEF+G	1189.8	K81UF+I+G	1876.9
TVMEF	2231.23	K80+I	1512.73	TIMEF+G	6541.13	TVM+I	2549.98	TIM+I	1190.38	K81+I+G	1876.96

16S		Cytb						PRLR			
		1st Pos		2nd Pos		3rd Pos		1st Pos		2nd + 3rd Pos	
Model	AICc	Model	AICc	Model	AICc	Model	AICc	Model	AICc	Model	AICc
TVMEF+I	2231.71	SYM+G	1513.35	TIMEF+I+G	6543.22	TIMEF+I	2551.19	TIM+G	1190.57	HKY+G	1878.67
SYM+I+G	2232.07	K81+I	1513.9	SYM+I	6544.96	K81UF+I	2551.93	K81UF+I+G	1190.73	TVMEF	1878.78
TVMEF+I+G	2233.18	TRNEF+G	1515.95	SYM	6548.26	K81+I	2552.45	TVM	1190.73	TRNEF+G	1878.88
TIMEF+G	2240.34	TVMEF+G	1516.93	K81+G	6548.36	TRN+I	2554.46	TRN+I	1190.81	SYM	1879.1
TIMEF+I	2241.06	TIMEF+G	1517.2	K81+I+G	6550.42	HKY+I	2556.7	TRN+G	1191.02	K80+G	1879.14
K81+G	2241.19	F81+I+G	1517.89	TVMEF+I	6553.21	TRNEF+I	2557.6	SYM+I	1191.13	TVM	1880.25
TIMEF	2241.21	F81+I	1519.47	TVMEF	6558.81	K80+I	2558.83	HKY+I+G	1191.17	TRN	1880.42
K81+I	2241.83	K80+G	1520.15	TRNEF+G	6564.37	JC+G	2638.64	SYM+G	1191.36	HKY+I	1880.64
TRNEF+G	2241.85	K81+G	1521.36	TIMEF+I	6566.35	JC+I+G	2640.53	TVMEF+I+G	1191.67	HKY+I+G	1880.75
K81	2242.07	F81+G	1526.44	TRNEF+I+G	6566.44	F81+G	2641.42	TVM+I	1192.45	K81UF	1880.77
TIMEF+I+G	2242.36	JC+I+G	1562.69	TIMEF	6568.68	F81+I+G	2643.34	GTR	1192.47	TRNEF+I	1880.85
TRNEF+I	2242.49	JC+I	1564.8	K81+I	6571.22	JC+I	2646.64	TIM+I+G	1192.6	TRNEF+I+G	1880.93
K80+G	2242.73	TVM	1570.35	K80+G	6571.36	F81+I	2649.46	TVM+G	1192.68	K81	1880.98
K81+I+G	2243.2	JC+G	1571.36	K80+I+G	6573.41	TVMEF	2654.35	TRN+I+G	1193.01	K80+I	1881.01
K80+I	2243.23	GTR	1572.45	K81	6575.21	SYM	2655.95	SYM+I+G	1193.34	K80+I+G	1881.18
TRNEF	2243.69	HKY	1574.2	TRNEF+I	6587.94	TVM	2664.82	GTR+I	1194.35	TIMEF	1881.19
TRNEF+I+G	2243.82	K81UF	1575.99	TRNEF	6590.54	GTR	2665.83	GTR+G	1194.5	HKY	1884.6
K80	2244.57	TRN	1576.26	K80+I	6592.79	K81	2672.8	TVM+I+G	1194.71	K80	1885.22
K80+I+G	2244.69	TIM	1578.06	K80	6597.1	TIMEF	2674.38	GTR+I+G	1196.62	TRNEF	1885.39
F81+G	2251.17	SYM	1601.48	F81+G	6874.54	K80	2678.52	JC	1205.04	F81+G	1918.24
F81+I	2251.73	TVMEF	1603.19	F81+I+G	6876.24	K81UF	2679.36	JC+I	1206.2	F81+I	1920.12
F81	2251.85	TRNEF	1606.23	F81+I	6892.42	TRNEF	2680.07	JC+G	1206.5	F81+I+G	1920.31
F81+I+G	2253.2	TIMEF	1607.66	F81	6880.59	TIM	2680.36	F81	1207.63	JC+G	1920.9
JC+G	2270.67	K80	1607.97	JC+G	6991.31	HKY	2684.82	JC+I+G	1208.27	JC+I	1922.72
JC	2271.2	K81	1609.38	JC+I+G	6993.34	TRN	2685.77	F81+I	1209.06	JC+I+G	1922.93
JC+I	2271.21	F81	1613.94	JC+I	7000.12	JC	2758.33	F81+G	1209.35	F81	1923.01
JC+I+G	2272.68	JC	1655.85	JC	7001.24	F81	2765.66	F81+I+G	1211.21	JC	1925.8

Appendix S5

Results of the Species Delimitation test with ABGD.

At the end, the group for the new species is highlighted in bold.

Results are shown for our dataset, according to the following parameters:

Pmin: 0.001 Pmax: 0.1 Steps: 10

X (relative gap width): 0.5

Nb bins (for distance distribution): 20

Simple Distance

Partition 1 : found 12 groups (prior maximal distance P= 0.001000)

Partition 2 : found 12 groups (prior maximal distance P= 0.001668)

Partition 3 : found 12 groups (prior maximal distance P= 0.002783)

Partition 4 : found 12 groups (prior maximal distance P= 0.004642)

Partition 5 : found 12 groups (prior maximal distance P= 0.007743)

Partition 6 : found 12 groups (prior maximal distance P= 0.012915)

Partition 7 : found 12 groups (prior maximal distance P= 0.021544)

Partition 8 : found 12 groups (prior maximal distance P= 0.035938)

Partition 9 : found 12 groups (prior maximal distance P= 0.059948)

Initial Partition with prior maximal distance P=5.99e-02

Distance Simple Dist MinSlope=0.500000

Group[1] n: 2 ;id: H_whitii_LJAMM-CNP_14387 H_whitii_LJAMM-CNP_14388

Group[2] n: 2 ;id: H_andicola_LJAMM-CNP_12493 H_andicola_LJAMM-CNP_12495

Group[3] n: 2 ;id: H_darwinii_LJAMM-CNP_11424 H_darwinii_LJAMM-CNP_9266

Group[4] n: 2 ;id: H_williamsii_LJAMM-CNP-4467 H_williamsii_LJAMM-CNP-6517

Group[5] n: 3 ;id: H_borellii_LJAMM-CNP_12116 H_borellii_LJAMM-CNP_12125 H_borellii_LJAMM_CNP_12119

Group[6] n: 3 ;id: H_uruguayensis_UFRGS-5769 H_uruguayensis_UFRGS-5770 H_uruguayensis_UFRGS-5771

Group[7] n: 2 ;id: H_rupicola_MNHNPA-2 H_rupicola_MNHNPA-1

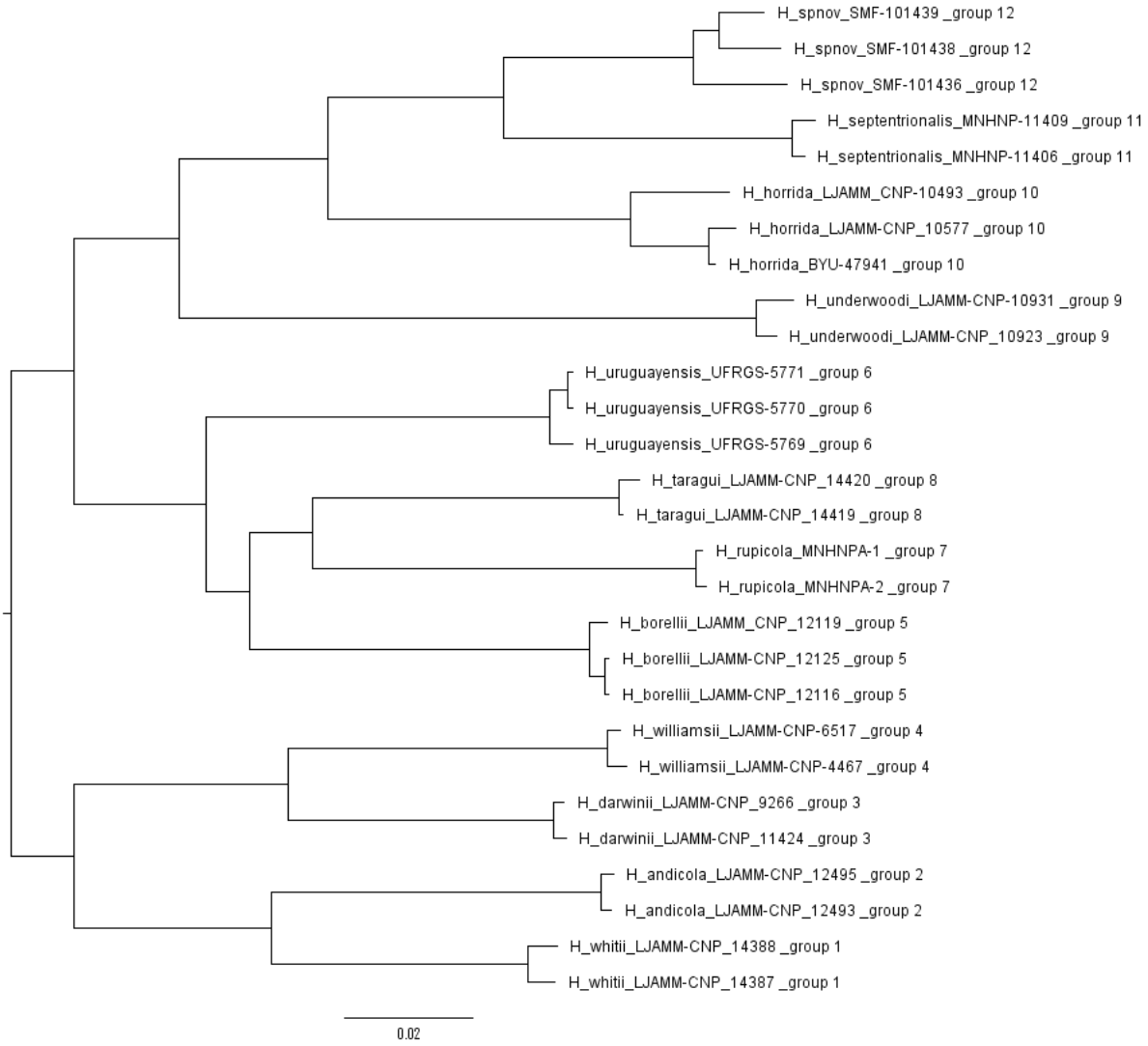
Group[8] n: 2 ;id: H_taragui_LJAMM-CNP_14419 H_taragui_LJAMM-CNP_14420

Group[9] n: 2 ;id: H_underwoodi_LJAMM-CNP_10923 H_underwoodi_LJAMM-CNP-10931

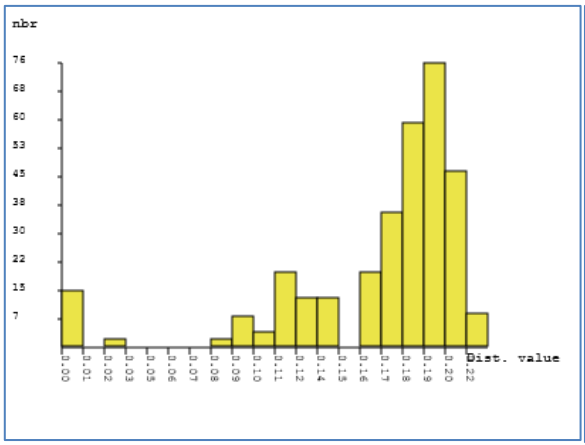
Group[10] n: 3 ;id: H_horrida_BYU-47941 H_horrida_LJAMM-CNP_10577 H_horrida_LJAMM_CNP-10493

Group[11] n: 2 ;id: H_septentrionalis_MNHNP-11406 H_septentrionalis_MNHNP-11409

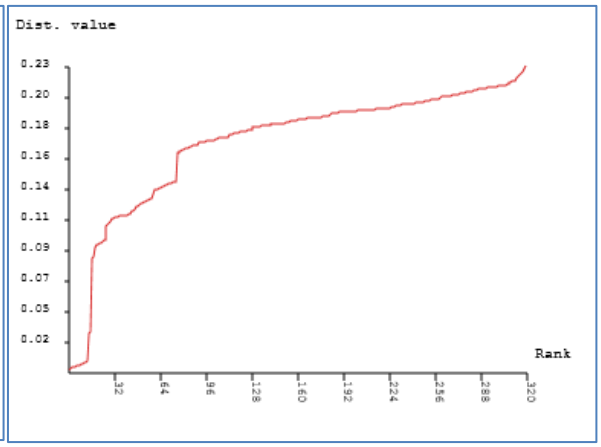
Group[12] n: 3 ;id: **H_spnov_SMF-101436 H_spnov_SMF-101438 H_spnov_SMF-101439**



Tree corresponding to the partition listed above.



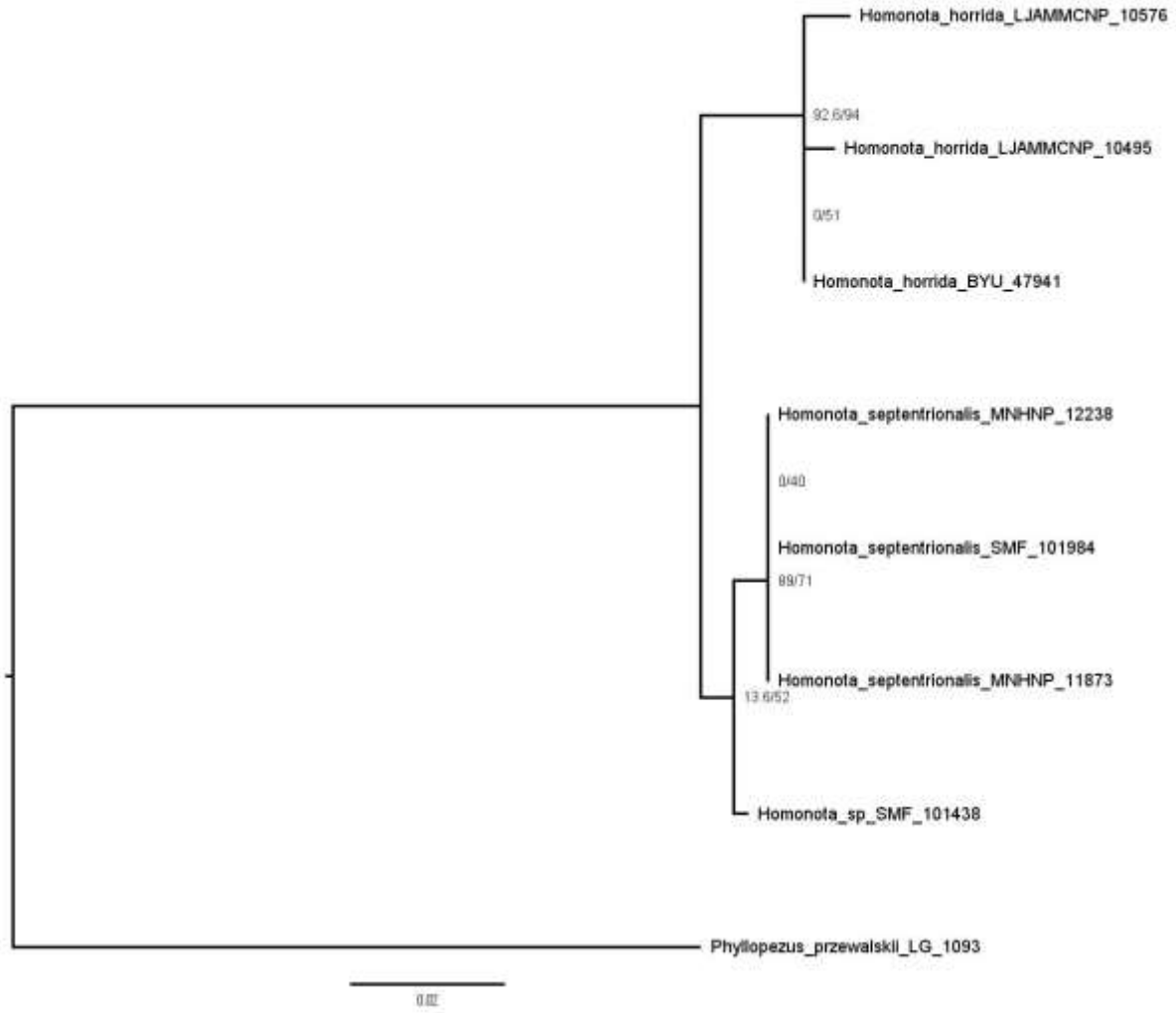
Histogram of distances



Ranked distances

Figure S1

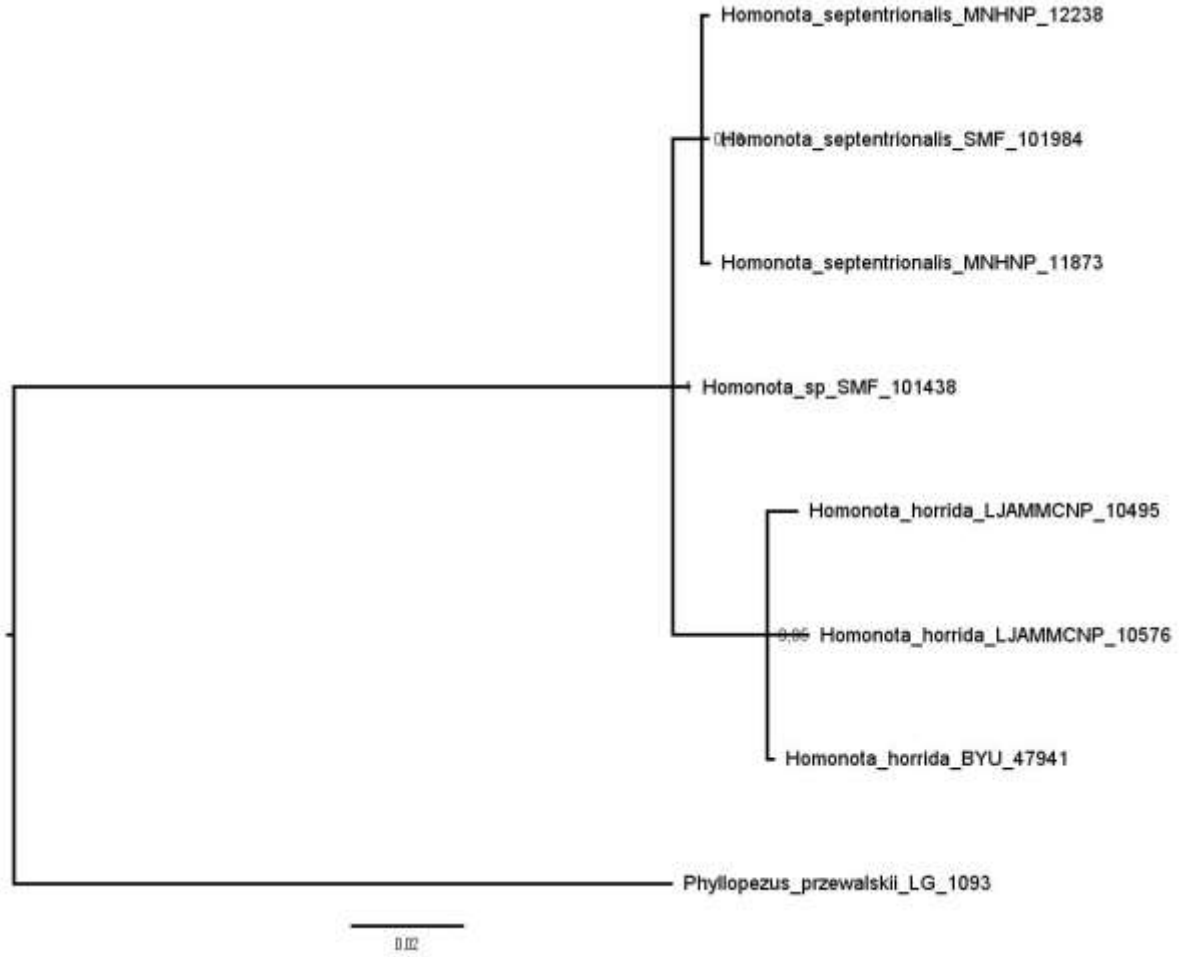
Maximum likelihood tree of 16S



Maximum Likelihood clusters of samples of *Homonota*, based on the rRNA gene 16S. Support values on nodes represent SH-aLRT/UFBoot (in percentages). Scale bar represents substitutions per site.

Figure S2

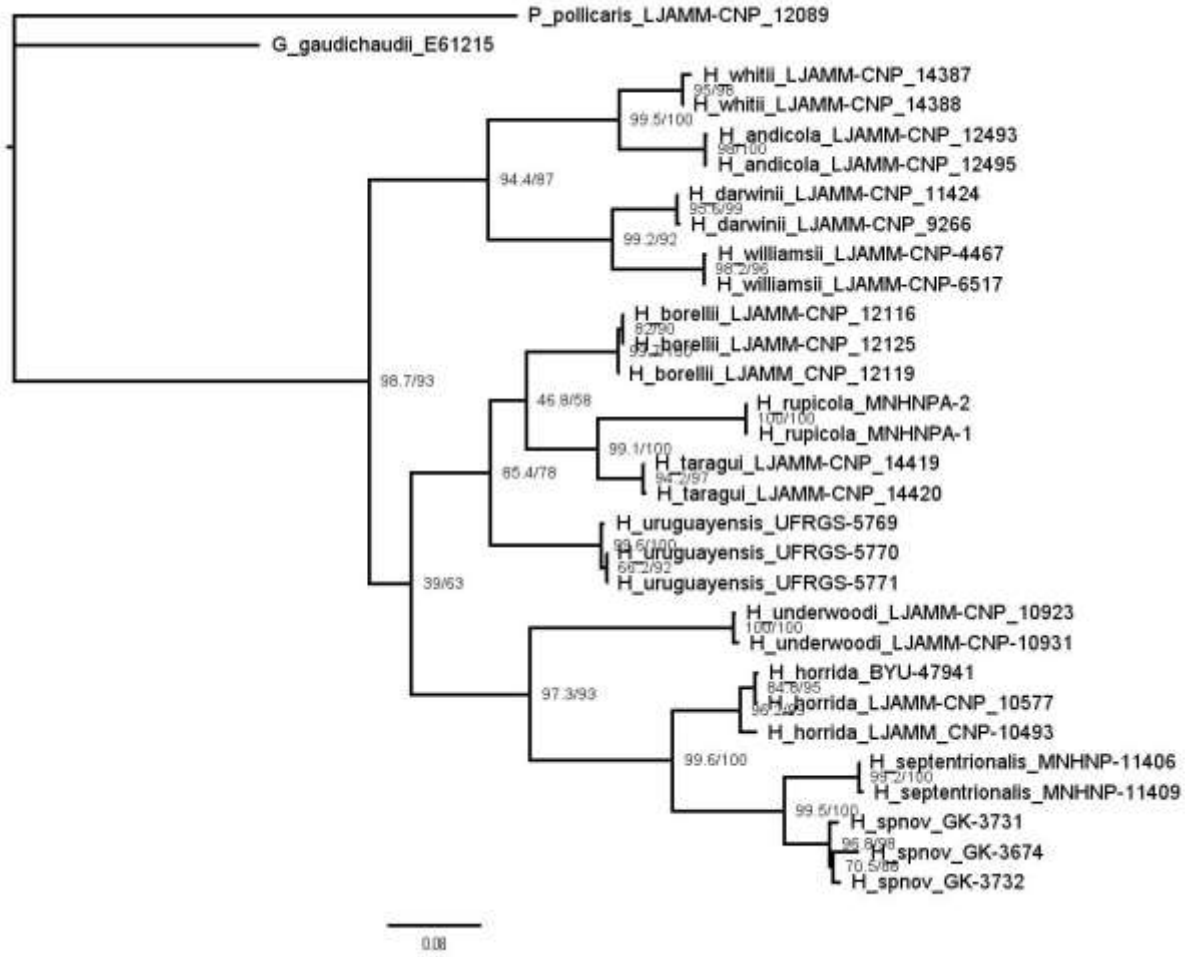
Bayesian tree of 16S



Clusters of the samples of *Homonota* based on a Bayesian inference, using the rRNA gene 16S. Support values on nodes represent posterior probability. Scale bar represents substitutions per site.

Figure S3

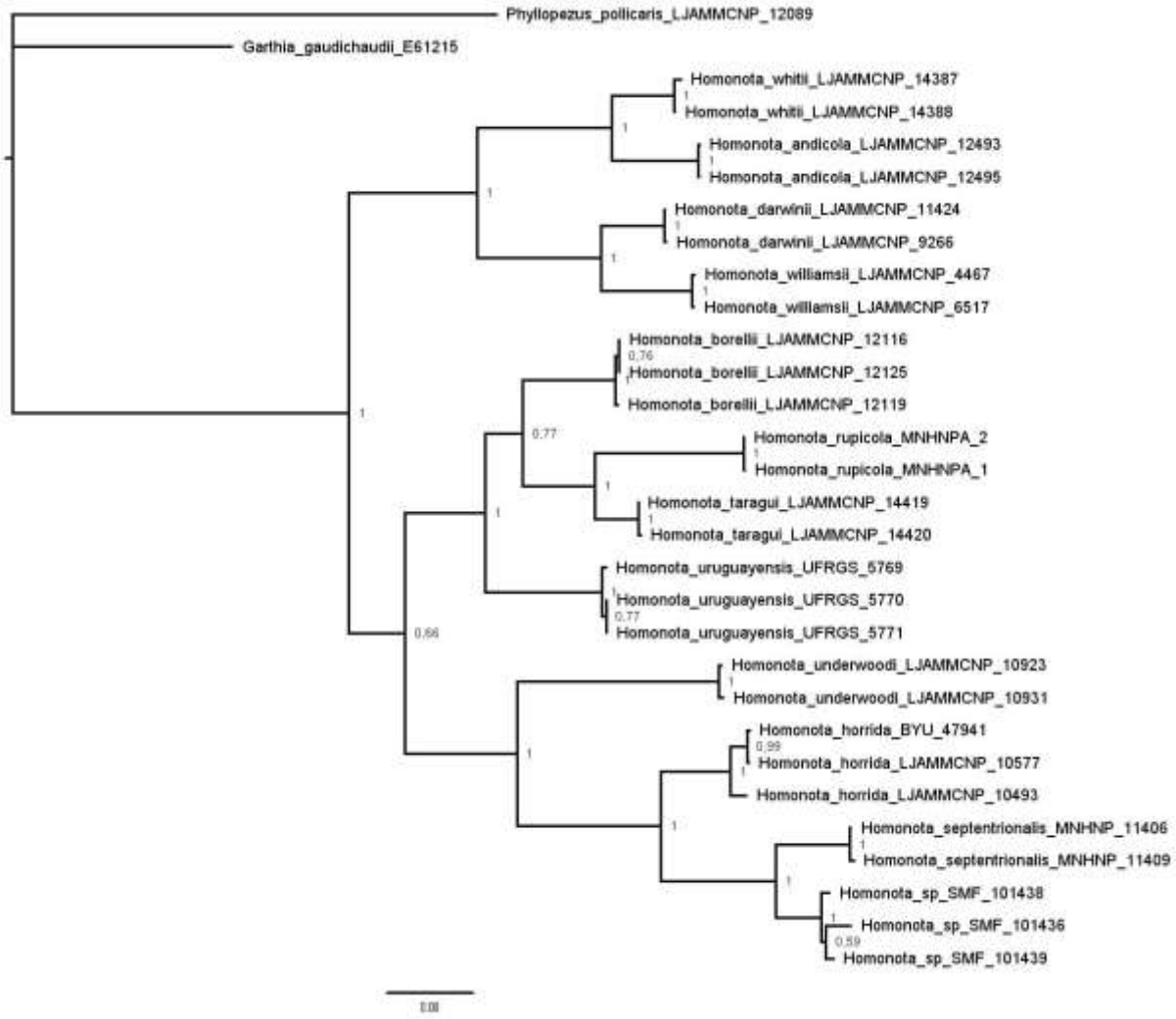
Maximum likelihood tree of Cytb



Maximum Likelihood gene tree of *Homonota*, based on the mitochondrial gene Cytb. Support values on nodes represent SH-aLRT/UFBoot (in percentages). Scale bar represents substitutions per site.

Figure S4

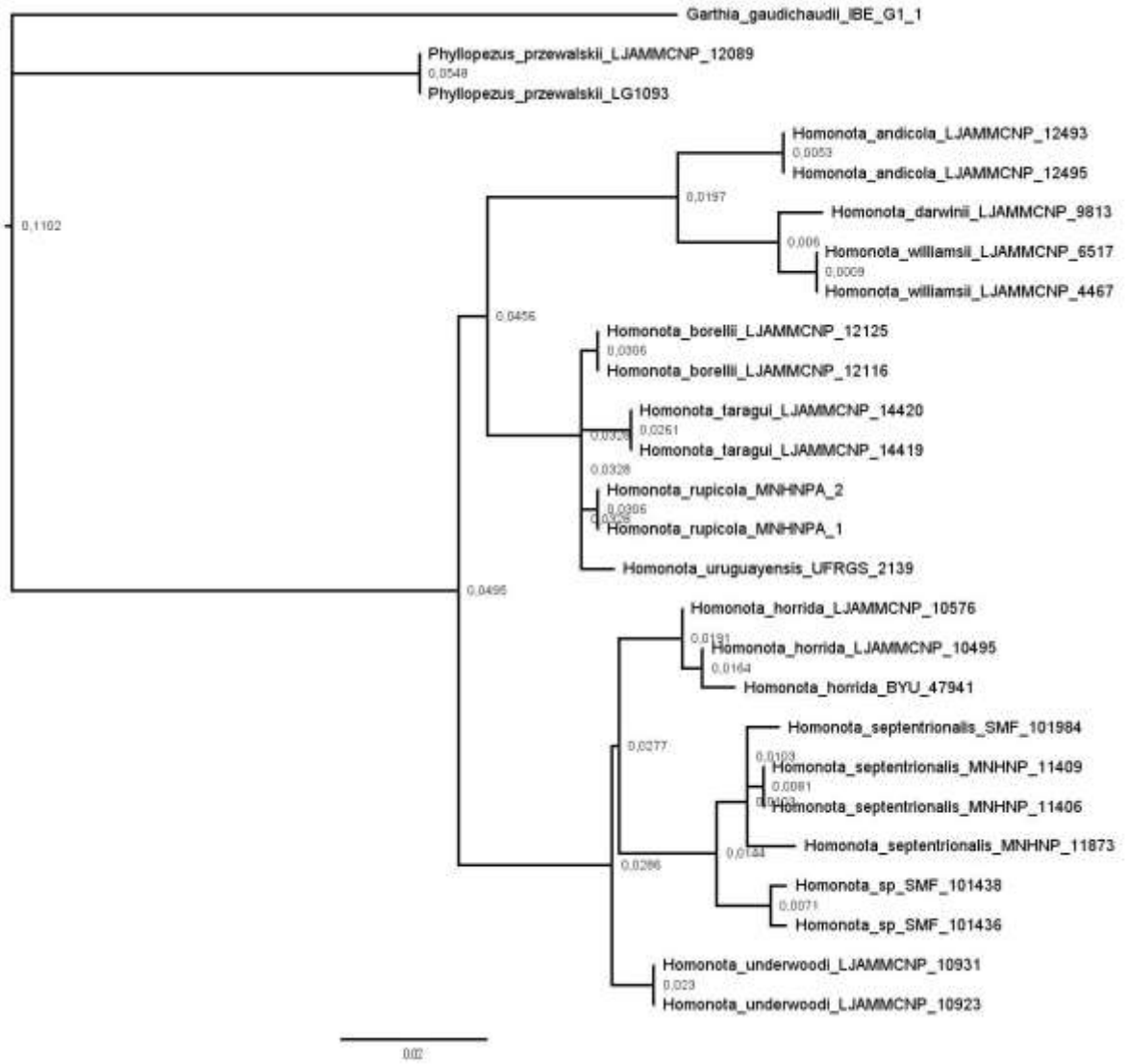
Bayesian tree of Cytb



Clusters of the samples of *Homonota* based on a Bayesian inference, using the mitochondrial gene Cytb. Support values on nodes represent posterior probability. Scale bar represents substitutions per site.

Figure S5

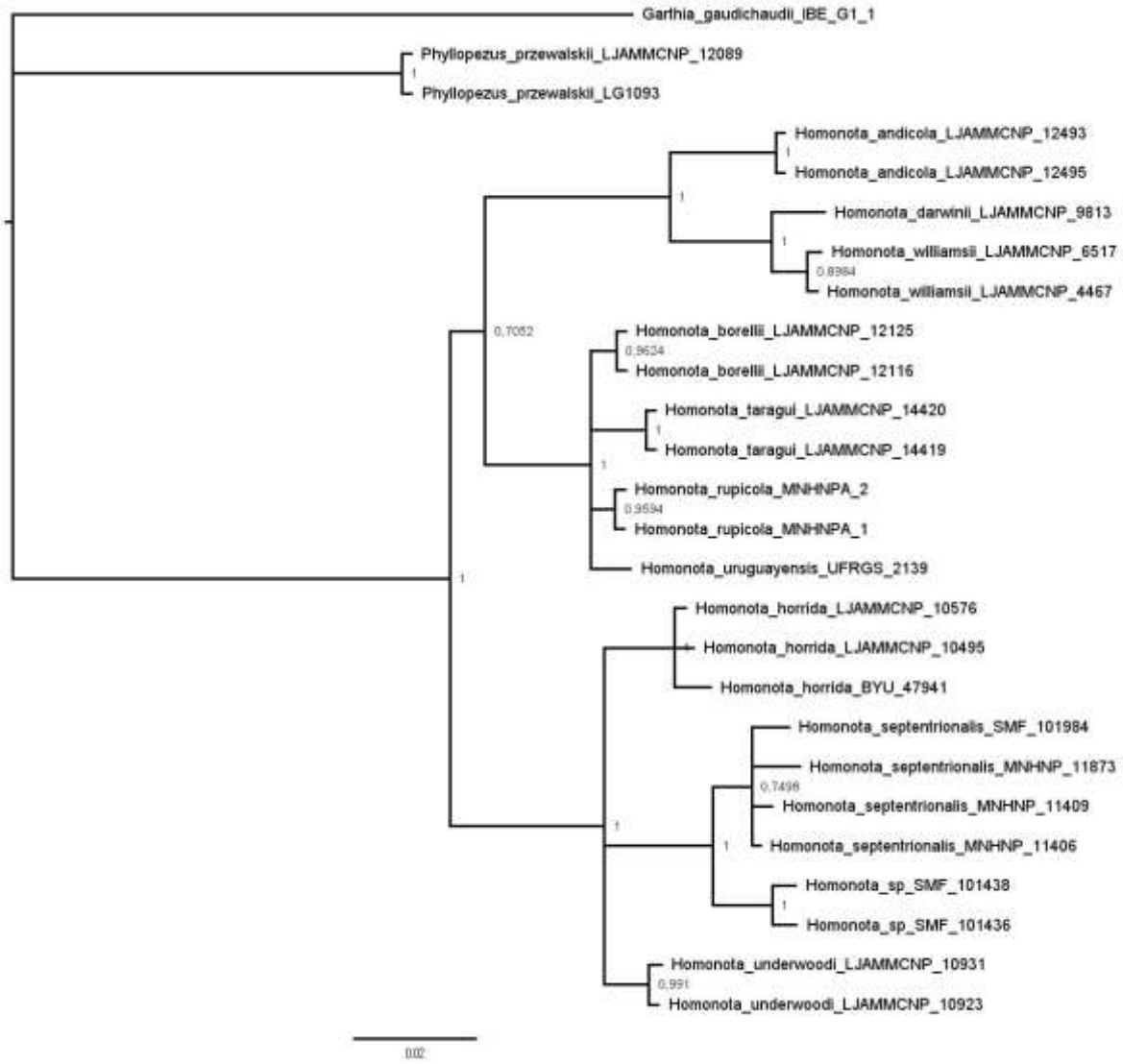
Maximum likelihood tree of PRLR



Maximum Likelihood gene tree of *Homonota*, based on the nuclear gene PRLR. Support values on nodes represent SH-aLRT/UFBoot (in percentages). Scale bar represents substitutions per site.

Figure S6

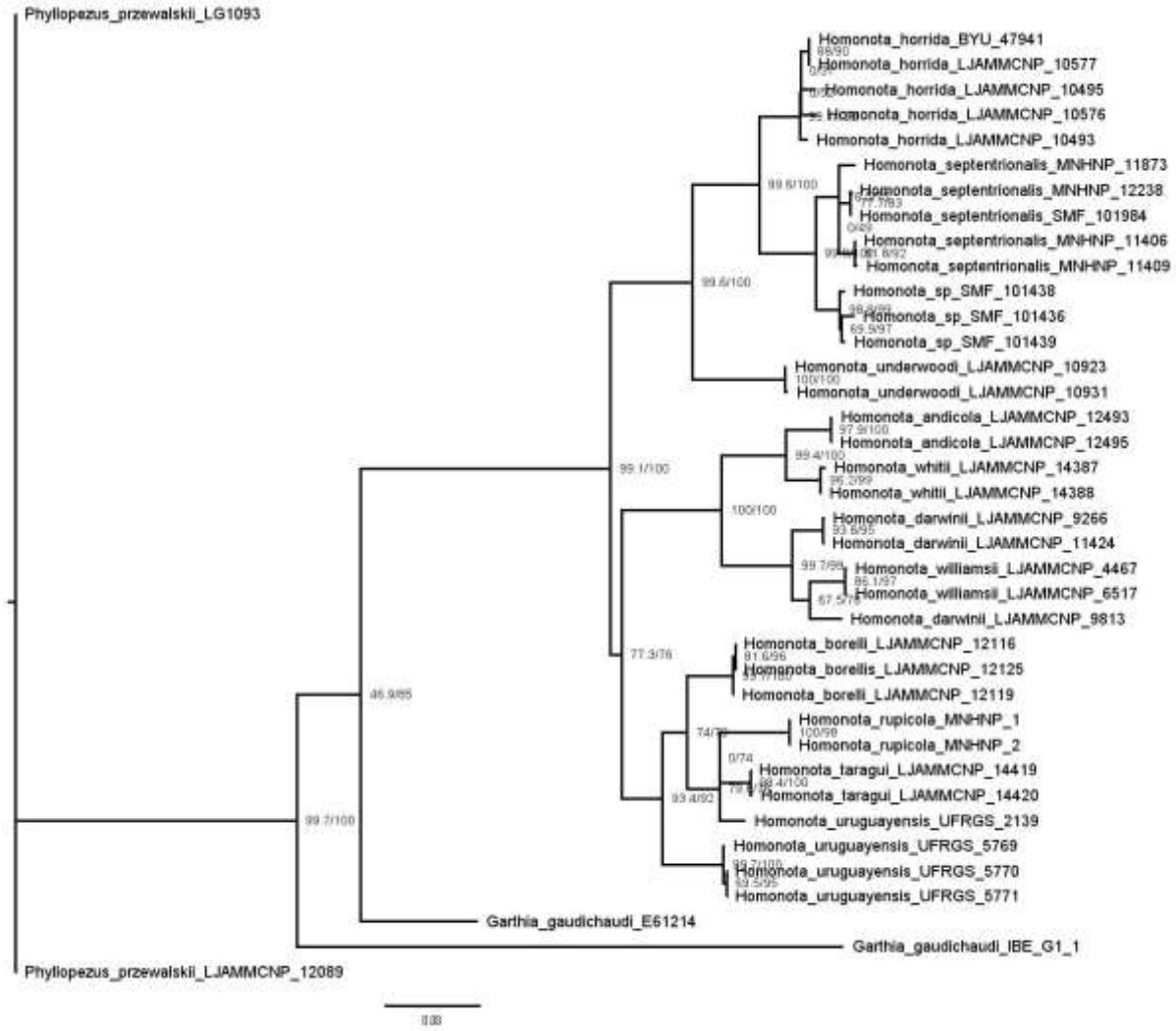
Bayesian tree of PRLR



Clusters of the samples of *Homonota* based on a Bayesian inference, using the nuclear gene PRLR. Support values on nodes represent posterior probability. Scale bar represents substitutions per site.

Figure S7

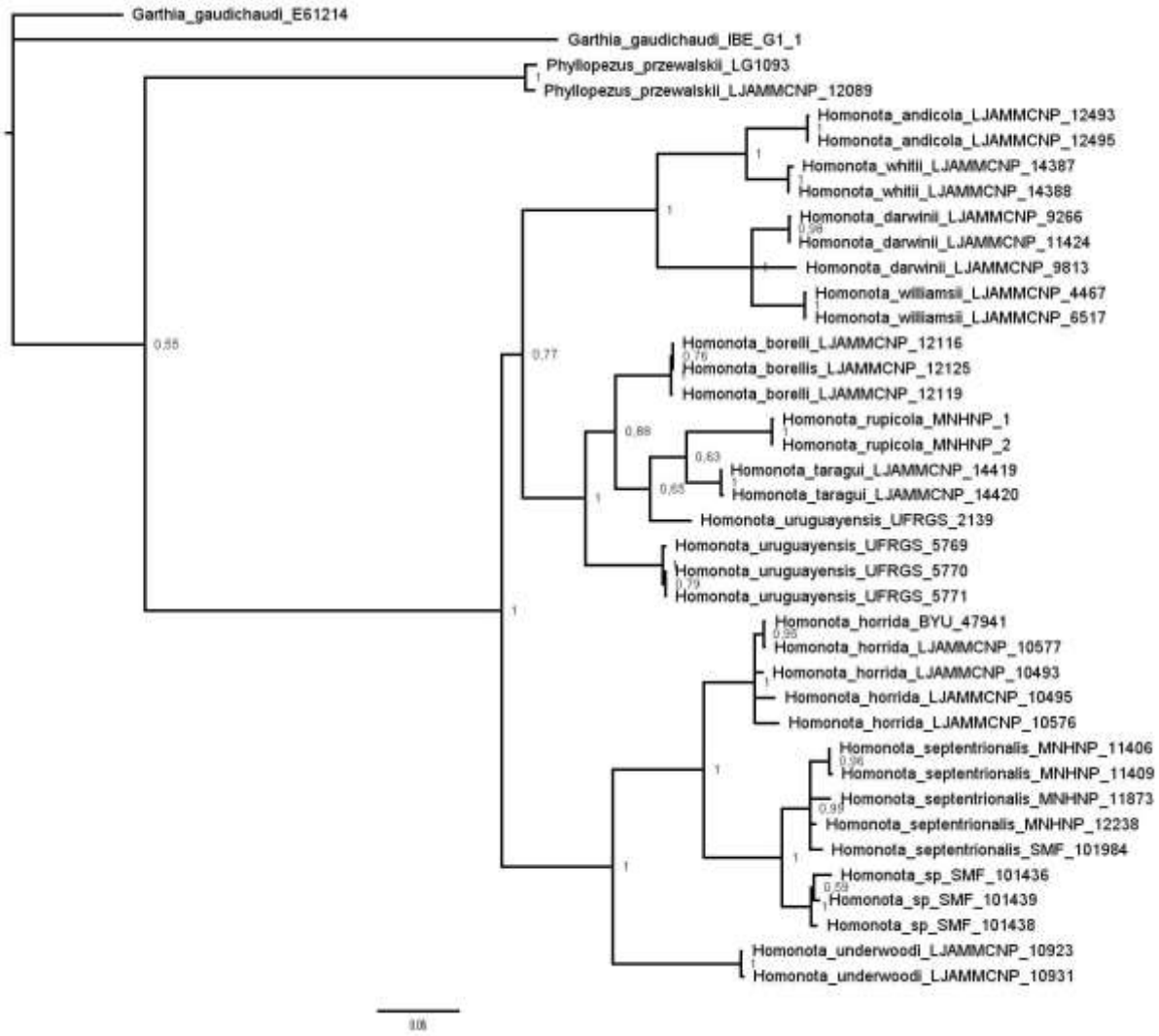
Maximum likelihood tree of concatenated genes



Maximum Likelihood gene tree of *Homonota*, based on concatenated genes (16S+Cytb+PRLR). Support values on nodes represent SH-aLRT/UFBoot (in percentages). Scale bar represents substitutions per site.

Figure S8

Bayesian tree of concatenated genes



Clusters of the samples of *Homonota* based on a Bayesian inference, based on concatenated genes (16S+Cytb+PRLR). Support values on nodes represent posterior probability. Scale bar represents substitutions per site.

Table S1

Raw metric data (in mm) used for statistical analyses. See Materials and Methods section for explanation of the characters. Missing data indicated with "?". Sex of specimens indicated in Table S2.

	Voucher	SVL	TrL	FL	TL	AL	HL	HW	HH	END	ESD	EMD	ID	IND
Homonota horrida	LJAMM-CNP 6520	57	24	10	9.2	12.2	12.8	8.6	6.7	4.3	5.3	4.8	3.8	1.7
	LJAMM-CNP 6530	64	29	11	12.5	15.4	14.6	10.4	7.6	4.7	6.3	5.3	4.8	1.7
	LJAMM-CNP 6532	56	23	9	9.6	?	13.5	10.5	6.9	4.6	5.8	5.2	5.2	1.8
	LJAMM-CNP 6533	60	26	?	11.2	13.4	13.9	11.1	7.1	4.8	6.1	4.9	4.9	2.3
	LJAMM-CNP 6535	53	21	11	9.6	?	?	?	?	?	?	?	?	?
	LJAMM-CNP 6967	42	18	7	8.3	11.9	10.5	10.4	5.8	3.5	4.1	4.4	4.5	1.7
	LJAMM-CNP 6968	46	19	10	9.0	13.7	?	8.9	?	?	?	?	4.6	1.6
	LJAMM-CNP 7670	55	23	11	11.3	?	10.5	8.6	5.8	4.2	4.9	4.6	4.5	1.8
	LJAMM-CNP 7674	51	23	10	9.9	11.9	11.8	8.3	6.3	4.1	4.8	4.7	4.5	1.6
	LJAMM-CNP 7804	52	21	12	10.2	12.1	9.8	10.1	6.1	2.9	3.6	4.7	5.3	2.3
	LJAMM-CNP 8713	56	25	9	10.5	?	?	10.6	?	?	?	?	4.8	2.2
	LJAMM-CNP 10493	53	22	11	10.4	14.4	11.7	8.7	?	3.7	4.6	4.5	4.5	2.0
	LJAMM-CNP 10496	51	23	9	9.4	12.9	12.5	9.9	5.5	4.0	4.8	5.1	4.4	1.8
	LJAMM-CNP 10523	58	23	?	10.3	14.5	14.5	12.4	6.7	4.8	6.0	5.9	5.8	1.8
	LJAMM-CNP 10526	65	28	12	12.0	16.8	14.3	10.8	6.3	4.9	6.1	5.5	5.2	1.9
	LJAMM-CNP 10576	51	18	9	10.1	12.7	13.2	10.8	6.1	4.5	5.8	5.3	4.6	1.8
	LJAMM-CNP 10577	42	17	9	8.3	11.8	10.6	8.5	4.9	3.7	4.2	4.2	4.1	1.2
	LJAMM-CNP 10578	47	17	9	9.5	14.2	14.1	9.2	6.7	4.8	6.0	5.5	4.4	2.2
	LJAMM-CNP 10579	53	20	?	10.4	13.1	13.4	9.9	6.4	4.1	5.3	5.3	4.1	2.0
	LJAMM-CNP 10584	60	24	9	11.4	14.7	16.1	10.4	7.8	5.0	6.6	6.5	5.1	2.3
	LJAMM-CNP 13948	44	20	10	8.9	12.8	10.7	8.2	5.5	3.2	4.3	4.4	4.1	1.7
	LJAMM-CNP 14551	52	24	9	9.9	12.8	12.0	10.0	?	3.9	5.0	4.7	4.8	1.5
Homonota septentrionalis	MNHNP 12238	60	26	11	10.8	14.1	14.8	13.3	7.9	4.6	6.6	5.1	5.5	2.5
	MNHNP 11860	51	23	10	9.4	13.1	12.9	9.7	6.8	4.9	5.8	4.6	4.6	1.9
	MNHNP 11850	53	25	9	9.8	12.2	13.3	10.6	7.9	4.7	5.9	4.5	5.1	2.5
	MNHNP 11855	58	28	11	11.1	13.6	13.7	11.2	8.4	4.6	6.4	5.6	5.3	2.3
	MNHNP 2821	61	28	12	9.9	14.6	14.4	11	7.7	4.5	5.9	5.3	4.8	2.4
	MNHNP 11872	48	20	9	8.4	11.8	11.5	8.9	6.4	4.5	4.9	3.8	3.8	1.9
	MNHNP 9038	51	23	9	9.8	13.1	13.1	10.2	7.1	4.3	5.1	4.9	4.4	2.0
	MNHNP 11423	45	19	9	9.1	11.6	11.6	9.0	6.4	3.4	4.5	4.0	4.0	1.6
	MNHNP 9037	65	28	12	11.3	15.0	17.3	12.4	8.6	5.8	6.8	6.0	5.2	2.5
	MNHNP 11421	45	17	9	8.1	11.0	10.9	9.2	6.7	3.7	5.0	3.9	3.8	1.7
	MNHNP 9131	41	20	9	8.4	11.8	11.4	9.5	6.4	3.9	3.6	4.4	4.2	1.4
	MNHNP 11410	39	15	8	7.2	10.2	10.7	8.1	5.8	3.7	4.4	3.6	3.7	1.7
Homonota sp. nov.	SMF 101441	56	25	11	9.6	13.3	13.6	10.8	8.3	4.2	5.9	4.7	4.6	2.0
	MNHNP 7832	59	24	11	10.2	14.0	13.5	11.2	7.4	4.5	6.1	5.0	5.4	1.7
	MNHNP 2810	53	23	8	9.3	12.7	12.7	10.5	6.7	4.5	5.7	4.3	4.9	1.5
	MNHNP 9726	51	23	10	9.8	12.4	12.9	10.3	7.4	4.2	5.2	4.4	5.1	1.8
	SMF 101438	45	21	9	8.3	11.4	11.4	9.8	6.9	4.0	5.3	3.9	5.1	1.6
	MNHNP 10534	56	26	11	10.1	13.7	13.8	11.2	7.6	4.6	5.8	4.8	5.3	2.1
	MNHNP 2795	52	23	9	9.4	11.7	13.1	10.8	6.9	4.6	5.6	4.4	4.8	1.6
	MNHNP 11791	54	22	8	8.8	13.3	12.8	10.1	6.9	4.1	5.7	4.7	5.2	1.6
	SMF 101442	57	25	9	9.1	12.9	13.1	10.5	8.0	4.4	5.9	4.1	4.7	2.1
	MNHNP 10744	53	24	11	9.8	13.4	13.1	10.5	6.9	5.1	6.0	4.6	5.8	1.7
	MNHNP 2798	56	27	10	8.7	12.8	12.5	11.0	7.5	4.3	5.6	4.3	5.0	1.9
	MNHNP 11790	50	23	8	10.1	11.7	12.1	10.1	6.8	3.8	4.9	4.1	4.5	1.6
	MNHNP 11793	45	20	7	8.3	11.2	11.1	9.1	6.0	4.1	5.1	3.9	4.4	1.4

Table S2

Raw meristic data used for statistical analyses. See Materials and Methods section for explanation of the characters. Missing data indicated with "?".

	Voucher	DT	TVS	LVS	4TL	3FL	IL	SL	Sex
Homonota horrida	LJAMM-CNP 6520	30	43	19	19	16	7	9	F
	LJAMM-CNP 6530	28	45	15	20	14	7	9	F
	LJAMM-CNP 6532	33	48	16	18	13	6	7	M
	LJAMM-CNP 6533	31	36	15	19	14	7	8	F
	LJAMM-CNP 6535	34	44	18	21	17	7	8	M
	LJAMM-CNP 6967	34	42	17	16	15	7	9	M
	LJAMM-CNP 6968	30	42	18	17	15	8	8	M
	LJAMM-CNP 7670	35	47	14	20	14	6	8	F
	LJAMM-CNP 7674	35	40	13	20	14	6	8	F
	LJAMM-CNP 7804	34	43	17	20	16	7	8	F
	LJAMM-CNP 8713	32	48	17	20	16	6	8	M
	LJAMM-CNP 10493	32	39	14	17	14	6	8	M
	LJAMM-CNP 10496	33	39	16	18	15	6	8	F
	LJAMM-CNP 10523	32	41	15	19	14	6	8	F
	LJAMM-CNP 10526	34	39	13	21	16	6	8	F
	LJAMM-CNP 10576	33	46	20	22	17	7	8	M
	LJAMM-CNP 10577	34	42	16	20	16	7	7	F
	LJAMM-CNP 10578	36	42	19	21	15	7	9	F
	LJAMM-CNP 10579	37	42	15	?	16	7	8	F
	LJAMM-CNP 10584	33	43	16	20	15	6	9	M
	LJAMM-CNP 13948	29	42	14	18	14	6	8	M
	LJAMM-CNP 14551	36	45	16	21	14	8	9	M
Homonota septentrionalis	MNHNP 12238	32	42	19	17	13	6	9	F
	MNHNP 11860	31	37	19	18	12	7	7	F
	MNHNP 11850	31	39	18	18	12	7	6	M
	MNHNP 11855	28	43	17	16	12	6	8	F
	MNHNP 2821	29	42	17	20	14	6	8	F
	MNHNP 11872	32	?	?	19	13	6	8	M
	MNHNP 9038	31	40	12	17	12	7	8	M
	MNHNP 11423	32	41	19	19	14	6	8	M
	MNHNP 9037	34	42	16	19	15	6	8	F
	MNHNP 11421	28	41	15	17	13	6	7	M
	MNHNP 9131	29	40	17	17	13	6	8	M
	MNHNP 11410	28	?	?	?	13	6	7	M
Homonota sp. nov.	SMF 101441	32	34	16	19	15	7	9	F
	MNHNP 7832	33	37	16	22	13	6	8	M
	MNHNP 2810	27	35	14	18	14	6	9	M
	MNHNP 9726	28	41	18	17	14	6	8	F
	SMF 101438	28	36	17	17	13	7	7	M
	MNHNP 10534	28	40	17	20	14	5	7	F
	MNHNP 2795	35	40	16	19	16	6	7	F
	MNHNP 11791	31	43	18	18	13	5	8	M
	SMF 101442	30	37	14	18	16	6	3	F
	MNHNP 10744	32	40	14	18	14	6	7	M
	MNHNP 2798	29	43	16	17	11	5	7	F
	MNHNP 11790	30	43	15	17	15	6	6	M
	MNHNP 11793	32	36	15	18	15	6	8	M

APPENDIX VI

Declaration on the contributions of authors

to the publication: Cryptic diversity in the Neotropical gecko genus *Phyllopezus* Peters, 1878 (Reptilia: Squamata: Phyllodactylidae): A new species from Paraguay
Status: In review.
Name of the journal:
Authors involved: Pier Cacciali (PC), Sebastian Lotzkat (SL), Tony Gamble (TG), Gunther Köhler (GK).

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor SL: 10%
Coauthor TG: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 70% – field work (collecting and documenting specimens), molecular analyses, morphological analyses, revision of museum vouchers.
Coauthor TG: 20% – molecular analyses.
Coauthor GK: 10% – field work (collecting and documenting specimens).

(3) to the creation of the data collection and figures

PhD candidate: 70% – created database, sequenced DNA barcodes, provided photographs, created figures, created maps.
Coauthor TG: 20% – created database, sequenced DNA barcodes.
Coauthor GK: 10% – provided photographs.

(4) to the analysis and interpretation of the data

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Cryptic diversity in the Neotropical gecko genus *Phyllopezus* Peters, 1878 (Reptilia: Squamata: Phyllodactylidae): A new species from Paraguay

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Abstract

The gecko genus *Phyllopezus* is distributed mainly along South America's "Dry Diagonal" (Caatinga, Cerrado, and Chaco). The genus has been the subject of recent taxonomic analyses and includes four described species and seven candidate species referred to here as *Phyllopezus pollicaris* sensu lato. In Paraguay, *Phyllopezus* is known from the Chaco and Cerrado where it is abundant, and also from a small isolated population from a rocky hill formation named "Cordillera de Los Altos" (Los Altos mountain range). Here we analyzed genetic samples from across its range, including new samples from Paraguay, using DNA barcoding analysis of the mitochondrial 16S gene and phylogenetic analyses using both Bayesian and Maximum Likelihood methods. We found genetic and morphological differences among geckos from the Los Altos mountain range and the remaining *Phyllopezus* populations. Using both molecular and morphological evidence we describe a new *Phyllopezus* species, sister to *P. przewalskii*. Genetic differentiation among described and putative *Phyllopezus* species is greater than their morphological differences, which likely accounts for these cryptic taxa remaining undescribed for so long.

1. Introduction

In South America, the so-called "Dry Diagonal" of dry seasonal woodland formations stretches from eastern Brazil to northern Argentina and includes the Caatinga, Cerrado, and Chaco ecoregions. The origin of these biomes and the evolution of their faunas has evoked much interest during the past decades [1–4], and is still a significant topic for understanding the evolution of the Neotropical biota [5–8]. As important as these "Dry Diagonal" ecoregions are, they remain poorly known with new species being described at an increasing rate [9–11]. Molecular data are now often used for species identification [12–14] and species delimitation [15–18] helping taxonomists to improve the global knowledge of alpha taxonomy. This led to the recognition of many cryptic phylogenetic linages of reptiles in the Neotropics [9, 19]. A good example of this undescribed diversity involves lizards of the genus *Phyllopezus* distributed mainly along the "Dry Diagonal" [10–11].

Phyllopezus is a genus of Neotropical geckos, which for decades was considered as monotypic with two subspecies: *P. pollicaris pollicaris* (Spix, 1825) and *P. p. przewalskii* Koslowsky, 1895 [20–21]. Later, *P. periosus* Rodrigues, 1986 was described from northeastern Brazil [22], and more recently *P. maranjonensis* Koch, Venegas & Böhme, 2006 from Peru [23]. Furthermore, a detailed phylogenetic study, based on a multi-locus genetic approach, showed a deep phylogenetic nesting of *Bogertia lutzae* within *Phyllopezus* and accordingly placed that species in the genus *Phyllopezus* to reestablish monophyly [10].

Phyllopezus are among the largest geckos in South America and they have a pattern of black or brown spots on gray or also whitish background color with a high intraspecific variation. Based on genetic analyses of *Phyllopezus* specimens (referred to as *P. pollicaris*) from the Chaco, Cerrado, and Caatinga ecoregions, a previous study found a high degree of genetic diversity in Cerrado and Caatinga (northeastern populations) specimens [10], followed by another study that proposed species status for *P. przewalskii* and designated seven additional taxa (within *P. pollicaris* sensu lato) as candidate species, thus, recognizing eleven tentative species-level units within this genus [11].

The distribution of *Phyllopezus pollicaris* sensu lato and *P. przewalskii* includes the "Dry Diagonal" [11], Chaco, Cerrado, and Caatinga, as well as another biome

recognized as “Seasonal Dry Tropical Forest” [24]. In the last phylogenetic study of the genus, three major genetic lineages of *Phyllopezus pollicaris* sensu lato were recognized [11]: a northeastern group from the Caatinga, a central clade from the Cerrado, and a southwestern group whose distribution matches that of the Chaco sensu lato (Dry Chaco + Humid Chaco) (Fig. 1). In Paraguay *P. przewalskii* is known from the Chaco [25–26], where the species is a common ground dweller, but also inhabits human dwellings. *Phyllopezus* is also present in the Paraguayan Cerrado [25–26], where it occurs in rocky hills. Furthermore, there is another isolated population inhabiting a rocky hill formation named “Cordillera de Los Altos” (Los Altos mountain range) in the Departments of Paraguarí [27] and Cordillera [26], with an environment completely different to that of the Paraguayan Chaco or Cerrado (details of environments presented in Appendix S1). Populations from “Cordillera de Los Altos” and Paraguayan Cerrado have not been included in previous genetic analyses.

In this work we first explored the genetic distinctiveness of *Phyllopezus* specimens from the “Cordillera de Los Altos” and the Paraguayan Cerrado within the framework of a project about barcoding of Paraguayan herpetofauna using the mitochondrial 16S rRNA gene. Genetic data indicates that *Phyllopezus* from “Cordillera de Los Altos” constitute a genetic lineage differentiated from the remaining Paraguayan populations. Next, we incorporated additional mitochondrial genes (Cytb and ND2) to our analyses in order to assess phylogenetic relationships of *Phyllopezus* from “Cordillera de Los Altos” with previously published conspecific data (cluster arrangement according to Werneck et al. [11]).

2. Materials and Methods

We sampled *Phyllopezus* from three localities of Paraguay (Fig. 1) and sequenced fragments of the mitochondrial 16S rRNA gene (GenBank accession numbers provided in Appendix S2) for comparison with sequences produced by Gamble et al [10] DNA was extracted from muscle stored in ethanol 98% at -27ºC using the standard glass fiber plate protocol Ivanova et al. [28]. Samples were washed for about 14 h in 50 µl of diluted PBS buffer (1:9 of buffer and water respectively). Tissues were digested with vertebrate lysis buffer (60 µl per sample) and proteinase K (6 µl per sample) at 56°C for around 14 h. After extraction, DNA samples were eluted in 50 µL TE buffer.

Amplification via double-stranded PCR of mitochondrial 16S rRNA fragments was performed using the Eurofins MWG Operon primers 16sar-L (forward: 5'–CGCCTGTTTATCAAAAACAT–3', also referred to as L2510) and 16sbr-H (reverse: 5'–CCGGTCTGAACTCAGATCACGT–3', also referred to as H3056) [29], in an Eppendorf Mastercycler® pro. The PCR conditions were: initial denaturation 2 min (94ºC) – denaturation 35 sec (94ºC)×40 – hybridization 35 sec (48.5ºC) – elongation 60 sec (72ºC) – final elongation 10 min (72ºC). Cycle-sequencing and sequencing (BigDye Terminator) were performed with the same forward and reverse primers mentioned above. Partial sequences of mtDNA from cytochrome b (Cytb) and NADH dehydrogenase subunit 2 (ND2) genes were amplified according to the procedures presented in Werneck et al. [11]. Inspection of DNA chromatograms and generation of consensus sequences were performed with SeqTrace 0.9.0 [30].

Sequences were aligned using MAFFT 7 [31–32] through the webserver (available at http://mafft.cbrc.jp/alignment/server/). We included the Q-INS-i search strategy for the secondary structure of 16S [33]. Results of alignment were visualized and exported with MSA Viewer [34]. According to the respective requirements of the different software applications, the formats of the sequences were converted using the online server Alter [35]. The best substitution model for each gene (analyzed separately) of our dataset was identified using PartitionFinder2 [36], with linked branch lengths (supported by most of the phylogenetic programs) via PhyML 3.0 analysis [37]. Model selection was detected using the corrected (for finite sample size) Akaike Information Criterion (AICc) [38]. Given that the correlation between gamma (+G) and invariant sites (+I) parameters, models that include both +G and +I are often inadequate [39–41]. Thus, we did not use models that included both parameters together. In all analyses, we used the *Phyllodactylus unctus* mitogenome (GenBank HQ896027) as an outgroup [42].

All of the following analyses were conducted for each gene individually, and the three genes concatenated. Sequences were concatenated in Mega 7.0.26 [43]. We performed Bayesian Inference analysis (BI) with MrBayes 3.2 [44–45]. BI analyses were performed setting 5 runs with 8 chains discarding the first 25% as the burn-in period and an initial set of 1,000,000 generations for MCMC with a sampling frequency of 500 generations; adding 500,000 generations until chains reached convergence. We considered convergence when the standard deviation of split frequencies was 0.015 or

less. Additionally, convergence was diagnosed by PRSF (Potential Scale Reduction Factor) which should approach 1.0 as runs converge [46].

We used the IQTree webserver [47] to run a Maximum Likelihood (ML) analysis using 10,000 ultrafast bootstrap approximation (UFBoot) replicates with 10,000 maximum iterations and Minimum correlation coefficient of 0.99 [48] plus 10,000 replicates of Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT), which proved to be accurate with a high statistical power [37]. We used FigTree 1.3.1 for tree viewing (http://tree.bio.ed.ac.uk/software/figtree/).

We estimated evolutionary genetic divergence for the 16S gene among sequences, computing uncorrected pairwise distances with Mega 7.0.26 to assess the degree of intra- and interspecific differences, using a Bootstrap estimation method of 10,000 replications. Data were compared with those available from Gamble et al. [10].

To assess the phylogenetic position of the "Cordillera de Los Altos" clade within *Phyllopezus*, we designed a species tree based on the three mtDNA gene sequences concatenated, using *BEAST [49] in BEAST 2.4.7 [50] under 1,000,000 generations for the mcmc model, visualizing the posterior probability in DensiTree 2.2.6 [51].

We performed an initial species delimitation analysis by visualizing barcode gaps in the pairwise distribution of each mtDNA gene separately (excluding the outgroup), using the automatic barcode gap discovery (ABGD) approach [52] through its webserver (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html); setting the use of Simple Distance, default values for Prior Intraspecific divergence, except for relative gap width (1.5) which does not work for some genes (as also noted by Kekkonen et al. [53]). Because high values in relative gap width tend to overly split species [54], we also used an intermediate value of 0.7.

In addition to the molecular genetics, we measured the following morphological characters: snout–vent length (SVL) from the tip of the snout to the anterior edge of the cloaca; head length (HL) from the tip of the snout to the anterior edge of the ear opening; head width (HW) measured at the widest section of the head; nares–eye distance (NED) taken from the posterior border of the nares to the anterior edge of the eye; eye diameter (ED) measured at the widest section of the eye; maximum diameter of the ear opening (EO) taken at the widest point of the opening; minimum distance between nares (DBN) measured from above; and tail diameter (TD) taken at the base of

the organ just posterior to the cloaca. Measurements were taken with digital calipers except SVL, which was taken with a meter stick. Meristic characters included the number of supralabials (SL) from the first scale behind the rostral scale to the last in contact with the mouth border; upper supralabial row (USL) from the first in contact with posterior nasal and first SL to the last scale before SL contact infraoculars; infralabials (IL) from the first scale contacting the mental to the last in contact with the mouth border; and scales between 4^{th} and 5^{th} digital pads from the first lateral scale of the 5^{th} toe (the distalmost lateral finger scale that is not situated on the raised portion of the terminal phalanx) to the first lateral scale of the 4^{th} toe (Fig. S1). When variation in the bilateral symmetry of morphological characters is present, a slash mark separates the respective values for left/right side. In the color descriptions, the capitalized colors and the color codes (in parentheses) are those of Köhler [55]. Specimens used for morphological comparison are listed in Appendix 1.

We also explored the value of diagnostic characters traditionally used to differentiate between *Phyllopezus pollicaris* and *P. przewalskii* [20], which are: post-cloacal tubercles at the sides of the vent; number of lamellae under the fourth toe; and number of longitudinal rows of ventral scales (counted transversally at midbody). To do this, we compared samples from Paraguayan Chaco and Cerrado (which belong to *P. przewalskii*) with topotypes of *P. pollicaris*. Institutional acronyms follow Sabaj Pérez [56].

The Secretaría del Ambiente in Paraguay authorized to euthanize and collect specimens, through the permits SEAM Nº 04/11, SEAM Nº 009/2014, and SEAM Nº 133/2015.

3. Results

The final alignments of 16S, Cytb, and ND2 were of 498, 907, and 886 nucleotide positions respectively for 64 *Phyllopezus* samples plus *Phyllodactylus unctus* as an outgroup (Appendix S2). Partition schemes were recorded as follows: 16S (GTR+G); Cytb (1^{st} pos HKY+I | 2^{nd} pos K81UF+I | 3^{rd} pos GTR+I); ND2 (GTR+I | HKY+G | TIM+I).

The trees obtained through BI and ML showed a high degree of concordance at well-supported nodes, with some differences in branch arrangement at poorly supported

nodes (Fig. S2–S7). The clade from "Cordillera de los Altos" always clustered as the sister taxon of *P. przewalskii* (Fig. S2–S7). Based on the concatenated genes, *P. maranjonensis* was the sister taxon to the remaining *Phyllopezus* taxa, followed by *P. lutzae*, and *P. periosus* as the sister clade to the remaining species within the genus. The Bayesian analysis (Fig. 2, S8) generally showed higher support values than ML (Fig. S9). The branches corresponding to *P. pollicaris* Clade I and *P. pollicaris* Clade II are clustered together. An important polyphyly is observed in *P. pollicaris* Clade III which appears as the sister clade to *P. pollicaris* Clade IV + *P. przewalskii*, and one sample is located as the sister group to the cluster *P. pollicaris* Clade VI + *P. pollicaris* Clade VII + *P. pollicaris* Clade VIII (Fig. 2). Both hypotheses (BI and ML) suggest a branch with the southwestern taxa (Los Altos population + *P. przewalskii* + *Phyllopezus pollicaris* Clade IV) differentiated from the remaining *P. pollicaris* clades. The *P. przewalskii* clade splits specimens from the Cerrado and from the Chaco separately; and specimens from "Cordillera de Los Altos" mountain range are recovered as a sister clade to *P. przewalskii*. The final alignment and trees (along with methods and parameters) are stored in TreeBASE (ID Pending) available at https://treebase.org/treebase-web/home.html.

The highest intraclade pairwise distance reaches 6.65% in *Phyllopezus pollicaris* Clade VII, and 4.4% in *P. przewalskii* (Appendix S3). The minimum pairwise distance between two clades usually is higher than 10% with the exception of the minimum distances between *P. pollicaris* Clade IV and *P. przewalskii* (9.9%), as well as between *P. pollicaris* Clade VIII with *P. przewalskii* (10.4%), *P. pollicaris* Clade VI (8.2%), and Clade VII (7.1%), respectively (Appendix S3).

Samples from Los Altos mountain range present high values for pairwise genetic distance, reaching a genetic distance of 16.8% with *P. periosus* (Appendix S3). The shortest genetic distance of the Los Altos mountain range population is 11.8% with *P. pollicaris* Clade IV (Appendix S3). Genetic distances between the Los Altos population and its sister clade, *P. przewalskii*, is 12.6–14.4%.

The species tree shows *P. periosus*, *P. maranjoensis* and *P. lutzae* clustered together and sister to the remaining *Phyllopezus* (Fig. 3). The highest support is observed in the clusters *P. pollicaris* Clade I + *P. pollicaris* Clade II, *P. pollicaris* Clade

VI + *P. pollicaris* Clade VII + *P. pollicaris* Clade VIII, and *P. przewalskii* + *Phyllopezus* sp. (Fig. 3).

Results from ABGD suggest the presence of a higher diversity within *Phyllopezus* than currently recognized (Fig. 4). The gene 16S seems to be the most conservative recognizing 11 different species (Clades II and III not included given the lack of samples), while ND2 recognized 15 species, and 18 species recognized by Cytb (Fig. 4). Full results of ABGD are found in Appendix S4. The mitochondrial Cytb gene suggests a higher diversity especially in the Clade VII. This gene, as well as ND2 distinguish the sample MTR 13452 (*P. pollicaris* Clade III) as a species separated from the remaining Clade III. Finally, the three genes are concordant in the recognition of the samples from "Cordillera de Los Altos" as a different taxon (Fig. 4, Appendix S4).

4. Taxonomic implications

The specimens from "Cordillera de Los Altos" mountain range exhibit a high genetic differentiation from all other studied populations of *Phyllopezus*. Moreover, the specimens from "Cordillera de Los Altos" are recovered as a clade phylogenetically distinct from the other clades. Finally, the population from "Cordillera de Los Altos" can be differentiated from the other species of this genus by subtle characters of coloration and scalation. Therefore, applying the general lineage species concept [57], we recognize the *Phyllopezus* population from the "Cordillera de Los Altos" as a separate species, described below.

This published work and the nomenclatural acts it contains have been registered in Zoobank: [Pending], and therefore are available under the International Code of Zoological Nomenclature. The Life Science Identifier (LSID) for this publication is: [Pending]. The LSID registration and any associated information can be viewed in a web browser by adding the LSID to the prefix "http://zoobank.org/."

***Phyllopezus heuteri* sp. nov.**

Holotype. SMF 100494 (original field number GK 3559), adult female (Fig. 5), collected on 12 September 2016 by G. Köhler, at the Cerro de Tobatí (25.2797º S,

57.0925º W, 428 masl), in the Cordillera de Los Altos mountain range, Cordillera Department, Paraguay (Fig. 6).

Paratypes. MVZ 110967 (unidentified sex), collected on 7 October 1972 by Phil Myers, at 1 mi S of Tobatí, Cordillera, Paraguay; UNNEC 1005 (unidentified sex), collected on 3 March 1998 by M. Culzzoni and B. Alvarez, at Chololó (25.55138º S, 57.0400º W, 272 masl), Paraguarí, Paraguay; MNHNP 11975 (adult male), collected on November 2014 by J. Méndez, at Cerro de Tobatí, Cordillera, Paraguay; MNHNP 12001 (adult male), collected on 23 April 2013 by J. Méndez, at Cerro de Tobatí, Cordillera, Paraguay; MNHNP 12111 (adult female) and MNHNP 12112 (adult male), collected on 27 January 2012 by J. Méndez, S. Escobar and T. López, at Cerro Hũ (25.6069º S, 57.1294º W, 365 masl), Paraguarí, Paraguay; SMF 100696 (adult female) collected on 16 April 2016 by P. Cacciali, F. Bauer and J. Méndez, at Cerro de Tobatí (25.2854º S, 57.0934º W, 157 masl), Cordillera, Paraguay.

Diagnosis. A medium-sized species of *Phyllopezus* with a color pattern consisting of irregular transversal bands or reticulations of dark colors on a grayish background, and large tubercles irregularly disposed on the body. Morphologically *P. heuteri* can be differentiated from *P. lutzae* by the irregular reticulated or banded coloration (vs dotted pattern in *P. lutzae*), and by the presence of tubercles on the body (vs absent) (Fig. S10). *Phyllopezus heuteri* is distinguished from *P. maranjonensis* by the smaller size (max. SVL 88 mm vs 115 mm in *P. maranjonensis*), presence of tubercles on the lateral surfaces of the body as well as between eyes and ear opening (vs absent from these regions) (Fig. S11), and spiny scales surrounding the ear opening (vs cycloids) (Fig. S12). *Phyllopezus heuteri* is differentiated from *P. periosus* by irregularly shaped elements of color pattern (vs well defined transversal bands in *P. periosus*), spiny scales surrounding the ear opening (vs cycloid scales) (Fig. S12), and by the contact between the two greatly enlarged postmentals (vs enlarged lateral postmentals separated by small median postmentals) (Fig. S13). *Phyllopezus heuteri* is distinguished from *P. pollicaris* by the presence of two to three larger scales (tubercle-shaped) at the mouth commissure (vs small homogeneous scales at the mouth commissure in *P. pollicaris*), lateral body tubercles reaching farther downwards (lowermost tubercle at 6–8 lateral scales from

ventrals vs 13–15), and presence of more tubercles (five to eight) between eye and ear opening (vs up to three) (Fig. S14). *Phyllopezus heuteri* can be distinguished from *P. przewalskii* by the presence of tubercles on the prescapular region and sides of the neck (vs homogeneous scalation in *P. przewalskii*) (Fig. S15), the presence of 36 to 39 scales between 4^{th} and 5^{th} toes (vs 33 to 36), and its large postmentals usually contacting only the first IL (vs contacting usually 1^{st} and 2^{nd} IL).

Description of the holotype. SVL 74 mm, tail incomplete, HL19.1 mm, HW 14.1, NED 6.9 mm, ED 4.6 mm, EO 1.6 mm, DBN 2.4 mm, TD 7.5 mm; rostral wide, with a median groove at the upper side of the scale; nares surrounded by rostral, nasorostral, supranasal, postnasal, and first SL; SL: 10/11; USL: 15/13; upper surface of the muzzle with a shallow median depression; lateral and upper surfaces of the head covered with granular juxtaposed scales, with scattered tubercles on the upper surface starting at the level of the posterior edge of the eye; supraocular scales spine-shaped posterior to the level of the center of the eye; IL: 9/10; mental bell-shaped with the narrower part posteriorly; two greatly enlarged postmentals contacting each other, the mental, the first IL, and a small portion of the second IL; postmentals followed by a row of five smaller scales; scales under the head gradually reducing in size posteriorly; dorsal and lateral parts of the neck covered with granular juxtaposed scales with irregular rows of tubercles; throat region with juxtaposed and cycloid homogeneous scales; dorsum of the body with granular scales and tubercles approximately 2.5 times the size of the surrounding granular scales, disposed in 8 or 9 irregular rows on each side reaching onto flanks; ventral scales cycloid and imbricate, arranged in 32 longitudinal rows at midbody; limbs covered with granular juxtaposed scales, except on the dorsal surface of the upper arms and thighs that present slightly imbricated scales; forelimbs more slender than hind limbs; infradigital lamellae of hands starting from pollex were recorded as follows: 5/6 - 10/9 - 10/11 - 10/10 - 9/8; infradigital lamellae of feet starting from hallux were recorded as follow: 6/5 - 10/9 - 11/11 - 10/12 - 8/9; claws enclosed by a sheath of six rows of scales; a single slightly developed post-cloacal tubercle on each side; tail with imbricate, cycloid scales that are smaller on the dorsal and larger on the lateral surfaces, and an enlarged median subcaudal row of scales covering most of the ventral surface.

Coloration in life. (Fig. 7) Dorsal ground color of body Pale Neutral Gray (296), with irregular transversal bands grading from Brownish Olive (292) into Raw Umber (280), bordered posteriorly by Cream White (52) and interrupted by a Beige (254) vertebral stripe. Lateral surfaces of the body Pale Neutral Gray (296) with scattered Raw Umber (280) speckling. Venter Cream White (52). Dorsal and lateral surfaces of the head Smoke Gray (267) with Cream White (52) and Raw Umber (280) speckles more concentrated on the occipital area. Ventral surface of the head Cream White (52) with Hair Brown (227) stipples concentrated in the infralabial area. Limbs Pale Neutral Gray (296) with reticula of Raw Umber (280) dorsally, and immaculate Cream White (52) ventrally. Ground color of the tail (only original portion of the tail described) Pale Gray (262) with irregular dorsal (transverse) bands reaching onto flanks that are Light Drab (269) grading into Raw Umber (280) posteriorly followed by Smoky White (261) halo. Ventral surface of the tail Pale Horn Color (11).

Coloration in preservative. (After five years in ethanol 70%). Dorsal Ground color Medium Neutral Gray (298), with irregular transversal bands Vandyke Brown (282), posteriorly bordered by Lavender (202); interrupted by a Pale Mauve (204) vertebral stripe. Lateral surfaces of the body Medium Neutral Gray (298) with scattered Dusky Brown (285) speckles. Venter Smoky White (261). Dorsal and lateral surfaces of the head Smoke Gray (267) with Pale Buff (1) and Raw Umber (280) speckles. Ventral surface of the head Smoky White (261) with Fawn Color (258) stipples concentrated in the infralabials. Limbs Light Neutral Gray (297) with reticulum of Brownish Olive (292) on the dorsal side of arms and Jet Black (300) on legs, and immaculate Smoky White (261) on the ventral surface. Ground color of the tail (only original portion of the tail described) Pale Gray (262) with irregular dorsal (transverse) diffused Smoke Gray (266) bands reaching onto flanks grading into Vandyke Brown (282) posteriorly followed by Smoky White (261) halo. Ventral surface of the tail Smoky White (261) with lateral suffusions of Smoke Gray (207).

Variation among the paratypes. SVL ranging from 60 to 73 mm in males and 77 to 88 mm in females (mean SVL 72.8 mm for both sexes combined); None of the tails of our

examined specimens is complete and original (being either regenerated or broken and incomplete); HL 16.5–21.8 mm (25.6–27.5 % of SVL in males and 24.4–25.8 % in females); HW 12.2–18.6 mm (0.65–0.82 proportion HW/HL in males and 0.73–0.85 in females); NED 6.1–8.3 mm; ED 3.8-5.0 mm (0.60–0.64 proportion ED/NED in males, and 0.59–0.66 in females); EO 1.6–2.0 mm (9.5–10.9 % of HL in males and 8.2–10.6 % in females); DBN 2.0–2.8 mm (14.5–18.8 % of HW in males and 14.2–17.0 % in females); TD 7.2–8.5 mm (11.6–12.0 % of SVL) in males, and 7.8–10.6 mm (10.1–12.0 % of SVL) in females; rostral scale always of a similar shape, but the median groove can extend more than half of the scale downwards; SL 9 or 10; USL from 10 to 15; IL 8 or 9; postmentals contacting only the first IL in all specimens except the holotype; one specimen (MNHNP 12001) with three large postmentals; 27 to 33 longitudinal rows of ventral scales at midbody; infradigital lamellar variation for hands and feet is presented in Table 1; post-cloacal tubercles from two to three per side.

The most remarkable aspects of variation in color pattern are visible on the dorsum. The largest examined specimen (MNHNP 12111) has no pattern other than the vertebral stripe present in all specimens. The remaining specimens show a pattern composed by bands similar to that of the holotype, but the bands are formed by a mottling in MNHNP 11975, and two specimens exhibit only very diffuse bands. One specimen (MNHNP 12001) has a paler ground color, and another (SMF 100696) exhibits a darker ground color.

Distribution. *Phyllopezus heuteri* is known from rocky outcrops at three localities along the “Cordillera de Los Altos” formation (Fig. 6) in the Paraguayan departments Cordillera and Paraguarí, at 268–428 m above sea level.

Natural History. Given the scarcity of records this gecko is not well known. It appears to be a nocturnal species found on sandstone rocky hills. It seeks shelter in caves or cracks in the rocks. Its coloration is mimetic with the lichens and mosses that cover the rocks’ surfaces. The area where *Phyllopezus heuteri* is present has a marked seasonality regarding rainfall and temperature (dry and cold season from May to September) with an annual precipitation of about 1200 to 1300 mm. The vegetation associated with the rocky environment is composed by thorny or thick plants such as *Polycarpaea*

hassleriana, *Cereus* sp., *Bromelia* sp. among others. Nothing is known about its feeding or reproductive habits, or any other aspects of its behavior.

Etymology. The specific name is a patronym for biologist Dr. Horst Heuter from Berlin, Germany, in recognition of the financial support of taxonomic research provided by Dr. Heuter through the BIOPAT initiative.

5. Discussion

The fact that *Phyllopezus przewalskii* was long considered as a subspecies of *P. pollicaris* can be attributed to the large overlaps in all proposed diagnostic characters: number of lamellae under the fourth toe (9 to 13 in *P. pollicaris*, and 8 to 11 in *P. przewalskii*), ventral scales at midbody (28 to 32 in *P. pollicaris*, and 26 to 29 in *P. przewalskii*), and post-cloacal tubercles at the sides of the vent (always present in *P. pollicaris*, not always present in adults of *P. przewalskii*) [20–21]. According to Werneck et al. [11] none of the *P. pollicaris* clades reaches Paraguay where only *P. przewalskii* is present, but the "diagnostic" characters of Paraguayan specimens show a variation beyond those established for either *P. pollicaris* or *P. przewalskii* (Table 1). Werneck et al. [11] provided evidence that the genus *Phyllopezus* is composed of multiple cryptic lineages, several of which are not formally described as species yet and therefore were not included in our morphological comparisons. The name *P. pollicaris* cannot confidently be assigned to any of the Werneck et al. [11] clades because the type locality *Thecadactylus pollicaris* ("*sylvis interioris Bahiae campestribus*") (Spix 1825: 17 [58]) is not precise enough. According to the distribution map of the *Phyllopezus* clades of Werneck et al. ([11]: last page of Supporting Information file) four candidate species are present in the Brazilian State of Bahia: *Phyllopezus pollicaris* clades I, III, VI, and VIII. Even though Bahia was much smaller at the time when the type specimen was collected, still three clades remain as possible candidates for the true *P. pollicaris* (Fig. 8).

To avoid confusion and get as closely as possible to the "real" *P. pollicaris* sensu stricto, our characterization of *P. pollicaris* is based on two paralectotypes that according to Müller and Brongersma [59] were part of the original type series that the description of Spix (1825 [58]) is based on, and came from the same locality as the

lectotype (ZSM 2510/0, considered lost, Michael Franzen *comm. pers.*). Nevertheless, paralectotypes have no legal status as name-bearing types, and therefore this issue remains to be solved.

Similarly, the taxonomic status of *Phyllopezus goyazensis* (Peters 1878) also needs to be accounted for. The *P. goyazensis* type locality of is stated as 'Goyaz' in Brazil [60], which, at the time, was larger than the current boundaries of modern Goiás state and included what is now Goiás and Tocantins. Three of the putative species from Werneck et al. [11] occur in this area, clades IV, VII, and VIII. Fortunately, the *P. goyazensis* type is still extant (ZMB 9079), which should make assigning that name easier than the aforementioned *P. pollicaris* problem.

Morphological differences are slight among the closest relative species of *Phyllopezus* (*P. pollicaris*, *P. przewalskii*, and *P. heuteri*), rendering it a genus with remarkable morphological crypsis. Nevertheless, *Phyllopezus heuteri* is relatively easily distinguishable from the phylogenetically most distant species: *P. lutzae*, *P. maranjonensis*, and *P. periosus*, showing more resemblance with *P. pollicaris* and *P. przewalskii*. We found high variation in traditional diagnostic characters for *P. przewalskii* (Table 1) which overlap with those proposed for *P. pollicaris* [20–21]. Nevertheless, we found some characters in head and body scalation, which allow distinguishing *P. heuteri* and *P. przewalskii*, respectively, from *P. pollicaris* sensu stricto. *Phyllopezus heuteri* and *P. przewalskii* are morphologically closely related, but there are differences in the number of scales between 4th and 5th toes, and in the shape of scales on the sides of the neck as well as prescapular region (Fig. S11). Additionally, *P. przewalskii* seems to have the cephalic tubercles more developed but we found some intraspecific variation in that character. With respect to coloration, like many other geckos *P. heuteri* is capable of metachrosis, which can cause it to mimic different substrates (Fig. 9).

Phyllopezus heuteri shows a high degree of genetic differentiation according to *p*-distances, having the lowest distance to its sister clade *P. przewalskii* (Appendix S3). *Phyllopezus pollicaris* Clade VII and *P. pollicaris* Clade VIII have the smallest distances (7.77–10.53%) among all pairs of clades. Werneck et al. [11] found higher genetic distances even at the intraspecific level with values that reach 27.5% between

haploclades of *P. pollicaris* Clade VIII; and genetic distances of 16.9 to 24.6% between *P. pollicaris* Clade VII and *P. pollicaris* Clade VIII.

6. Conclusions

For almost a century, the genus *Phyllopezus* was considered as monotypic with two subspecies, until two more species were described and more recent works revealed an even higher species-level diversity [10–11]. We add to this growing knowledge with the description of a new species from a poorly sampled area at the southern margin of the distributional range of the genus. We highlight the importance of a morphological analysis that can put practicable names on the candidate species recognized by genetic data. *Phyllopezus heuteri* is a rock dweller as many other members of the genus, and the split between this species and *P. przewalskii* (its closest relative) occurred before the latter species colonized the xerophytic Chaco. This paper represents another contribution oriented to resolve the taxonomy of the genus *Phyllopezus*, and we hope that the unnamed clades can be morphologically diagnosed in further researches.

Data availability

Sequences used for this study are stored in GenBank. Numbers pending.

Sequence matrices and trees are deposited in TreeBASE. Number pending.

Conflicts of interests

The authors declare that they have no conflicts of interests.

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685

Appendix 1

Examined specimens

Phyllopezus lutzae (3)

Brazil: Bahia: São Salvador (AMNH 65381, MCZ 46190 [Syntype], UMMZ 115644 [Syntype]).

Phyllopezus maranjonensis (3)

Peru: Amazonas: Quebrada Honda (ZFMK 84995–7 [Paratypes]).

Phyllopezus periosus (3)

Brazil: Paraíba: Cabeceiras (AMNH 131825, MCZ 172929–30).

Phyllopezus pollicaris (2)

Brazil: Bahia (no more specific locality data) (ZSM 165/0/1–2 [Paralectotypes]).

Phyllopezus przewalskii (59)

Argentina: Chaco: Fuerte Esperanza (LJAMM-CNP 12094–5). Formosa: Ingeniero Juárez (LJAMM-CNP 12071, 12084; MACN 3230). Santiago del Estero: Pampa de los Guanacos (MACN 4999). **Bolivia:** Santa Cruz: San Antonio de Parapeti (MACN 47006–7, 47009–10). Tarija: Villamontes (SMF 29259–64). **Paraguay:** (No additional data) MNHNP 11174, 11176. Alto Paraguay: Bahía Negra (MNHNP 10202, 11691); Colonia Potrerito (MNHNP 3371); Parque Nacional Defensores del Chaco (MNHNP 2850 , 4298); Puerto Ramos (MNHNP 3243–6, 3248–9). Amambay: Parque Nacional Cerro Corá (MNHNP 6983, 7046, 7640–4, 11919). Boquerón: Establecimiento Ko'e Pyahu (MNHNP 11069); Estancia Agropil S.A. (MNHNP 8042); Estancia Amistad (SMF 100495–6); Estancia Jabalí (MNHNP 8043–4, 8071); Estancia Mbutú Retã (MNHNP 3818); Filadelfia (MNHNP 2851); Parque Nacional Teniente Enciso (MNHNP 2853, 3253, 4300, 11797, 11847, 11857). Concepción: Vallemí (MACN 12860–6).

Figure 1

Figure 1. Distribution of the genus *Phyllopezus* according to Koch et al. [23] and Werneck et al. [11] indicating collection localities of our specimens. Red oval: Tobatí (in Los Altos mountain range). Square: Estancia La Amistad. Triangle: Parque Nacional Cerro Corá.

Figure 2

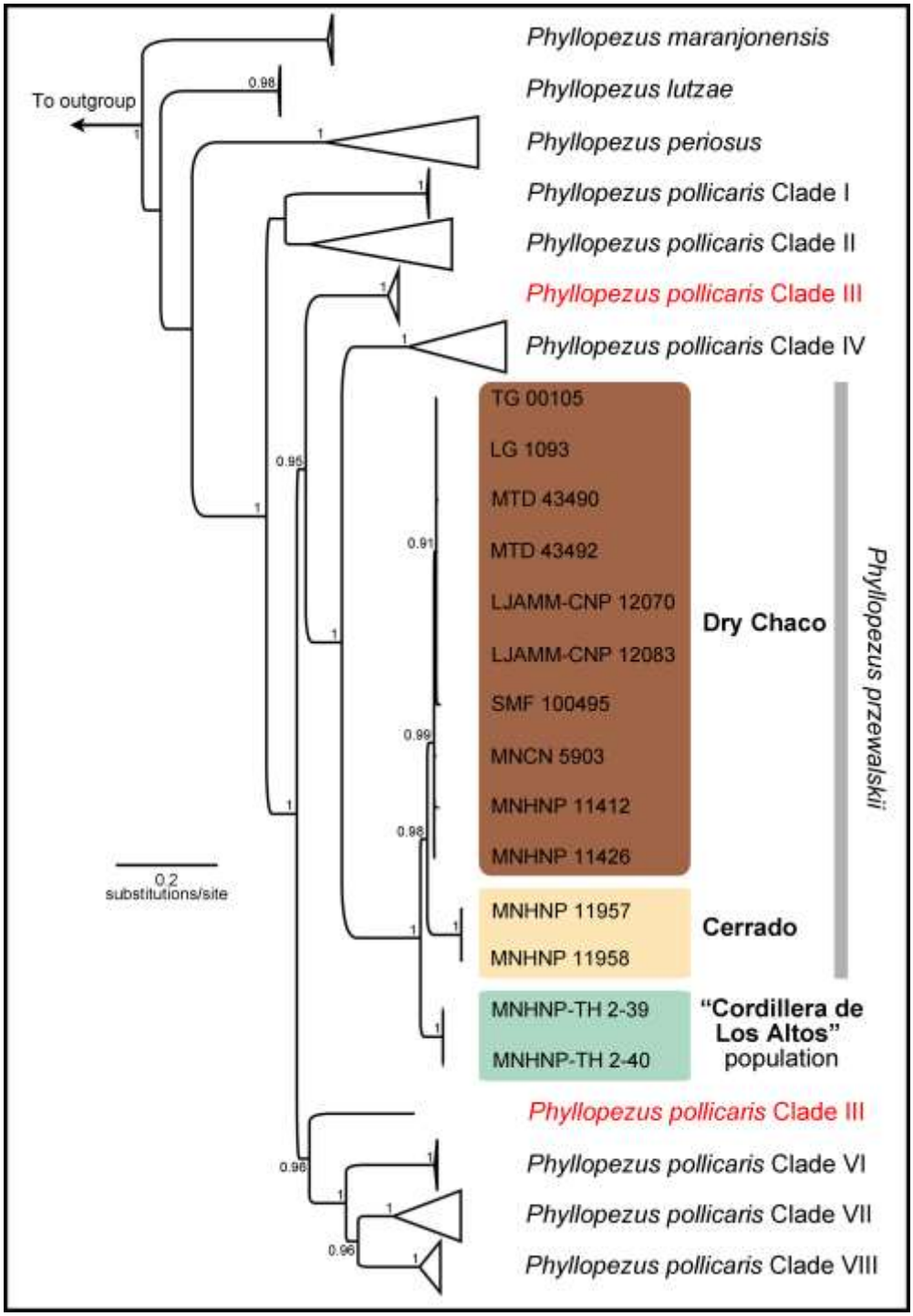
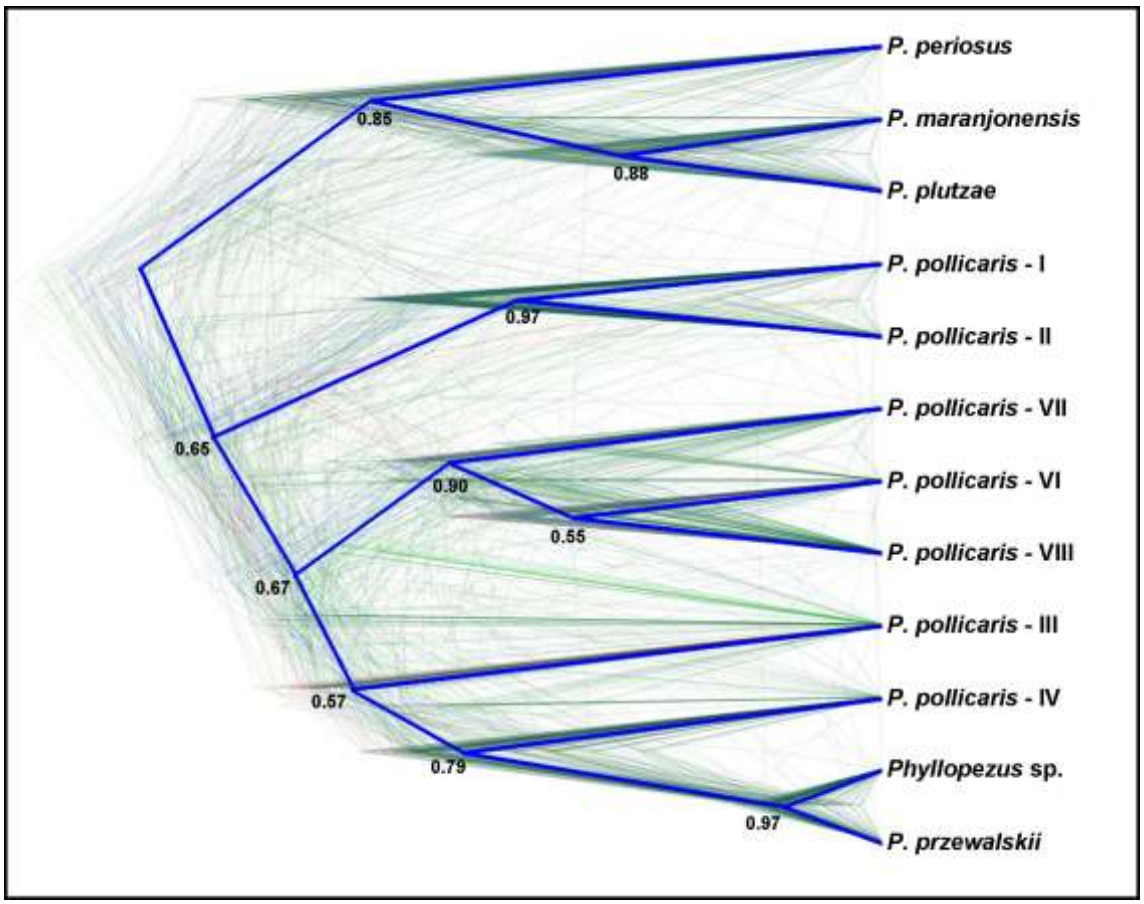


Figure 2. Bayesian tree obtained from concatenated mitochondrial genes (16S, Cytb, and ND2) of the gecko genus *Phyllopezus*. Numbered clades of *P. pollicaris* represent the groups recognized by Werneck et al. [11]. Most of clades (with the exception of *P. przewalskii*) are collapsed. For details on specimens' allocation see Figure S8. In red, there is a clade that appears to be polyphiletic. Samples from Dry Chaco are shown in a brown box, from Cerrado in a yellow box, and in a green box specimens from "Cordillera de los Altos". Only PP values higher than 0.9 are shown. Outgroup: *Phyllodactylus unctus*.

731 **Figure 3**



732

733 **Figure 3.** Species tree inferred with *BEAST showing density of trees proportional to
734 frequency of occurrence (thin lines) drawn in DensiTree, and the consensus tree (blue lines)
735 with the posterior probability for each node.

736

Figure 4

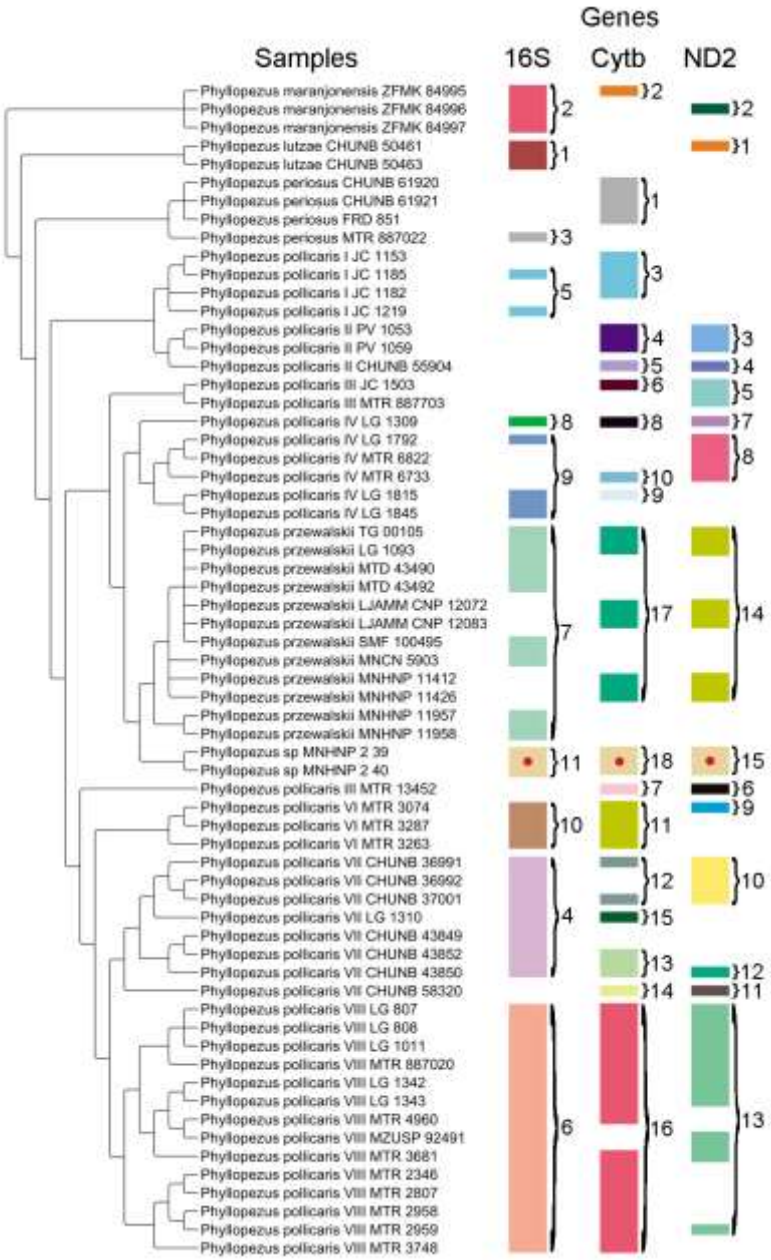
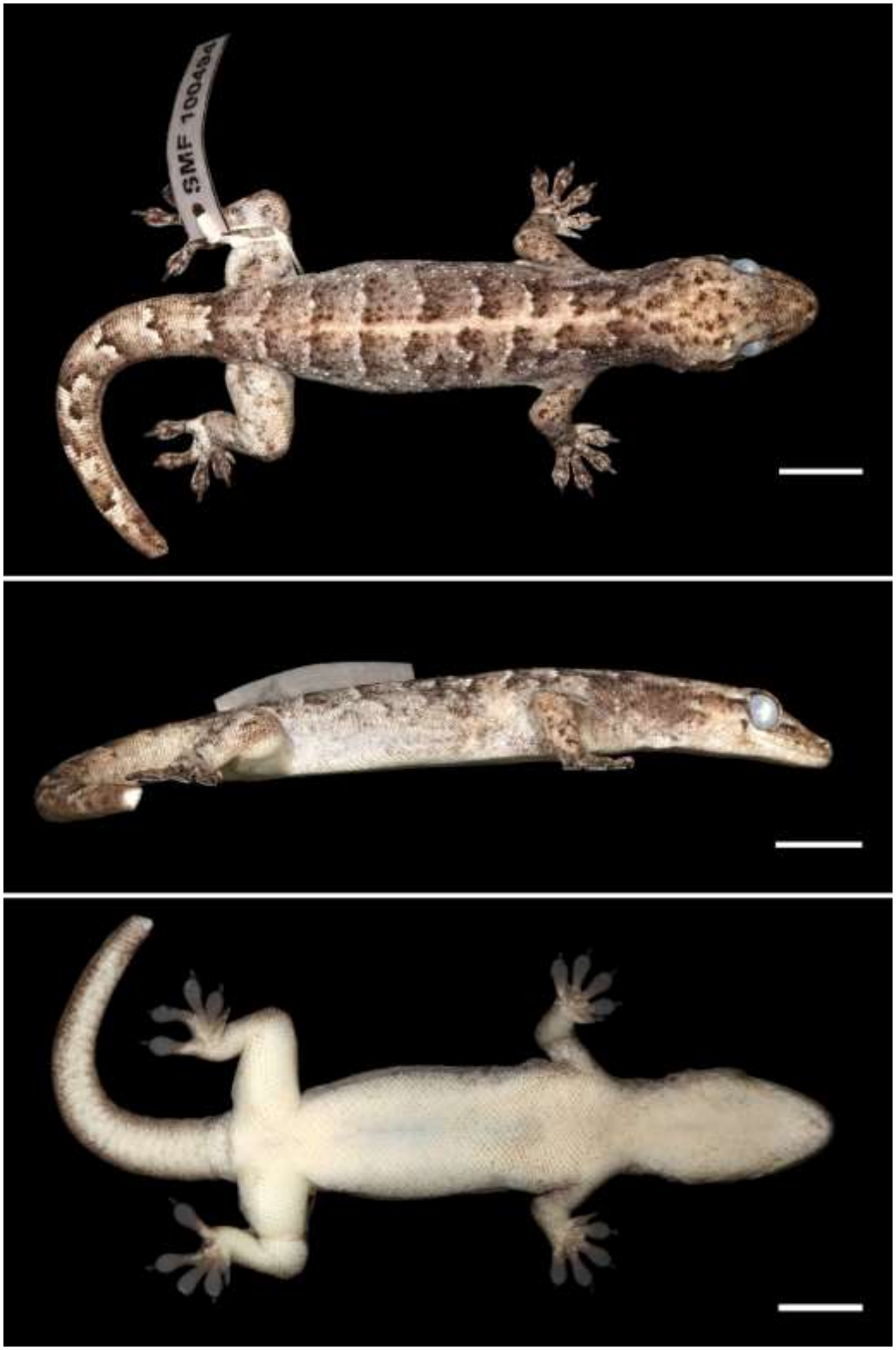


Figure 4. Results of ABGD analysis for each gene. Colors represent groups identified by ABGD for each gene, with group numbers presented at the right. As seen, not all samples were available for all genes. Group colors do not represent the same grouping among genes, except for samples from "Cordillera de Los Altos" which are identified with a cream colored box with a red central dot. Base phylogeny (at the left) same as for Figure 2.

745 **Figure 5**



746

747 **Figure 5.** Dorsal (above), lateral (middle), and ventral (below) views of the holotype (SMF
748 100494) of *Phyllopezus heuteri*. Scale bars = 10 mm.

749

Figure 6

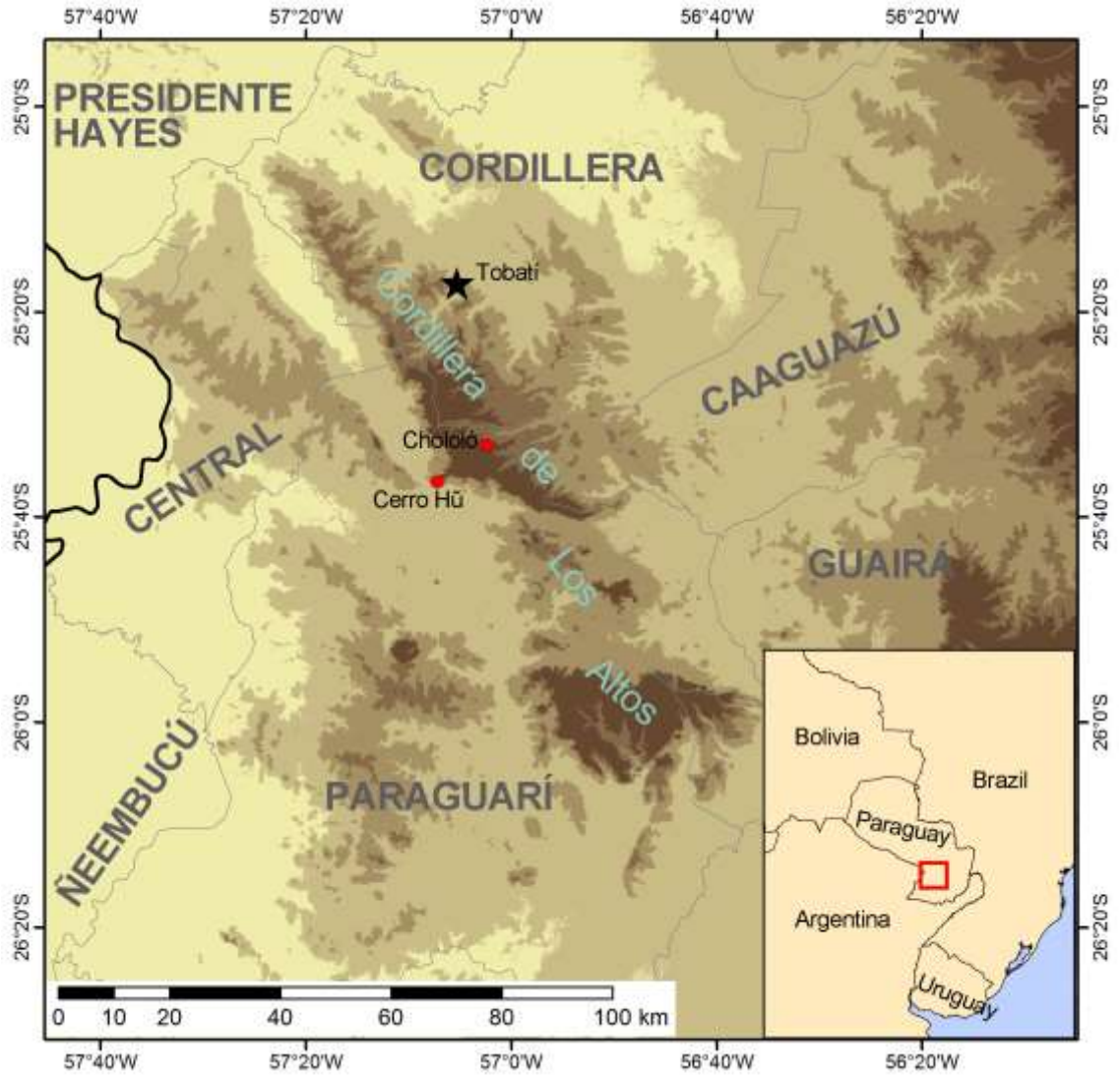


Figure 6. Known records of *Phyllopezus heuteri* showing the type locality (black star) and additional localities (red dots). Capitalized names refer to Paraguayan Departments.

Figure 7

Figure 7. Coloration in life of the holotype (SMF 100949) of *Phyllopezus heuteri*.

Figure 8

Figure 8. Candidate species present in the Brazilian State of Bahia (type locality for *P. pollicaris*), considering current and old borders.

Figure 9

Figure 9. Differences in coloration of *P. heuteri* between animals on light green lichens and pinkish rock substrates (above), and animals on rocks fully covered by dark green lichens (below).

Table 1

Morphological variation among *Phyllopezus heuteri*, *P. pollicaris*, and *P. przewalskii*.

	Hands					Feet					
	1	2	3	4	5	1	2	3	4	5	Vent
Ppol	6–7	10–12	11	9–11	9–10	6–7	10–11	11–12	9–12	10–11	28–30
Pprz	5–6	8–9	9–11	9–11	8–9	5–6	8–10	10–12	10–11	8–10	24–33
Pheu	3–6	7–10	9–11	9–11	7–9	5–6	9–10	10–12	9–11	8–9	27–33

Infradigital lamellar variation (including holotype) of *Phyllopezus heuteri* (Pheu) and the most closely related species, *P. pollicaris* (Ppol) and *P. przewalskii* (Pprz). Variation is presented from the pollex (1) to the 5th finger of the hands, and from the hallux (1) to the 5th toe of the feet. Also presented is the range of longitudinal rows of ventral scales (Vent).

Supplemental Information

Appendix S1: Environmental traits of *Phyllopezus* habitats in Paraguay.

Appendix S2: Genbank accession numbers of sequences used in this work.

Appendix S3: Pairwise genetic distances (16S).

Appendix S4: Results of the ABGD assessment.

Figure S1: Scales between 4^{th} and 5^{th} digital pads.

Figure S2: Phylogenetic tree inferred from 16S using BI approach.

Figure S3: Phylogenetic tree inferred from 16S using ML approach.

Figure S4: Phylogenetic tree inferred from Cytb using BI approach.

Figure S5: Phylogenetic tree inferred from Cytb using ML approach.

Figure S6: Phylogenetic tree inferred from ND2 using BI approach.

Figure S7: Phylogenetic tree inferred from ND2 using ML approach.

Figure S8: Phylogenetic tree inferred from concatenated genes, using BI approach.

Figure S9: Phylogenetic tree inferred from concatenated genes, using ML approach.

Figure S10: Dorsal scalation.

Figure S11: Head and lateral body scalation.

Figure S12: Ear opening.

Figure S13: Mental scalation.

Figure S14: Lateral scalation of the head.

Figure S15: Prescapular scalation.

Appendix S1

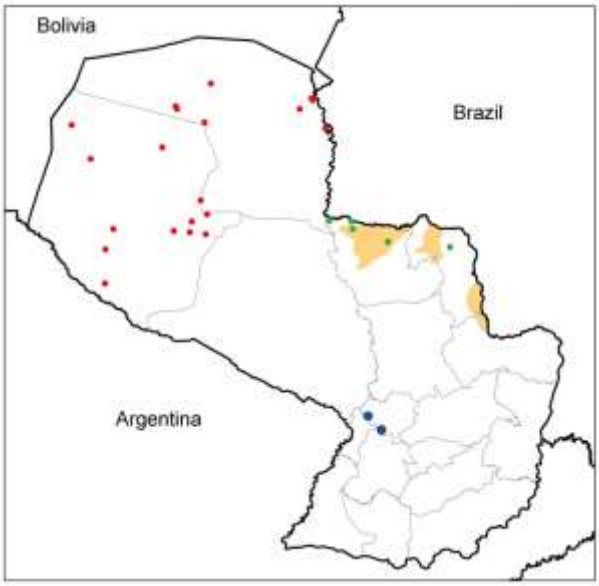
Environmental traits of *Phyllopezus* habitats in Paraguay

Dry Chaco

The Dry Chaco is a xerophytic environment adapted to low precipitation (400–700 mm/yr). The vegetation is implanted on clayish soils, with a dominance of thorny shrubs and cacti, and no herbaceous stratum. In this environment, *Phyllopezus* is mostly a ground dweller, but also frequently seen on trees.

Distribution of Dry Chaco in Paraguay (brown area), showing the different populations of *Phyllopezus*: Dry Chaco (red dots), Cerrado population (green dots), and Cordillera de los Altos population (blue dots).

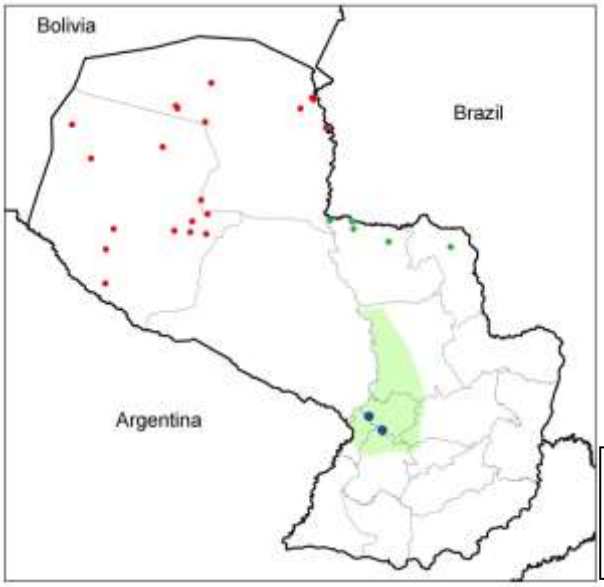
Cerrado



The Cerrado is a Neotropical savanna dominated by grasses adapted to periodic fires. Associated to this environment, there are islands of tortuous small trees with cork-like bark. There are also tall green forests along the rivers or streams present in the area.
In this environment, *Phyllopezus* is exclusively a ground dweller, inhabiting the rocky hills scattered on the area.

Distribution of Cerrado in Paraguay (orange area), showing the different populations of *Phyllopezus*: Dry Chaco (red dots), Cerrado population (green dots), and Cordillera de los Altos population (blue dots).

Cordillera de Los Altos (Litoral Central ecoregion)



Cordillera de Los Altos is a chain of rocky hills of up to 450 masl, located in the south of the Litoral Central ecoregion. The environment has components of the Humid Chaco, with grasses and shrubs covering most of the area, tall forests and abundance of the Paraguayan coconut palm *Acrocomia aculeata*.

In this environment, *Phyllopezus* is a ground dweller, inhabiting the rocky hills where the lizard is mimetic with the lichens on the stones.

Distribution of Litoral Central in Paraguay (green area), showing the different populations of *Phyllopezus*: Dry Chaco (red dots), Cerrado population (green dots), and Cordillera de los Altos population (blue dots).

Appendix S2

Genbank accession numbers of sequences used in this work.

Species	Voucher	16S	Cytb	ND2
Phyllodactylus unctus		HQ896027		
Phyllopezus lutzae	CHUNB 50461	JN935548	MD	MD
Phyllopezus lutzae	CHUNB 50463	JN935550	MD	MD
P. maranjonensis	ZFMK 84995	JN935555	JQ826820	MD
P. maranjonensis	ZFMK 84996	JN935556	MD	JX041416
P. maranjonensis	ZFMK 84997	JN935557	MD	MD
P. periosus	CHUNB 61920	MD	JQ826827	MD
P. periosus	CHUNB 61921	MD	JQ826826	MD
P. periosus	FRD 851	MD	JQ826824	MD
P. periosus	MTR 887022	JN935552	MD	MD
P. pollicaris Clade I	JC 1153	MD	JQ826836	MD
P. pollicaris Clade I	JC 1182	MD	JQ826834	MD
P. pollicaris Clade I	JC 1185	JN935553	JQ826838	MD
P. pollicaris Clade I	JC 1219	JN935554	MD	MD
P. pollicaris Clade II	PV 1053	MD	JQ826840	JQ825289
P. pollicaris Clade II	PV 1059	MD	JQ826841	JQ825290
P. pollicaris Clade II	CHUNB 55904	MD	JQ826842	JQ825288
P. pollicaris Clade III	JC 1503	MD	JQ826958	JQ825294
P. pollicaris Clade III	MTR 13452	MD	JQ826955	JQ825291
P. pollicaris Clade III	MTR 887703	MD	MD	JQ825293
P. pollicaris Clade IV	LG 1309[B1]	JN935567	JQ826845	JQ825492
P. pollicaris Clade IV	LG 1792[B2]	JN935572	MD	JQ825495
P. pollicaris Clade IV	LG 1815	JN935573	JQ826982	MD
P. pollicaris Clade IV	LG 1845[B3]	JN935574	MD	MD
P. pollicaris Clade IV	MTR 6733	MD	JQ826849	JQ825494
P. pollicaris Clade IV	MTR 6822	MD	MD	JQ825497
P. pollicaris Clade VI	MTR 3074	JN935584	JQ826963	JQ825369
P. pollicaris Clade VI	MTR 3263	JN935585	JQ826960	MD
P. pollicaris Clade VI	MTR 3287	JN935586	JQ826964	MD
P. pollicaris Clade VII	CHUNB 36991	JN935559	JQ827012	JQ825354
P. pollicaris Clade VII	CHUNB 36992	JN935560	MD	JQ825351
P. pollicaris Clade VII	CHUNB 37001	JN935561	JQ827011	JQ825352
P. pollicaris Clade VII	CHUNB 43849	JN935562	MD	MD
P. pollicaris Clade VII	CHUNB 43850	JN935563	JQ827039	JQ825317
P. pollicaris Clade VII	CHUNB 43852	JN935564	JQ827033	MD
P. pollicaris Clade VII	CHUNB 58320	MD	JQ826993	JQ825333
P. pollicaris Clade VII	LG 1310	JN935568	JQ827049	MD
P. pollicaris Clade VIII	LG 807	JN935575	JQ827102	JQ825393
P. pollicaris Clade VIII	LG 808	JN935576	JQ827107	JQ825394
P. pollicaris Clade VIII	LG 1011	JN935566	JQ827100	JQ825390
P. pollicaris Clade VIII	LG 1342	JN935570	JQ827123	JQ825423
P. pollicaris Clade VIII	LG 1343	JN935571	JQ827124	JQ825424
P. pollicaris Clade VIII	MTR 2346	JN935580	JQ827056	MD
P. pollicaris Clade VIII	MTR 2807	JN935581	JQ827057	MD
P. pollicaris Clade VIII	MTR 2958	JN935582	JQ827053	MD
P. pollicaris Clade VIII	MTR 2959	JN935583	JQ827054	JQ825489
P. pollicaris Clade VIII	MTR 3681	JN935587	JQ827073	JQ825374
P. pollicaris Clade VIII	MTR 3748	JN935588	JQ827133	MD

P. pollicaris Clade VIII	MTR 4960	JN935589	JQ827130	MD
P. pollicaris Clade VIII	MTR 887020	JN935558	JQ827087	JQ825376
P. pollicaris Clade VIII	MZUSP 92491	JN935590	MD	JX041417
P. przewalskii	TG 00105	JN935565	JQ826885	JQ825594
P. przewalskii	LG 1093[B9]	JN935569	JQ826890	JQ825593
P. przewalskii	MTD 43490[B10]	JN935578	MD	MD
P. przewalskii	MTD 43492[B11]	JN935579	MD	MD
P. przewalskii	MNCN 5903[B12]	JN935577	MD	JQ825362
P. przewalskii	LJAMM-CNP 12072	MD	JQ826895	JQ825577
P. przewalskii	LJAMM-CNP 12083	MD	JQ826901	JQ825583
P. przewalskii	**SMF 100495**[B8]	pending	MD	MD
P. przewalskii	MNHNP 11412	MD	JQ826880	JQ825595
P. przewalskii	MNHNP 11426	MD	JQ826879	JQ825596
P. przewalskii	**MNHNP 11957**[B6]	pending	MD	MD
P. przewalskii	**MNHNP 11958**[B7]	pending	MD	MD
Phyllopezus sp. nov.	**MNHNP-TH 2-39**[B5]	pending	pending	pending
Phyllopezus sp. nov.	**MNHNP-TH 2-40**[B4]	pending	pending	pending

819

820

Appendix S3

Pairwise genetic distances (in percentages) – 16S

	P. lutzae	*P. maranjonensis*	*P. periosus*	*P. przewalskii*	*P. pollicaris* – I	*P. pollicaris* – IV	*P. pollicaris* – VI	*P. pollicaris* – VII	*P. pollicaris* – VIII	*Phyllopezus* – Los Altos
Phyllopezus lutzae	0									
P. maranjonensis	11.5 11.8	<0.01 0.3								
P. periosus	14.6	13.7 14.0	-							
P. przewalskii	14.8 15.4	13.7 14.6	15.4 17.0	<0.01 4.4						
P. pollicaris – I	13.2	13.7 14.6	15.9	11.5 12.1	0					
P. pollicaris – IV	15.4 16.8	14.0 14.3	16.2	9.9 11.0	13.7 14.8	<0.01 0.3				
P. pollicaris – VI	15.1 15.4	14.0 16.5	16.2 16.5	9.6 10.4	13.2	12.1 13.2	<0.01 0.5			
P. pollicaris – VII	15.4 16.5	15.9 17.3	15.9 17.9	11.5 14.0	13.2 15.1	13.2 15.7	9.1 12.4	<0.01 6.6		
P. pollicaris – VIII	14.6 15.1	13.7 14.8	16.2 17.3	10.4 13.2	12.4 14.3	11.3 12.6	8.2 9.9	7.1 9.9	<0.01 3.3	
Phyllopezus – Los Altos	16.2	15.9 16.2	16.8	12.6 14.3	13.2	11.8	12.4 12.6	12.6 14.3	11.8 12.1	<0.01

Genetic uncorrected p-distances among clades (species formally described plus unnamed taxa according to Werneck et al. [19]), showing minimum and maximum values.

Appendix S4

Results of the ABGD assessment

16S

Initial Partition with prior maximal distance P=2.15e-02

Distance Simple Dist MinSlope=0.700000

Group[1] n: 2 ;id: Phyllopezus_lutzae_CHUNB-50461 Phyllopezus_lutzae_CHUNB-50463

Group[2] n: 3 ;id: Phyllopezus_maranjonensis_ZFMK-84995 Phyllopezus_maranjonensis_ZFMK-84996 Phyllopezus_maranjonensis_ZFMK-84997

Group[3] n: 1 ;id: Phyllopezus_periosus_MTR-887022

Group[4] n: 7 ;id: Phyllopezus_pollicaris_VII_CHUNB-36991 Phyllopezus_pollicaris_VII_CHUNB-36992 Phyllopezus_pollicaris_VII_CHUNB-37001 Phyllopezus_pollicaris_VII_CHUNB-43849 Phyllopezus_pollicaris_VII_CHUNB-43850 Phyllopezus_pollicaris_VII_CHUNB-43852 Phyllopezus_pollicaris_VII_LG-1310

Group[5] n: 2 ;id: Phyllopezus_pollicaris_I_JC-1185 Phyllopezus_pollicaris_I_JC-1219

Group[6] n: 14 ;id: Phyllopezus_pollicaris_VIII_LG-807 Phyllopezus_pollicaris_VIII_LG-808 Phyllopezus_pollicaris_VIII_LG-1011 Phyllopezus_pollicaris_VIII_LG-1342 Phyllopezus_pollicaris_VIII_LG-1343 Phyllopezus_pollicaris_VIII_MTR-2346 Phyllopezus_pollicaris_VIII_MTR-2807 Phyllopezus_pollicaris_VIII_MTR-2958 Phyllopezus_pollicaris_VIII_MTR-2959 Phyllopezus_pollicaris_VIII_MTR-3681 Phyllopezus_pollicaris_VIII_MTR-3748 Phyllopezus_pollicaris_VIII_MTR-4960 Phyllopezus_pollicaris_VIII_MTR-887020 Phyllopezus_pollicaris_VIII_MZUSP-92491

Group[7] n: 8 ;id: Phyllopezus_przewalskii_LG-1093 Phyllopezus_przewalskii_MNCN-5903 Phyllopezus_przewalskii_MTD-43490 Phyllopezus_przewalskii_MTD-43492 Phyllopezus_przewalskii_TG-00105 Phyllopezus_przewalskii_MNHNP-11957 Phyllopezus_przewalskii_MNHNP-11958 Phyllopezus_przewalskii_SMF-100495

Group[8] n: 1 ;id: Phyllopezus_pollicaris_IV_LG-1309

Group[9] n: 3 ;id: Phyllopezus_pollicaris_IV_LG-1792 Phyllopezus_pollicaris_IV_LG-1815 Phyllopezus_pollicaris_IV_LG-1845

Group[10] n: 3 ;id: Phyllopezus_pollicaris_VI_MTR-3074 Phyllopezus_pollicaris_VI_MTR-3263 Phyllopezus_pollicaris_VI_MTR-3287

Group[11] n: 2 ;id: Phyllopezus_sp_MNHNP-2-40 Phyllopezus_sp_MNHNP-2-39

Tree

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:0.000780,Phyllopezus_pollicaris_IV_LG-1792 _group 9   :0.002398)
:0.038772,Phyllopezus_pollicaris_IV_LG-1309 _group 8   :0.041760)
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_group 5   :0.004856,Phyllopezus_pollicaris_I_JC-1185 _group 5   :-
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3   :0.083999,(((Phyllopezus_maranjonensis_ZFMK-84997 _group 2
:0.001053,Phyllopezus_maranjonensis_ZFMK-84995 _group 2   :0.001053)
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:0.001048,Phyllopezus_lutzae_CHUNB-50461 _group 1   :0.001048)
:0.059645)            :0.007278)            :0.019520)
:0.011039)            :0.007160,((Phyllopezus_pollicaris_VI_MTR-3287
_group 10  :0.001053,Phyllopezus_pollicaris_VI_MTR-3074 _group 10
:0.001053)            :0.002826,Phyllopezus_pollicaris_VI_MTR-3263
_group 10  :0.002437)           :0.050835)
:0.013014,((Phyllopezus_pollicaris_VII_LG-1310 _group 4
:0.027765,(Phyllopezus_pollicaris_VII_CHUNB-37001 _group 4
:0.001053,(Phyllopezus_pollicaris_VII_CHUNB-36992 _group 4
:0.001053,Phyllopezus_pollicaris_VII_CHUNB-36991 _group 4   :0.001053)
:-0.000000)           :0.034742)
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:0.002476,Phyllopezus_pollicaris_VII_CHUNB-43850 _group 4   :0.002799)
:0.025206)            :0.018747)
:0.020750,(Phyllopezus_pollicaris_VIII_MTR-3748 _group 6
:0.014115,((Phyllopezus_pollicaris_VIII_MTR-2959 _group 6
:0.001046,Phyllopezus_pollicaris_VIII_MTR-2958 _group 6   :0.001046)
:0.002493,(Phyllopezus_pollicaris_VIII_MTR-2807 _group 6
:0.001086,Phyllopezus_pollicaris_VIII_MTR-2346 _group 6   :0.003098)
:0.001672)            :0.009835)            :0.001751)
:0.003941,Phyllopezus_pollicaris_VIII_MTR-3681 _group 6   :0.012086)
:0.001059);
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Cytb

Partition with prior maximal distance P=3.59e-02

Distance Simple Dist MinSlope=0.700000

Group[1] n: 3 ;id: Phyllopezus_periosus_CHUNB-61920 Phyllopezus_periosus_CHUNB-61921 Phyllopezus_periosus_FRD-851

Group[2] n: 1 ;id: Phyllopezus_maranjonensis-ZFMK84995

Group[3] n: 3 ;id: Phyllopezus_pollicaris_I_JC-1153 Phyllopezus_pollicaris_I_JC-1182 Phyllopezus_pollicaris_I_JC-1185

Group[4] n: 2 ;id: Phyllopezus_pollicaris_II_PV-1053 Phyllopezus_pollicaris_II_PV-1059

Group[5] n: 1 ;id: Phyllopezus_pollicaris_II_CHUNB-55904

Group[6] n: 1 ;id: Phyllopezus_pollicaris_III_JC-1503

Group[7] n: 1 ;id: Phyllopezus_pollicaris_III_MTR-13452

Group[8] n: 1 ;id: Phyllopezus_pollicaris_IV_LG-1309

Group[9] n: 1 ;id: Phyllopezus_pollicaris_IV_LG-1815

Group[10] n: 1 ;id: Phyllopezus_pollicaris_IV_MTR-6733

Group[11] n: 3 ;id: Phyllopezus_pollicaris_VI_MTR-3074 Phyllopezus_pollicaris_VI_MTR-3263 Phyllopezus_pollicaris_VI_MTR-3287

Group[12] n: 2 ;id: Phyllopezus_pollicaris_VII_CHUNB-36991 Phyllopezus_pollicaris_VII_CHUNB-

37001
Group[13] n: 2 ;id: Phyllopezus_pollicaris_VII_CHUNB-43850 Phyllopezus_pollicaris_VII_CHUNB-43852
Group[14] n: 1 ;id: Phyllopezus_pollicaris_VII_CHUNB-58320
Group[15] n: 1 ;id: Phyllopezus_pollicaris_VII_LG-1310
Group[16] n: 13 ;id: Phyllopezus_pollicaris_VIII_LG-807 Phyllopezus_pollicaris_VIII_LG-808 Phyllopezus_pollicaris_VIII_LG-1011 Phyllopezus_pollicaris_VIII_LG-1342 Phyllopezus_pollicaris_VIII_LG-1343 Phyllopezus_pollicaris_VIII_MTR-2346 Phyllopezus_pollicaris_VIII_MTR-2807 Phyllopezus_pollicaris_VIII_MTR-2958 Phyllopezus_pollicaris_VIII_MTR-2959 Phyllopezus_pollicaris_VIII_MTR-3681 Phyllopezus_pollicaris_VIII_MTR-3748 Phyllopezus_pollicaris_VIII_MTR-4960 Phyllopezus_pollicaris_VIII_MTR-887020
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Group[18] n: 2 ;id: Phyllopezus_sp_MNHNP-2_39 Phyllopezus_sp_MNHNP-2_40
Tree

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:0.001324,(Phyllopezus_pollicaris_VIII_LG-808 _group 16
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:0.002421,Phyllopezus_pollicaris_VIII_LG-1342 _group 16  :0.001826)
:0.015853)            :0.005850,Phyllopezus_pollicaris_VIII_MTR-3681
_group 16  :0.022826)
:0.001869,Phyllopezus_pollicaris_VIII_MTR-3748 _group 16  :0.021920)
:0.000579)            :0.007370,((Phyllopezus_pollicaris_VIII_MTR-2959
_group 16  :0.000531,Phyllopezus_pollicaris_VIII_MTR-2958 _group 16
:0.000531)            :0.005988,(Phyllopezus_pollicaris_VIII_MTR-2807
_group 16  :0.001428,Phyllopezus_pollicaris_VIII_MTR-2346 _group 16
:0.000695)            :0.002448)            :0.029300)
:0.050777,(((Phyllopezus_pollicaris_VII_LG-1310 _group 15
:0.065087,((Phyllopezus_pollicaris_VII_CHUNB-43852 _group 13
:0.004761,Phyllopezus_pollicaris_VII_CHUNB-43850 _group 13  :0.003732)
:0.038563,(Phyllopezus_pollicaris_VII_CHUNB-37001 _group 12
:0.002206,Phyllopezus_pollicaris_VII_CHUNB-36991 _group 12  :0.000978)
:0.057551)            :0.005182)
:0.006168,Phyllopezus_pollicaris_VII_CHUNB-58320 _group 14  :0.062351)
:0.018437,((Phyllopezus_pollicaris_VI_MTR-3287 _group 11
:0.000531,Phyllopezus_pollicaris_VI_MTR-3074 _group 11  :0.000531)
:0.002027,Phyllopezus_pollicaris_VI_MTR-3263 _group 11  :0.002750)
:0.085075)            :0.000235)
:0.012226,((Phyllopezus_pollicaris_II_CHUNB-55904 _group 5
:0.099794,((Phyllopezus_pollicaris_II_PV-1059 _group 4
:0.000531,Phyllopezus_pollicaris_II_PV-1053 _group 4   :0.000531)
:0.097655,((Phyllopezus_pollicaris_I_JC-1185 _group 3
:0.000742,Phyllopezus_pollicaris_I_JC-1153 _group 3   :0.002467)
:0.001495,Phyllopezus_pollicaris_I_JC-1182 _group 3   :0.002137)
:0.098043)            :0.004011)
:0.002778,(Phyllopezus_maranjonensis-ZFMK84995 _group 2
:0.120459,(Phyllopezus_periosus_FRD-851 _group 1
:0.000531,(Phyllopezus_periosus_CHUNB-61921 _group 1
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:0.000531,Phyllopezus_periosus_CHUNB-61920 _group 1   :0.000531)
:-0.000000)   :0.119987)   :0.013589)
:0.005672)   :0.001034,(((Phyllopezus_sp_MNHNP-2_40 _group
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:0.029664,((Phyllopezus_przewalskii_MNHNP-11412 _group 17
:0.001760,Phyllopezus_przewalskii_MNHNP-11426 _group 17 :0.002487)
:0.001402,(((Phyllopezus_przewalskii_LJAMM-CNP-12083 _group 17
:0.001714,Phyllopezus_przewalskii_LJAMM-CNP-12072 _group 17
:0.001471)   :0.000259,Phyllopezus_przewalskii_LG-1093 _group
17 :0.001343)   :0.000633,Phyllopezus_przewalskii_TG-00105
_group 17 :0.001990)   :0.003245)   :0.021419)
:0.064860,((Phyllopezus_pollicaris_IV_MTR-6733 _group 10
:0.065292,Phyllopezus_pollicaris_IV_LG-1309 _group 8   :0.059558)
:0.025754,Phyllopezus_pollicaris_IV_LG-1815 _group 9   :0.082294)
:0.006389)   :0.003559);

ND2

Partition with prior maximal distance P=3.59e-02

Distance Simple Dist MinSlope=0.700000

Group[1] n: 1 ;id: Phyllopezus_lutzae_CHUNB-50461

Group[2] n: 1 ;id: Phyllopezus_maranjonensis_ZFMK-84996

Group[3] n: 2 ;id: Phyllopezus_pollicaris_II_PV-1053 Phyllopezus_pollicaris_II_PV-1059

Group[4] n: 1 ;id: Phyllopezus_pollicaris_II_CHUNB-55904

Group[5] n: 2 ;id: Phyllopezus_pollicaris_III_JC-1503 Phyllopezus_pollicaris_III_MTR-887703

Group[6] n: 1 ;id: Phyllopezus_pollicaris_III_MTR-13452

Group[7] n: 1 ;id: Phyllopezus_pollicaris_IV_LG-1309

Group[8] n: 3 ;id: Phyllopezus_pollicaris_IV_LG-1792 Phyllopezus_pollicaris_IV_MTR-6733 Phyllopezus_pollicaris_IV_MTR-6822

Group[9] n: 1 ;id: Phyllopezus_pollicaris_VI_MTR-3074

Group[10] n: 3 ;id: Phyllopezus_pollicaris_VII_CHUNB-36991 Phyllopezus_pollicaris_VII_CHUNB-36992 Phyllopezus_pollicaris_VII_CHUNB-37001

Group[11] n: 1 ;id: Phyllopezus_pollicaris_VII_CHUNB-58320

Group[12] n: 1 ;id: Phyllopezus_pollicaris_VII_CHUNB-43850

Group[13] n: 9 ;id: Phyllopezus_pollicaris_VIII_LG-807 Phyllopezus_pollicaris_VIII_LG-808 Phyllopezus_pollicaris_VIII_LG-1011 Phyllopezus_pollicaris_VIII_MTR-887020 Phyllopezus_pollicaris_VIII_LG-1342 Phyllopezus_pollicaris_VIII_LG-1343 Phyllopezus_pollicaris_VIII_MTR-3681 Phyllopezus_pollicaris_VIII_MZUSP-92491 Phyllopezus_pollicaris_VIII_MTR-2959

Group[14] n: 6 ;id: Phyllopezus_przewalskii_LG-1093 Phyllopezus_przewalskii_TG-00105 Phyllopezus_przewalskii_LJAMM-CNP-12072 Phyllopezus_przewalskii_LJAMM-CNP-12083 Phyllopezus_przewalskii_MNHNP-11412 Phyllopezus_przewalskii_MNHNP-11426

Group[15] n: 2 ;id: Phyllopezus_sp_MNHNP-2_39 Phyllopezus_sp_MNHNP-2_40

Tree

((Phyllopezus_pollicaris_III_MTR-13452 _group 6
:0.110150,(((Phyllopezus_pollicaris_II_PV-1059 _group 3
:0.000564,Phyllopezus_pollicaris_II_PV-1053 _group 3   :0.000564)
:0.108517,Phyllopezus_pollicaris_II_CHUNB-55904 _group 4   :0.122296)
:0.005838,(Phyllopezus_maranjonensis_ZFMK-84996 _group 2
:0.166941,Phyllopezus_lutzae_CHUNB-50461 _group 1   :0.119366)
:0.012657)   :0.005728)
:0.002404,((((Phyllopezus_pollicaris_VIII_MTR-2959 _group 13
:0.021406,((Phyllopezus_pollicaris_VIII_MZUSP-92491 _group 13

```
:0.013924,(Phyllopezus_pollicaris_VIII_MTR-3681 _group 13
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:0.010252)            :0.003126)
:0.003253,(Phyllopezus_pollicaris_VIII_MTR-887020 _group 13
:0.012280,(Phyllopezus_pollicaris_VIII_LG-1011 _group 13
:0.000564,(Phyllopezus_pollicaris_VIII_LG-808 _group 13
:0.000564,Phyllopezus_pollicaris_VIII_LG-807 _group 13  :0.000564)
:-0.000000)            :0.011986)            :0.007038)
:0.006171)            :0.063475,Phyllopezus_pollicaris_VI_MTR-3074
_group 9   :0.079965)
:0.006292,((Phyllopezus_pollicaris_VII_CHUNB-43850 _group 12
:0.069086,((Phyllopezus_pollicaris_VII_CHUNB-37001 _group 10
:0.000564,Phyllopezus_pollicaris_VII_CHUNB-36992 _group 10  :0.000564)
:0.000773,Phyllopezus_pollicaris_VII_CHUNB-36991 _group 10  :0.002049)
:0.061389)            :0.005968,Phyllopezus_pollicaris_VII_CHUNB-58320
_group 11  :0.065865)            :0.023282)
:0.018137,(Phyllopezus_pollicaris_III_MTR-887703 _group 5
:0.021337,Phyllopezus_pollicaris_III_JC-1503 _group 5   :0.013691)
:0.080173)              :0.005596,(((Phyllopezus_sp_MNHNP-2_40 _group
15  :0.000566,Phyllopezus_sp_MNHNP-2_39 _group 15  :0.000566)
:0.020474,((Phyllopezus_przewalskii_MNHNP-11426 _group 14
:0.001945,Phyllopezus_przewalskii_MNHNP-11412 _group 14  :0.001441)
:0.001771,(Phyllopezus_przewalskii_LJAMM-CNP-12083 _group 14
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:0.000564,(Phyllopezus_przewalskii_TG-00105 _group 14
:0.003449,Phyllopezus_przewalskii_LG-1093 _group 14  :-0.002028)
:0.002593)            :-0.000000)            :0.002781)
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_group 8   :0.004932,Phyllopezus_pollicaris_IV_LG-1792 _group 8
:0.007483)            :0.004305,Phyllopezus_pollicaris_IV_MTR-6733
_group 8   :0.005087)
:0.050058,Phyllopezus_pollicaris_IV_LG-1309 _group 7   :0.059956)
:0.044443)            :0.014650);
```

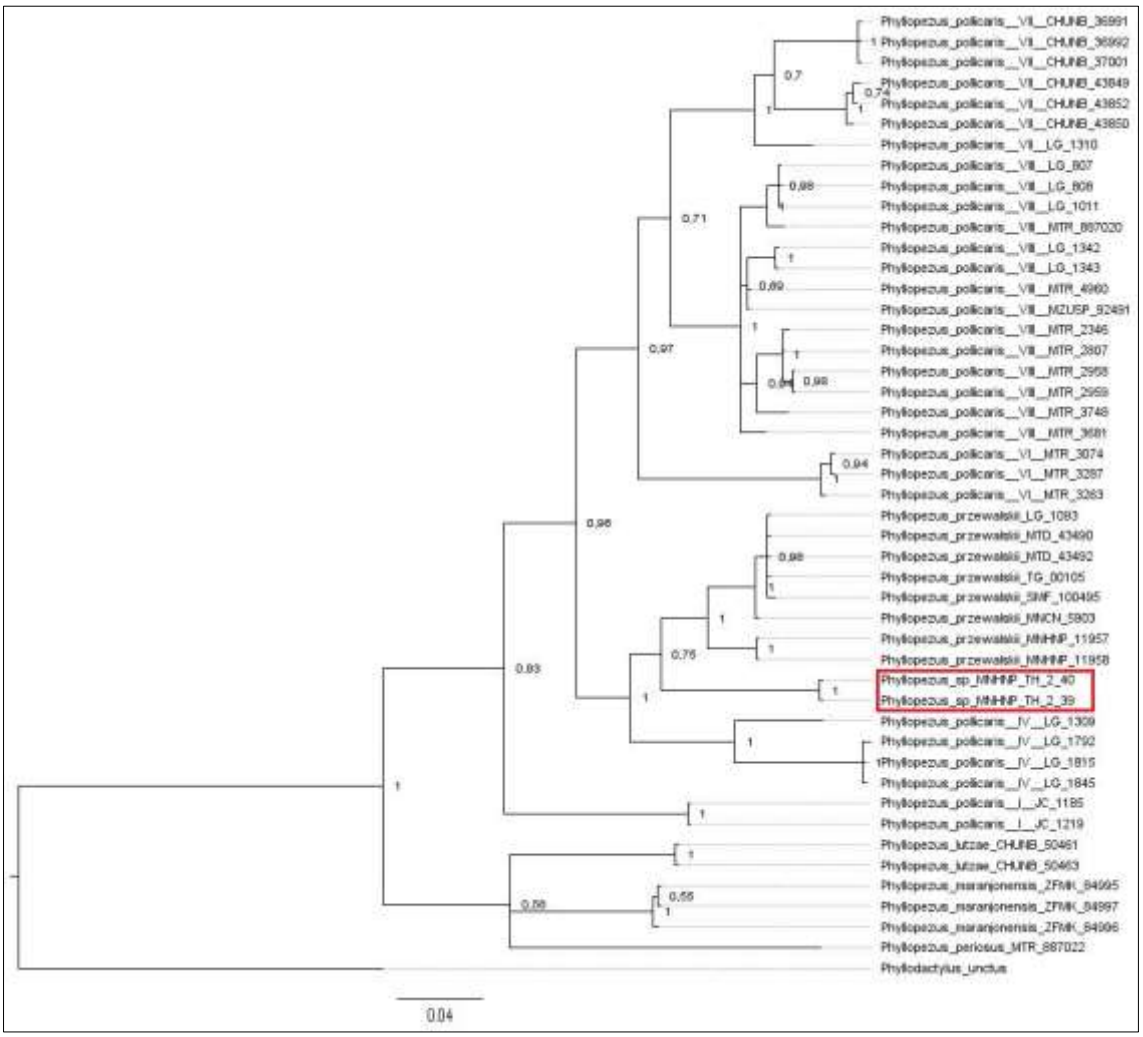
Figure S1

Scales between 4th and 5th digital pads

Detail of the right foot of *Phyllopezus* (SMF 100494) showing the line of lateral scales between the 4th and 5th digital pads. Scale bar = 5 mm.

Figure S2

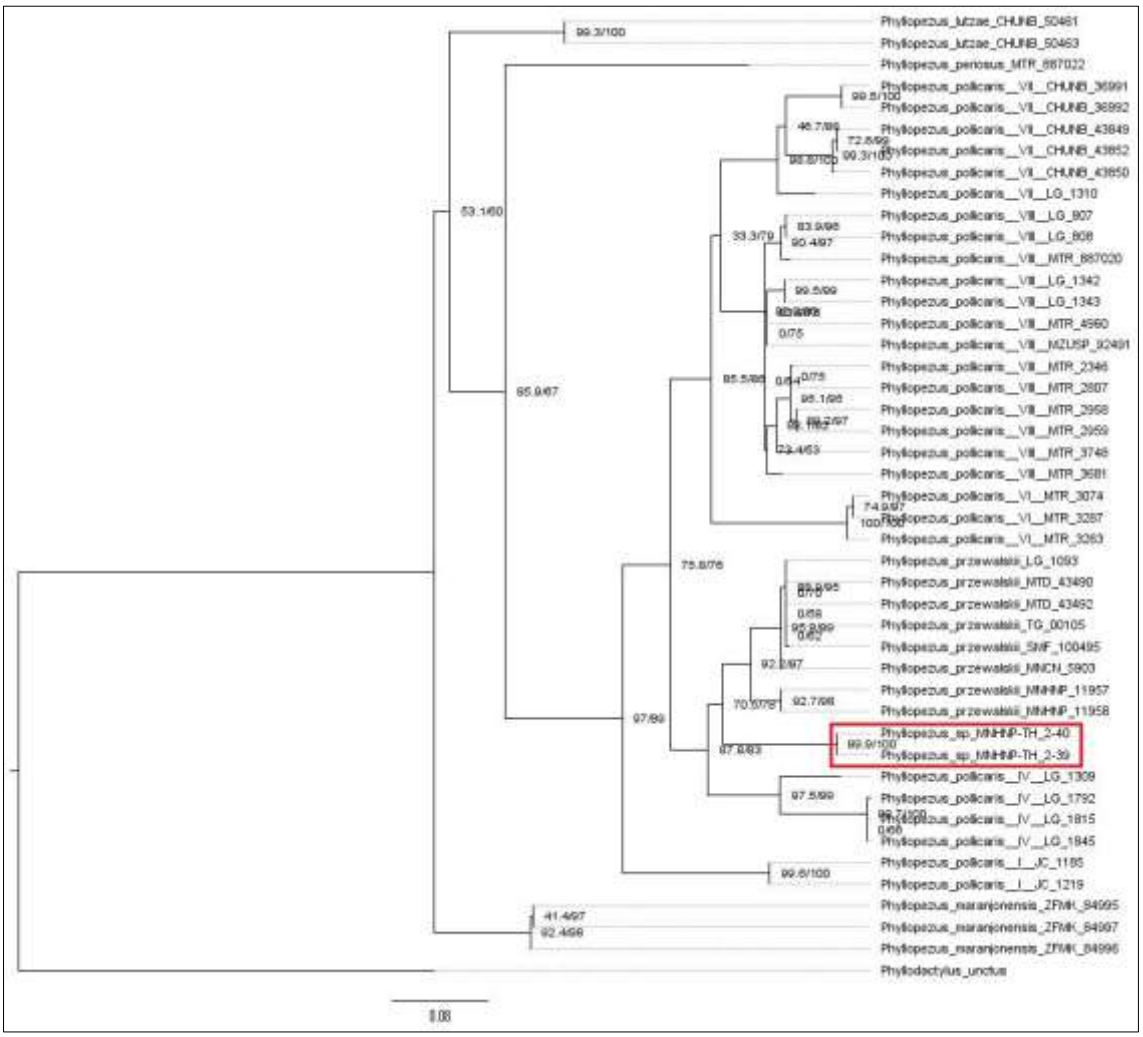
Phylogenetic tree inferred from 16S using BI approach



Phylogeny of the genus *Phyllopezus* using BI based on the mtDNA gene 16S. Numbers at the right of the nodes denote posterior probability. Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S3

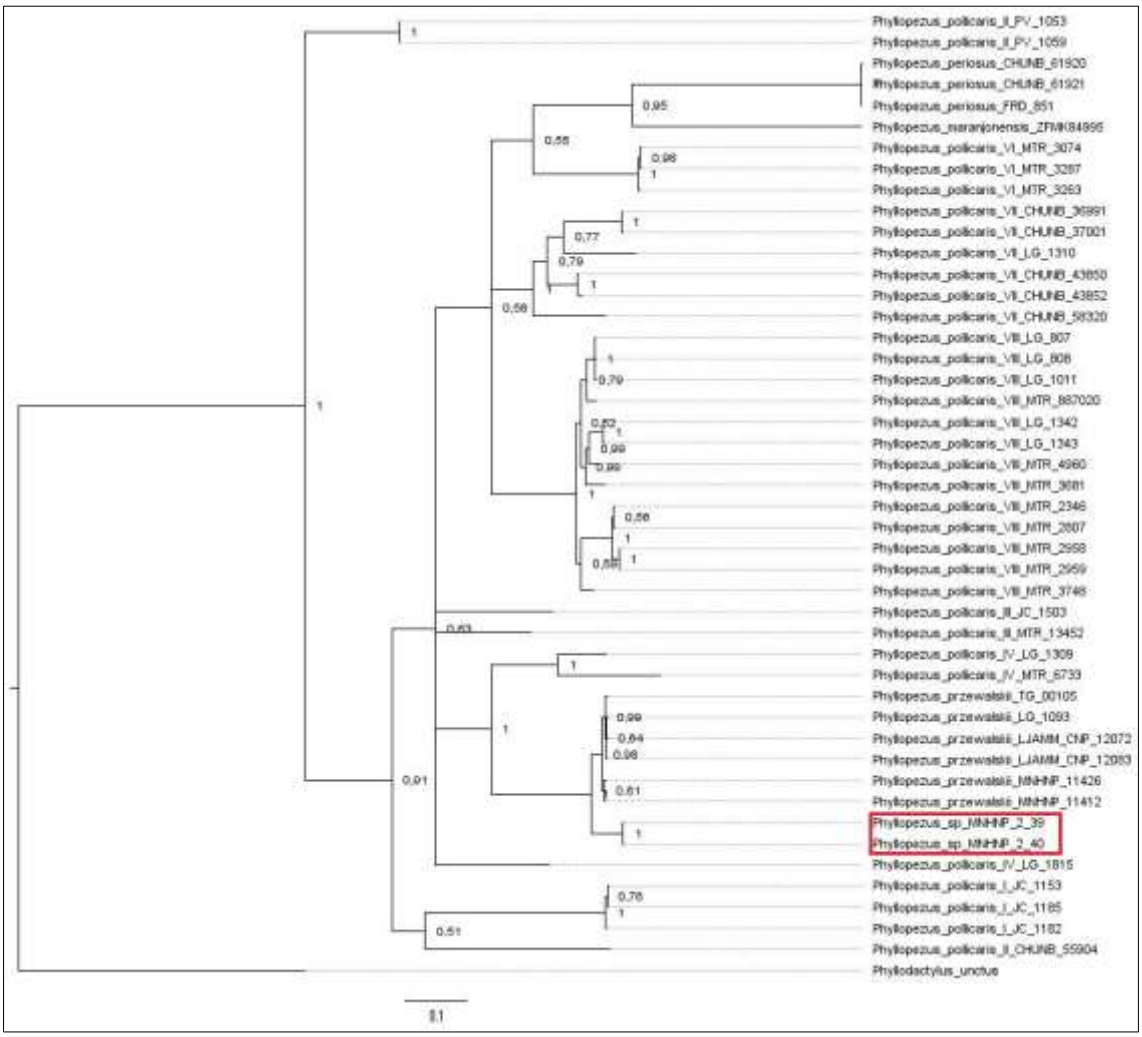
Phylogenetic tree inferred from 16S using ML approach



Phylogeny of the genus *Phyllopezus* using ML based on the mtDNA gene 16S. Numbers at the right of the nodes denote SH-aLRT/UFBoot (in percentages). Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S4

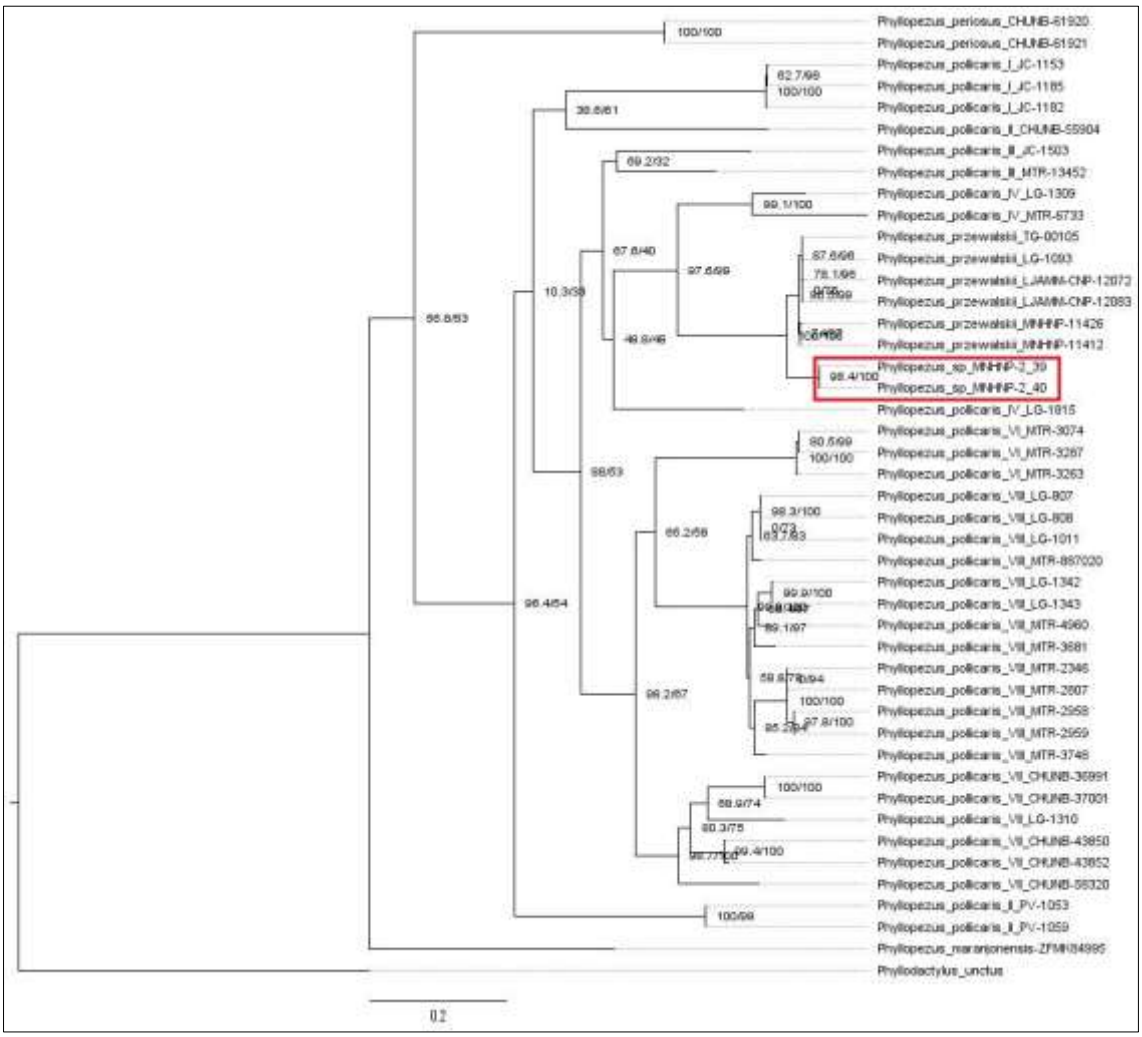
Phylogenetic tree inferred from Cytb using BI approach



Phylogeny of the genus *Phyllopezus* using BI based on the mtDNA gene Cytb. Numbers at the right of the nodes denote posterior probability. Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S5

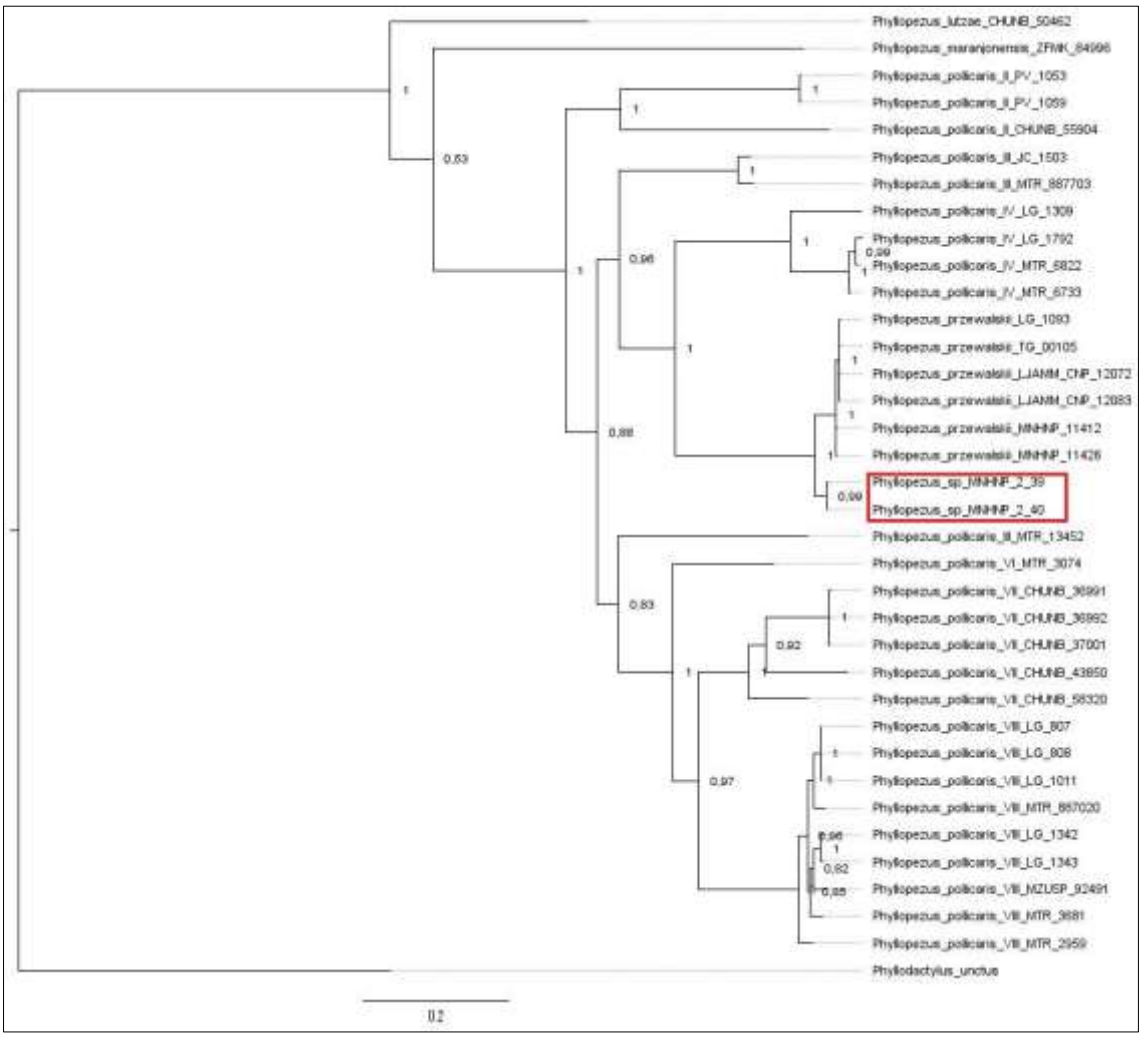
Phylogenetic tree inferred from Cytb using ML approach



Phylogeny of the genus *Phyllopezus* using ML based on the mtDNA gene Cytb. Numbers at the right of the nodes denote SH-aLRT/UFBoot (in percentages). Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S6

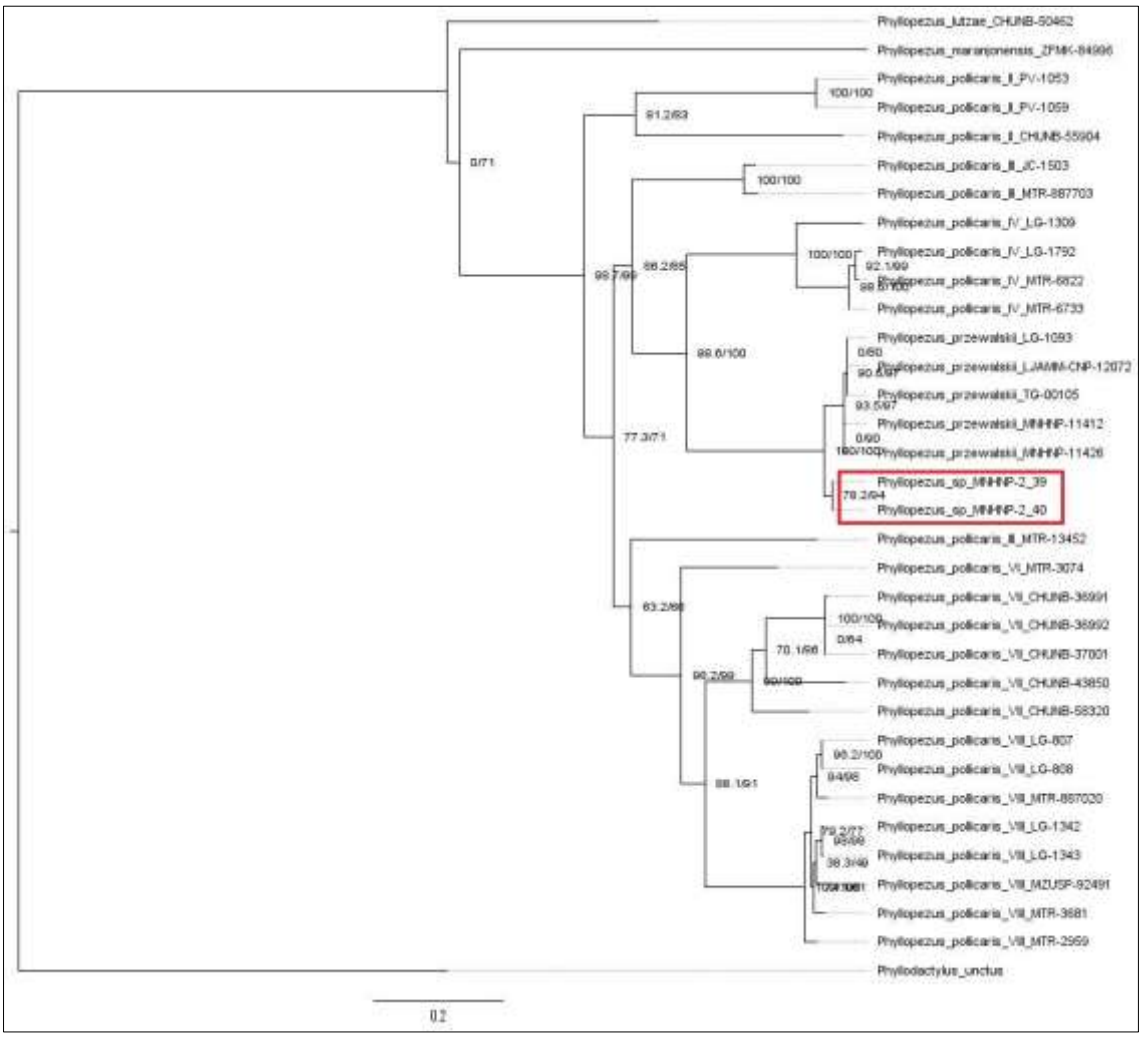
Phylogenetic tree inferred from ND2 using BI approach



Phylogeny of the genus *Phyllopezus* using BI based on the mtDNA gene ND2. Numbers at the right of the nodes denote posterior probability. Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S7

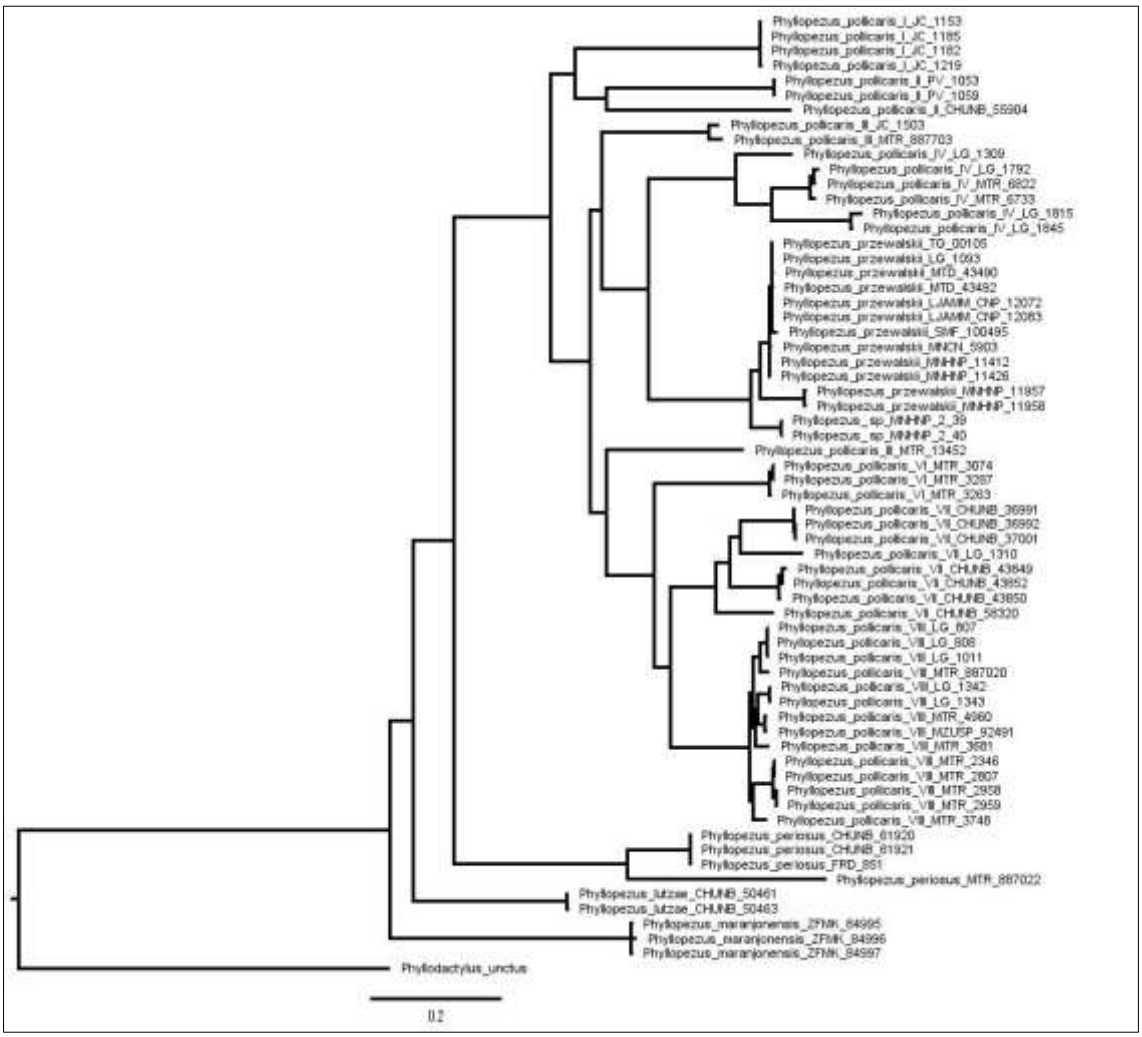
Phylogenetic tree inferred from ND2 using ML approach



Phylogeny of the genus *Phyllopezus* using ML based on the mtDNA gene ND2. Numbers at the right of the nodes denote SH-aLRT/UFBoot (in percentages). Bar at the bottom represents substitutions per site. Specimens from "Cordillera de los Altos" highlighted in the red box.

Figure S8

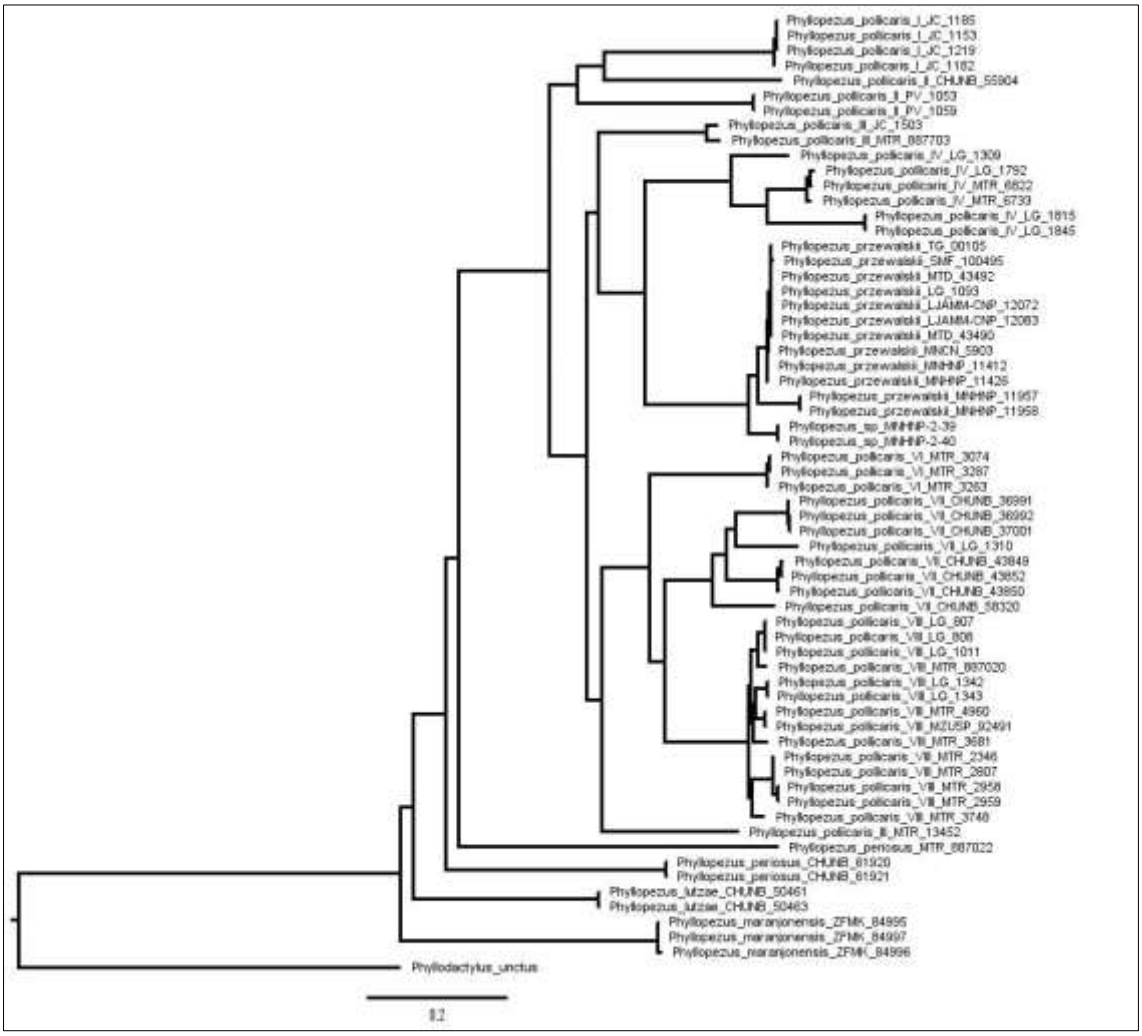
Phylogenetic tree inferred from concatenated genes, using BI approach



Phylogeny of the genus *Phyllopezus* using BI based on concatenated mtDNA genes 16S, Cytb, and ND2. Bar at the bottom represents substitutions per site.

Figure S9

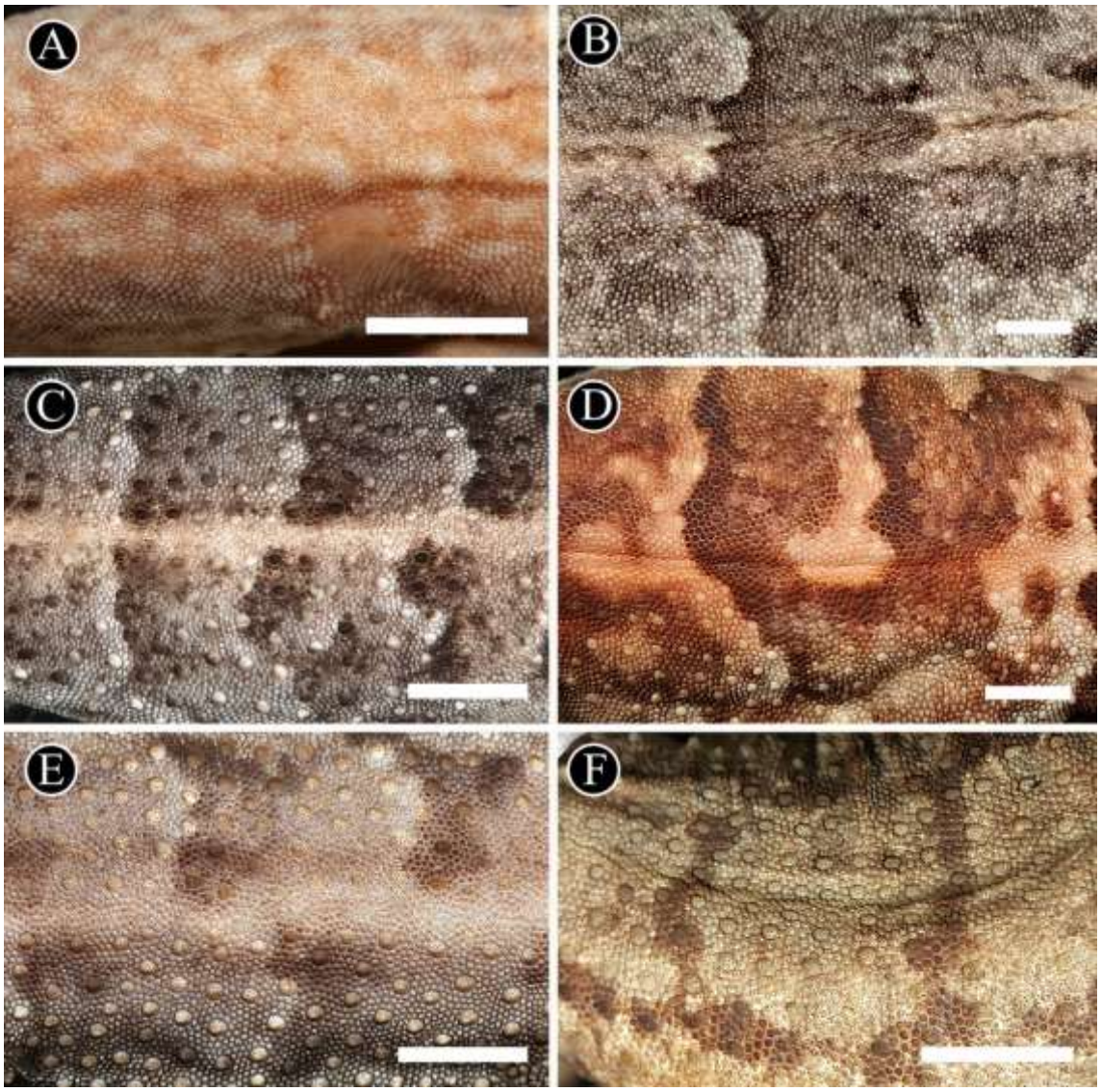
Phylogenetic tree inferred from concatenated genes, using ML approach



Phylogeny of the genus *Phyllopezus* using ML based on concatenated mtDNA genes 16S, Cytb, and ND2. Bar at the bottom represents substitutions per site.

Figure S10

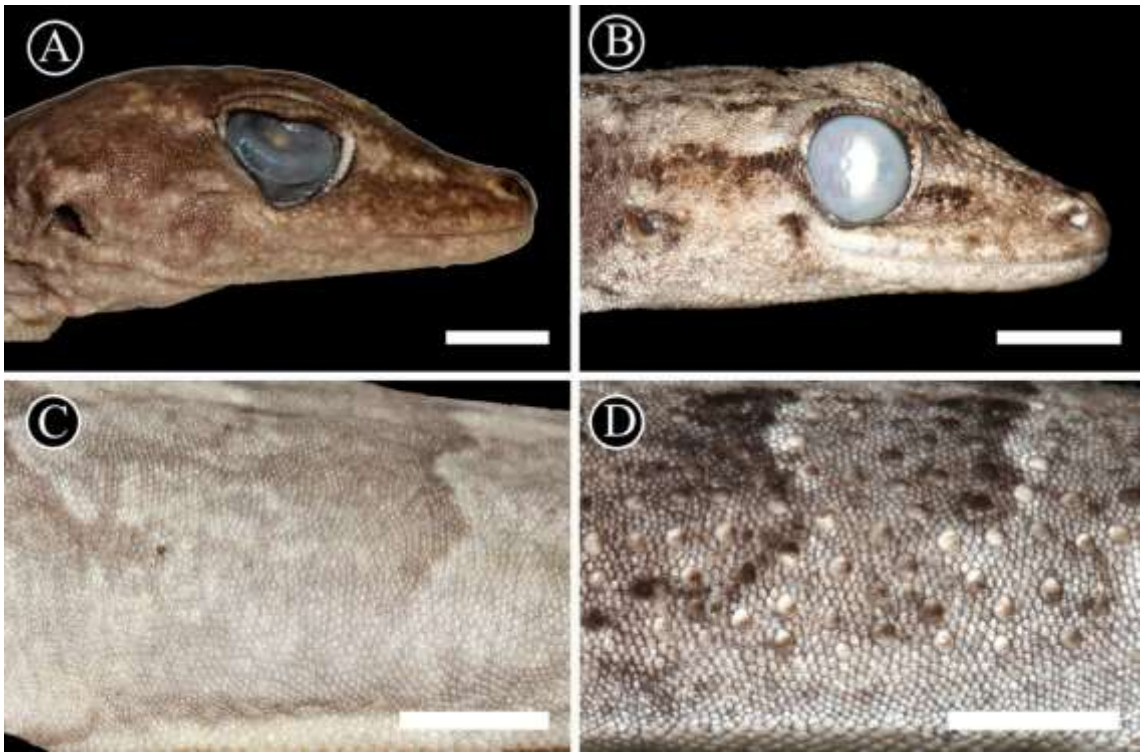
Dorsal scalation



Dorsal scalation pattern of the named species of *Phyllopezus*: A- *P. lutzae* (MCZ 46190), B- *P. maranjonensis* (ZFMK 84995), C- *P. heuteri* (SMF 100494), D- *P. periosus* (MCZ 172930), E- *P. przewalskii* (SMF 100496), F- *P. pollicaris* (ZSM 165/0/1). Heads oriented to the right. Scale bars = 5 mm.

Figure S12

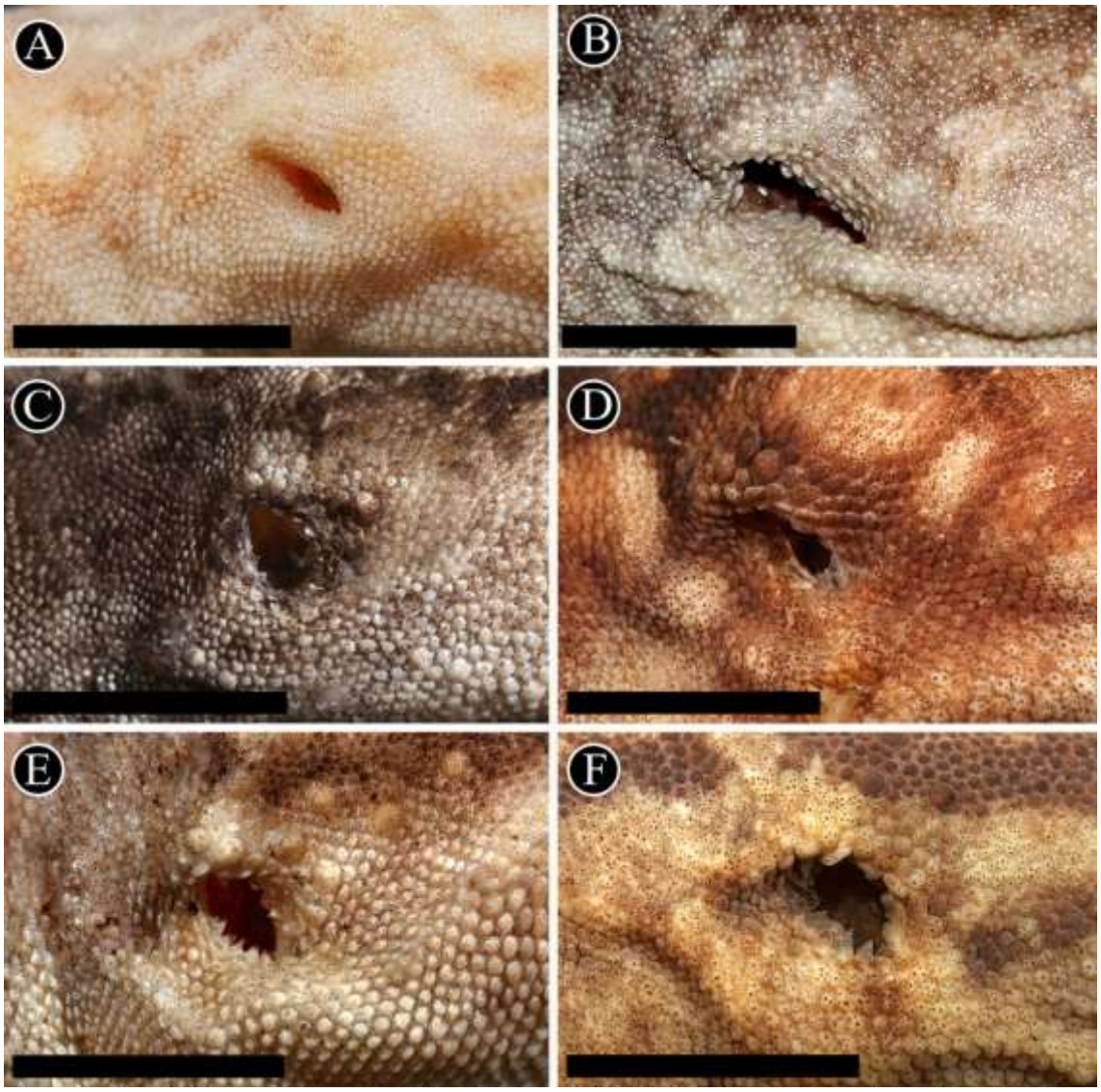
Head and lateral body scalation



Differences in head scalation (above) and lateral body scalation (below) between *Phyllopezus maranjonensis* (A, C; ZMFK 84996) and *P. heuteri* (B, D; SMF 100494). In C and D heads oriented to the left. Scale bars = 5 mm.

Figure S13

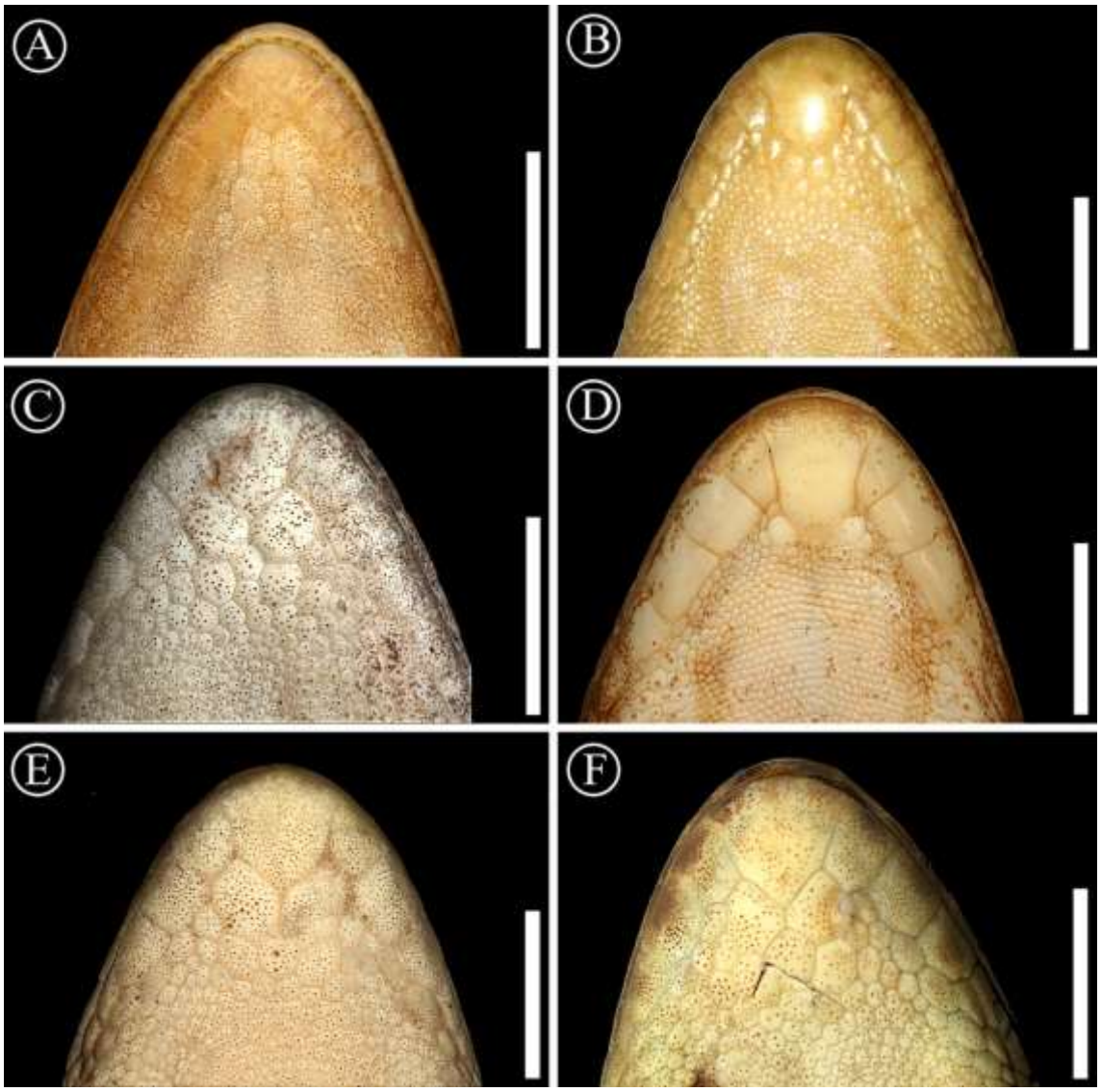
Ear opening



Scalation pattern around the ear opening of: A- *P. lutzae* (MCZ 46190), B- *P. maranjonensis* (ZFMK 84997), C- *P. heuteri* (SMF 100494), D- *P. periosus* (MCZ 172930), E- *P. przewalskii* (SMF 100496), and F- *P. pollicaris* (ZSM 165/0/1). Heads oriented to the right. Scale bars = 5 mm.

Figure S14

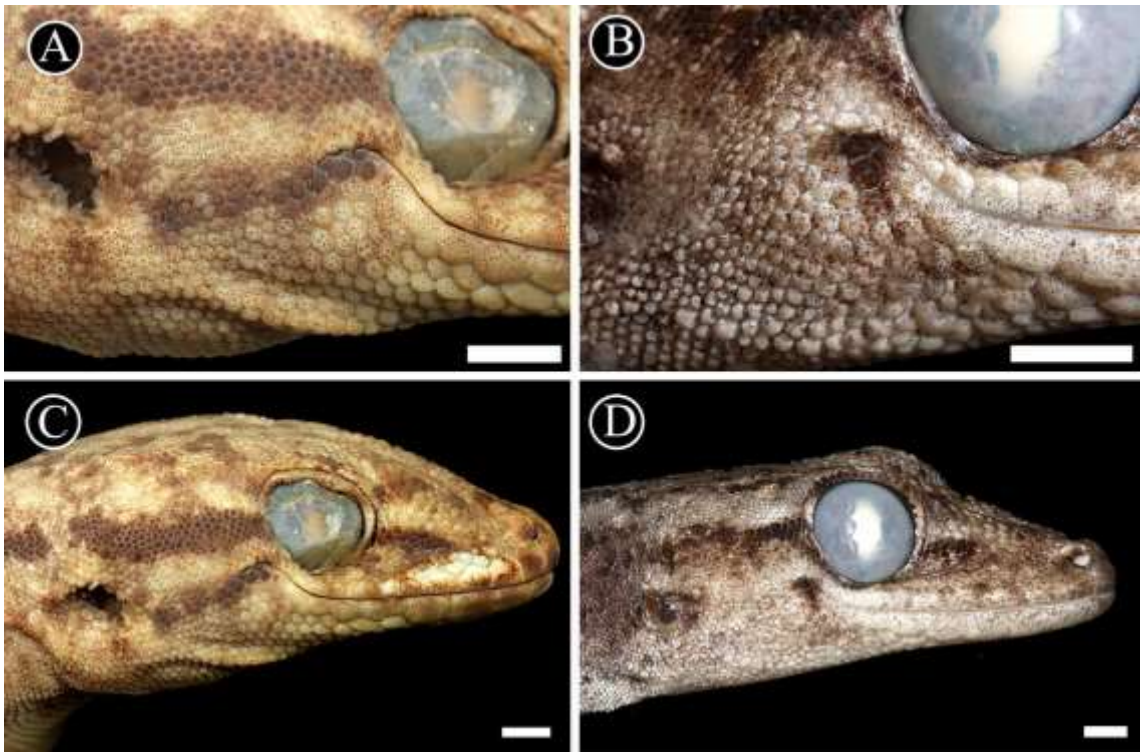
Mental scalation



Detailed view of the chin region of: A- *P. lutzae* (MCZ 46190), B- *P. maranjonensis* (ZFMK 84997), C- *P. heuteri* (SMF 100494), D- *P. periosus* (MCZ 172929), E- *P. przewalskii* (SMF 100496), and F- *P. pollicaris* (ZSM 165/0/1). Scale bars = 5 mm.

Figure S15

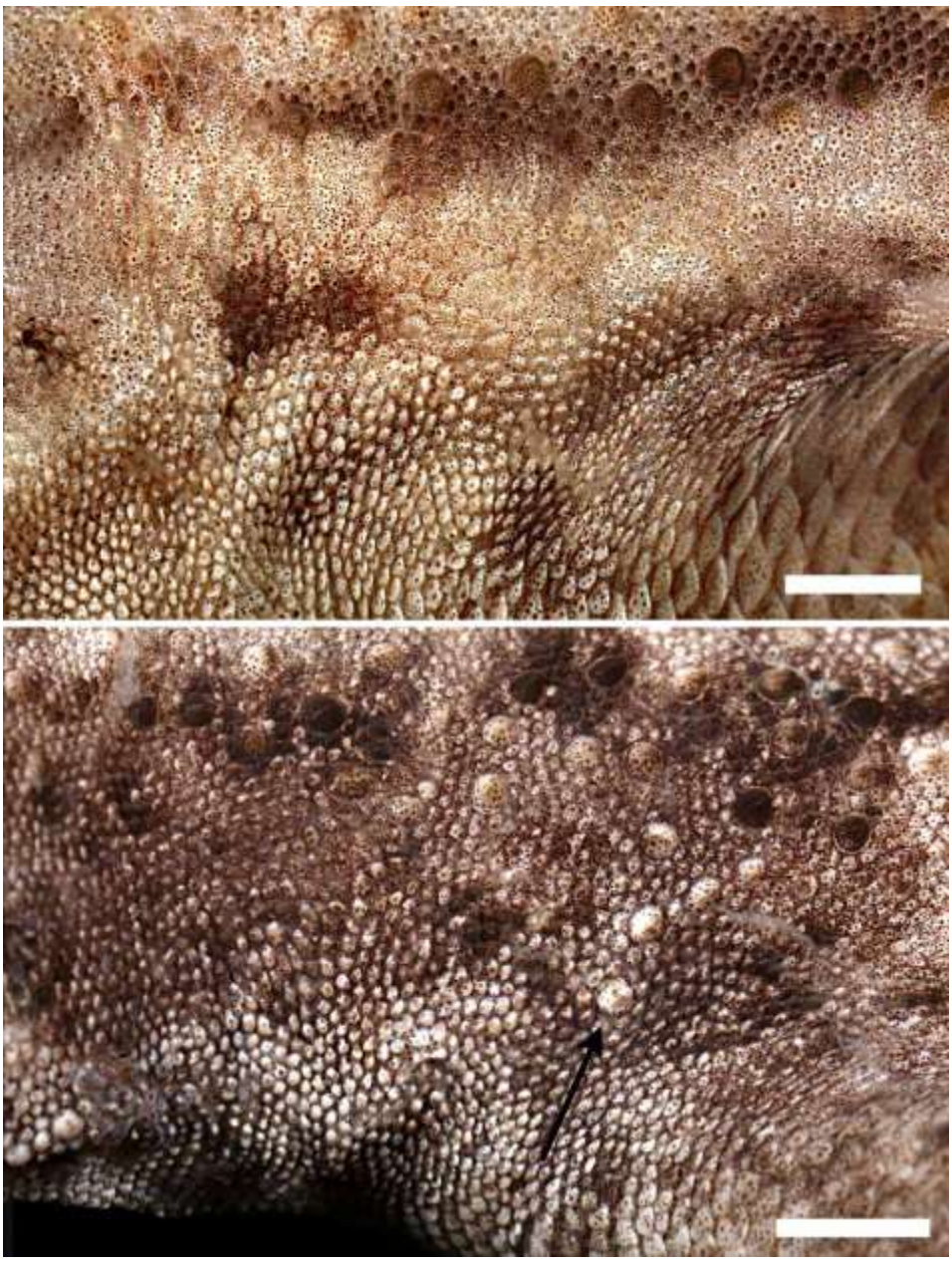
Lateral scalation of the head



Differences in head scalation between *P. pollicaris* (A, C; ZSM 165/0/1) and *P. heuteri* (B, D; SMF 100494), showing homogeneous scalation in the mouth commissure of *P pollicaris* (A) compared to the presence of some bigger scales in *P. heuteri*, and fewer tubercles between eye and ear opening in *P. pollicaris* (C) compared with *P. heuteri* (D). Scale bars = 2 mm.

Figure S16

Prescapular scalation



Differences in prescapular scalation of *P. przewalskii* (above, SMF 100496) and *P. heuteri* (below, SMF 100494). Note the tubercles intermingled with smaller scales in *P. przewalskii* compared with the homogeneous scalation in *P. heuteri*). Scale bars = 2 mm.

APPENDIX VII

Declaration on the contributions of authors

to the publication: On the distribution of the genus *Teius* Merrem, 1820 (Reptilia: Squamata: Teiidae).
Status: Published (2016).
Name of the journal: Zootaxa 4136.
Authors involved: Pier Cacciali (PC), Mariana Morando (MM), Gunther Köhler (GK), Luciano J. Avila (LJA).

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 60%
Coauthor MM: 10%
Coauthor GK: 10%
Coauthor LJA: 20%

(2) to the implementation of the respective studies and experiments

PhD candidate: 75% – field work (collecting and documenting specimens), performed climatic and distributional analyses.
Coauthor MM: 5% – field work (collecting and documenting specimens).
Coauthor MM: 5% – field work (collecting and documenting specimens).
Coauthor LJA: 15% – field work (collecting and documenting specimens).

(3) to the creation of the data collection and figures

PhD candidate: 90% – created database, provided photographs, created figures, created maps.
Coauthor LJA: 10% – created database.

(4) to the analysis and interpretation of the data

PhD candidate: 70% – analysis and interpretation of biogeographical data.
Coauthor MM: 5% – contributed to data analysis and interpretation.
Coauthor GK: 10% – contributed to data analysis and interpretation.
Coauthor LJA: 15% – contributed to data analysis and interpretation.

(5) to writing the manuscript

PhD candidate: 85%
Coauthor MM: 2.5%
Coauthor GK: 7.5%
Coauthor LJA: 5%

Date/place: _____

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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On the distribution of the genus *Teius* Merrem, 1820 (Reptilia: Squamata: Teiidae)

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[4]*Grupo de Herpetología Patagónica. IPEEC-CENPAT-CONICET. Puerto Madryn, Chubut, Argentina*

Abstract

The lizard genus *Teius* is composed by three species: *Teius teyou*, *T. oculatus*, and *T. suquiensis* and is distributed in South America, east of Andes. *Teius teyou* and *T. oculatus* have wide parapatric distributions with contact zones. *Teius suquiensis* is present in a small range along a sympatric area of the former species. In this work we analyze the distribution of the three species of *Teius* across its whole geographic range, examining its relationships with climatic parameters. We based our analysis on specimens in collections and literature records. Our analysis shows that the genus *Teius* is distributed from central Bolivia southwards to north of Río Negro Province in northern Patagonia, Argentina. *Teius teyou* reaches the northernmost range limit of the genus whereas *T. oculatus* occupies the southernmost limit. *Teius oculatus* is related to open and moist environments whereas *T. teyou* is more adapted to xeric and forested areas. *Teius suquiensis* is present in xerophytic areas of Dry Chaco and Espinal. Climatic factors in the distribution of the distribution of the two widespread species show marked differences and seasonality.

Key words: climate, deforestation, ecoregions, habitat loss, South America, sympatry

Introduction

The South American lizard genus *Teius* Merrem, 1820 currently includes three species: *Teius teyou* (as *Lacerta teyou*) Daudin, 1802; *T. oculatus* (as *Ameiva oculata*) D'Orbigny & Bibron, 1837; and *T. suquiensis* Avila & Martori, 1991. *Teius* belongs to the Teiinae subfamily (Estes *et al*. 1988), and as many other members of the subfamily, individuals of *Teius* have a bright green coloration, a long tail with autotomy, are extremely agile and exceptional runners (Cei 1993; Avila 2002; Carreira *et al*. 2005).

The three species of *Teius* have an eastern-Andean distribution. Avila (2002) studied the chorology of the genus, showing that the bisexual species (*Teius oculatus* and *T. teyou*) have wide distributions, whereas the parthenogenetic *T. suquiensis* is restricted to a narrow contact zone between the bisexual species, in the provinces of Córdoba, San Luis, and Santa Fe (Avila & Martori 1991; Avila 2002), being sympatric with either one of the sexual species or even with both of them (Guerreiro *et al*. 1998; Avila 2002; Cabrera & Monguillot 2007).

An important fact of *Teius* is that these lizards have wide trophic ranges, they feed opportunistically on the available prey that varies depending on the season (Acosta *et al*. 1991; Avila *et al*. 1992; Cappellari *et al*. 2007). Even when these lizards consume mainly insects, they are omnivorous animals eating also fruits and therefore being considered a seed disperser in the Dry Chaco (Varela & Bucher 2001). All these dietary traits plus the fact that they can tolerate rather well anthropogenic alterations, make these lizards the most common reptiles within its chorological range.

Avila (2002) published a detailed revision of the distribution of the genus and found a sympatric diagonal gradient between *Teius oculatus* and *T. teyou*, highlighting areas where even the three species seem to be sympatric.

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Knowledge of the biodiversity distribution is not only important for conservation but also to understand the different species distribution patterns facing the growing and rapid process of habitat loss. In this work we analyzed the distribution of the genus *Teius* across its whole geographic range, and discuss the chorology as well as its relationships with climatic parameters.

Method

Our work is based on an exhaustive revision of scientific collections and web resources including Species Link (http://splink.cria.org.br/) from Brazil, and VertNet (http://vertnet.org/index.php) from the USA. Additionally, we conducted fieldwork in different areas of the distribution of the genus that resulted in the addition of new distributional data for species of this genus. Acronyms used in the text follow Sabaj Pérez (2014) except CHC-L (Colección Herpetológica de Corrientes – Lagartijas, actualmente UNNEC) and CZPLT (Colección Zoológica Para La Tierra, San Pedro, Paraguay). Bibliographic records were obtained from Acosta & Murúa (1998), Álvarez *et al*. (1995), Briguera *et al*. (2005), Carreira *et al*. (2005), Céspedez *et al*. (2001), Dirksen & de la Riva (1999), Etchepare *et al*. (2013), Gallardo (1966; 1969), Gallardo *et al*. (1985), Guerreiro *et al*. (1998; 2005), Halloy *et al*. (2007), Hellmich (1960), Kacoliris *et al*. (2006), Leynaud & Bucher (2005), Lions *et al.* (1997), Lopez & Prado (2008), Mertens (1929), Padial *et al*. (2003), Pelegrin & Bucher (2015), Peracca (1897), Pérez *et al.* (2011), Scrocchi *et al*. (2010), Souza *et al*. (2010), Winck *et al*. (2011), and Zaracho *et al*. (2014).

We generated distribution maps with DIVA-GIS 7.5 and ArcMap 10.3, and we used ecoregional maps from http://maps.tnc.org/gis_data.html based on Olson *et al*. (2001).

We obtained climatic information from WorldClim (Global Climate Data) based on Hijmans *et al*. (2005). For high resolution elevation maps we used SRTM30 (30 seconds resolution) datasets taken from Consortium for Spatial Information (CGIAR-CSI) available on http://www.diva-gis.org/gdata (Jarvis *et al*. 2008).

We plotted the records of *Teius* on elevation and ecoregional maps using ArcMap 10.3, and precipitation and temperature maps using DIVA-GIS 7.5. To visualize sympatric areas, we conducted a richness analysis using grids of 0.5 square degrees, and simple "Point to grid procedure".

Results

We analyzed 2020 specimens (917 *T. oculatus*, 302 *T. suquiensis* and 801 *T. teyou*) from 1071 localities (Appendix 1), including all the type specimens of the three species.

Our analysis shows that the genus *Teius* is distributed from central Bolivia southwards to north of Río Negro Province in northern Patagonia, Argentina; occupying an area in the center and north of the southern cone of South America, east of Andes (Fig. 1). The whole distribution extends from high elevation areas in the "pre-cordillera" (up to 2587 masl, San Francisco, Tarija Province, Bolivia; Dirksen & De la Riva 1999) to the Atlantic coast (near sea level).

Teius oculatus reaches the southernmost range limit of the genus (Fig. 1). The latitudinal range extends from 24º04'12"S (Reserva Natural del Bosque Mbaracayá, Canindeyú, Paraguay; MNHNP 10860) to 40º54'03"S (Río Negro Province, Argentina; Scrocchi *et al*. 2010) southwards; and from 51º01'19"W (Viamão, Rio Grande do Sul, Brazil; MCP 4657) to 68º28'15"W (San Rafael, Mendoza, Argentina; several specimens at the MHN SR H listed in Appendix 1) of longitude west. These and other localities mentioned in the text, are shown in Figure 2. The southern limit, south of the Colorado River still needs effective confirmation with voucher specimens; only observations of a few specimens in the area between the Colorado and Negro rivers are available. This species is present in eastern Paraguay, northeastern, eastern and central Argentina, Uruguay, and Rio Grande do Sul State in Brazil.

The ecoregional distribution of *Teius oculatus* is related to Low Monte, Espinal, Pampas, Parana Flooded Savanna, Uruguayan Savanna, Southern Cone Mesopotamian Savanna, and Humid Chaco (Fig. 3). Furthermore it is present in the southeastern edge of the Dry Chaco, and there are some records of this species in the Araucaria Moist Forest (one record from Mormaço, Rio Grande do Sul, MCP 17718) and Alto Paraná Atlantic Forest. Nevertheless, it is important to note that even when this species is present in this ecoregion, it is not associated with the forest. For example, a detailed observation at Tuparendi (Fig. 4A) in Rio Grande do Sul (Brazil; 27º45'23"S, 54º28'54"W) or Campo Viera (Fig. 4B) in Misiones (Argentina; 27º19'41"S, 55º03'36"W) reveals that those areas

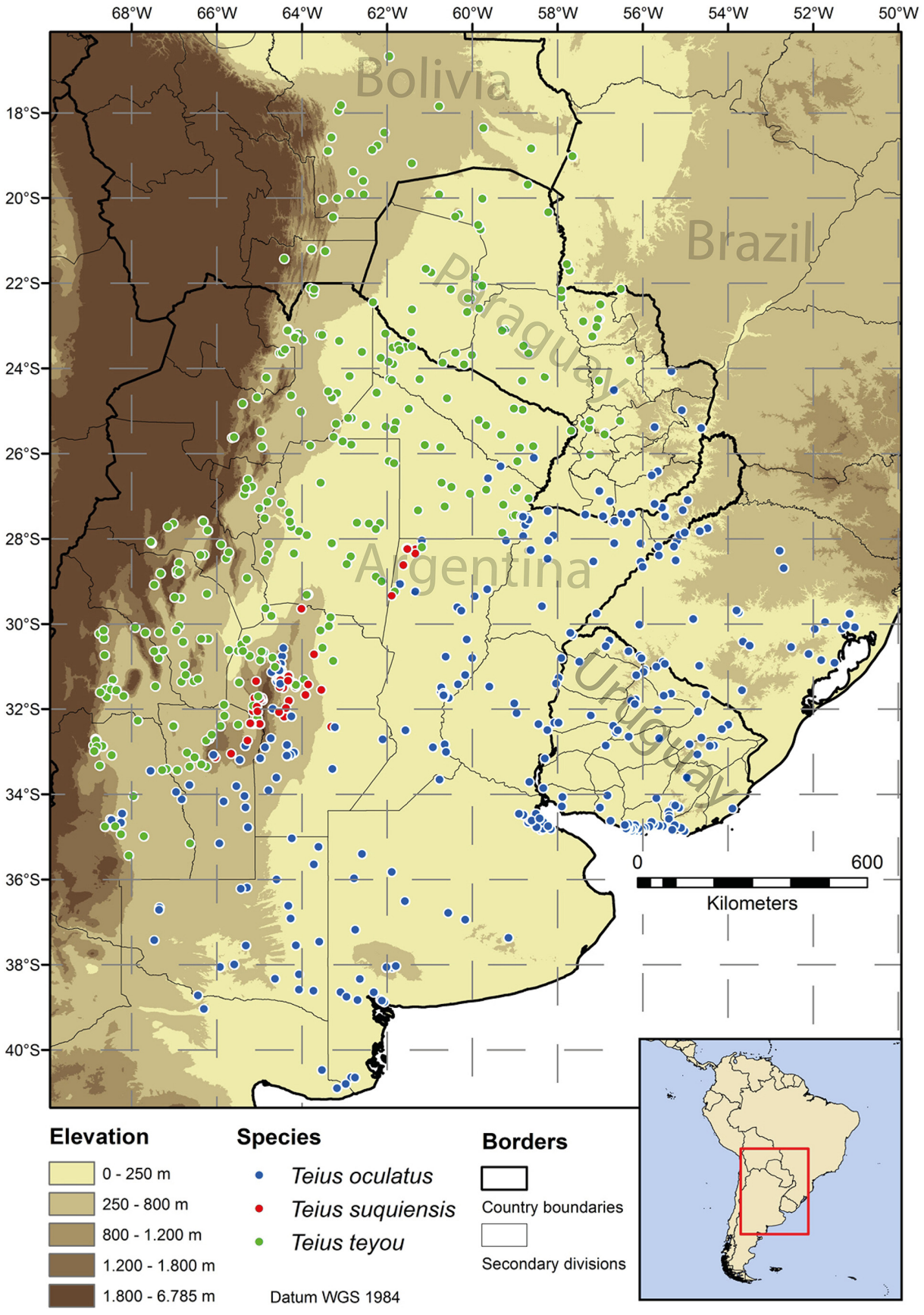

FIGURE 1. Geographic distribution of the genus *Teius*.

FIGURE 2. Important localities mentioned in the text.

actually lack forest, and most of the area is covered by grasslands or crop fields. We performed a field survey at San Rafael National Park (Itapúa, Paraguay; 26º30'42"S, 55º47'20"W), and found that even when the study area has two main biomes (forest and grassland, Fig. 4C) *T. oculatus* is only present in the grasslands (Fig. 4D). Given that *Teius oculatus* shows tolerance for modified environments it can be found in natural or introduced grasslands; nevertheless, when the degrading impact is too high, *T. oculatus* is absent. For example, in a field survey at Itakyry (Alto Paraná, Paraguay; 24º58'59"S, 55º04'58"W) (Fig. 4E) we found this species in shrub-lands with some degree of anthropogenic perturbations (Fig. 4F), but absent in crop fields. In the southwestern limits of its distribution, in areas were vegetation is typical of Monte (eastern Mendoza, southwestern San Luis and western La Pampa), *Teius oculatus* is found in dry and shrubby environments that are commonly occupied by *T. teyou*. This entire region is part of the Atuel and Salado Chadileuvu rivers drainage, a southern portion of the Desaguadero basin, which became drier when water use increased for agricultural and human consumption around Jachal, San

Juan, Mendoza and San Rafael cities. Some chronicles mention the advance of the "jarilla" (*Larrea* spp.) on grasslands areas, but there is no scientific information about the effect of the lack of water on the landscape and biodiversity. Probably populations of *T. oculatus* are relicts of a more westernmost distributions of this genus in central Argentina. Present population density seems to be low, but in the last 10 years we were able to collected several specimens and observe its presence along roads of western La Pampa and southern San Luis, extending its known southwestern distribution at least 100-200 km SW from previous records. *Teius oculatus* is not the only "pampasic" (Gallardo 1966) species in this region, *Liolaemus wiegmannii* is in periurban areas surrounding San Juan, Mendoza and San Rafael-General Alvear cities in San Juan and Mendoza provinces (Avila et al. 1998; Corbalan and Debandi 2008), and some other species of vertebrates presently only found in eastern Argentina were registered several years ago (e.g., *Synbranchus marmoratus*, Murúa and Acosta 2007).

The altitudinal range of *T. oculatus* varies from near sea level in coastal areas of Uruguay and Buenos Aires (Argentina) to 1292 masl in central Argentina in "Sierras de Córdoba", Córdoba, Argentina (2 km E of Characato, CM 72486) (Fig. 1). The climatic conditions in the distribution area of this species ranges from a minimum mean temperature of -1.1ºC (July) in Mendoza (Argentina), to a maximum mean temperature of 35.8ºC (January) in Santa Fe (Argentina) (Fig. 5). Most of the locality records of this species are in areas with an annual mean temperature ranging from 14 to 23ºC (Fig. 6). With respect to rainfalls, *T. oculatus* is distributed in areas with values from 200 to 1800 mm/yr (Fig. 7). Figure 5 shows that some areas of Uruguay and southern Brazil where *T. oculatus* is present, hold almost equal levels of precipitation over the year; while towards the west of its distribution, during the dry season values of rainfalls can be less than 10 mm/month between June and August (Locality 5, Fig. 5).

Teius suquiensis is present in two relatively small areas, one in the northeastern corner of Santa Fe Province, and another in central and western Córdoba Province, following the "Sierras" and reaching the northeastern area of San Luis Province (Fig. 1). The range extends from 28º14'20"S (Estancia El Nochero, Santa Fe, Argentina; several specimens at the CENAI listed in Appendix 1) to 33º07'59"S (La Florida, San Luis, Argentina; Guerreiro *et al*. 1998) southwards, and from 61º20'24"W (45 km NE of Villa Minetti, Santa Fe, Argentina; MACN 4176) to 66º01'59"W (La Florida, San Luis, Argentina; Guerreiro *et al*. 1998). This species is present in the southernmost edge of the Humid Chaco, being more abundant in Dry Chaco and Espinal (Fig. 8). The northeastern areas of its distribution are lowlands (from 68 to 74 masl), while the populations that inhabit the surroundings of "Sierras de Córdoba" have an altitudinal range from 210 to 1210 masl. This species is present in areas with a temperature range from 0.1ºC (July) in San Luis (Argentina) to a maximum of 35.8ºC (January) in Santa Fe (Argentina) (Fig. 5). The annual mean temperature in areas where *T. suquiensis* is present ranges from 14 to 20ºC in the southwestern population (Córdoba and San Luis provinces) and from 20 to 23ºC in the northeastern population (Santa Fe Province) (Fig. 6). The annual rainfall range is between 400 and 1000 mm/yr (Fig. 7) with records of less than 10 mm/month in the dry season (June-August) to maximum peaks of more than 100 mm/month in the rainy season (October-March) (Fig. 5).

Teius teyou, the most boreal species of the genus (Fig. 1), is present from 16º40'08"S (Los Troncos, Santa Cruz Province, Bolivia; Hellmich 1960) to 35º26'09"S (Prov. Route 179, 9.2 km N of intersections with Routes 179 and 190, Mendoza, Argentina; LJAMM-CNP 5036) in the south, and the longitudinal range extends from 56º17'59"W (Laguna Blanca, San Pedro, Paraguay; several specimens at the CZPLT listed in Appendix 1) to 68º53'42"W (El Challao, Mendoza, Argentina; IBAUNC 147 and 849). This species is present from south-central Bolivia through the western portion of Paraguay (west of Paraguay River), with some records in the eastern portion of the country, and in the western border of the Brazilian State of Paraná, to central western Argentina, reaching the Precordillera de los Andes (Fig. 1).

The ecoregional affinities of *T. teyou* correspond mainly to xeric environments in Chiquitano Dry Forest, Dry Chaco, edge of Southern Andean Yungas, Humid Chaco, Low as well as High Monte, and Espinal. Furthermore, there are some records in Pantanal, Cerrado, and Paraná Flooded Savanna, but only marginally near the border with the Humid Chaco (Fig. 9). In Paraguay the species has a single record in the Alto Paraná Atlantic Forest (Fig. 9).

Teius teyou is the species that has the broadest vertical range, being present from lowlands in the Chaco (approx. between 50 and 100 masl) to 2595 masl in San Francisco (Tarija, Bolivia). Additional records above 1000 masl include several along the western edge of its distribution. The majority of the distributional range of *T. teyou* is in areas with an annual mean temperature of 20 to 26ºC, with some records of 14

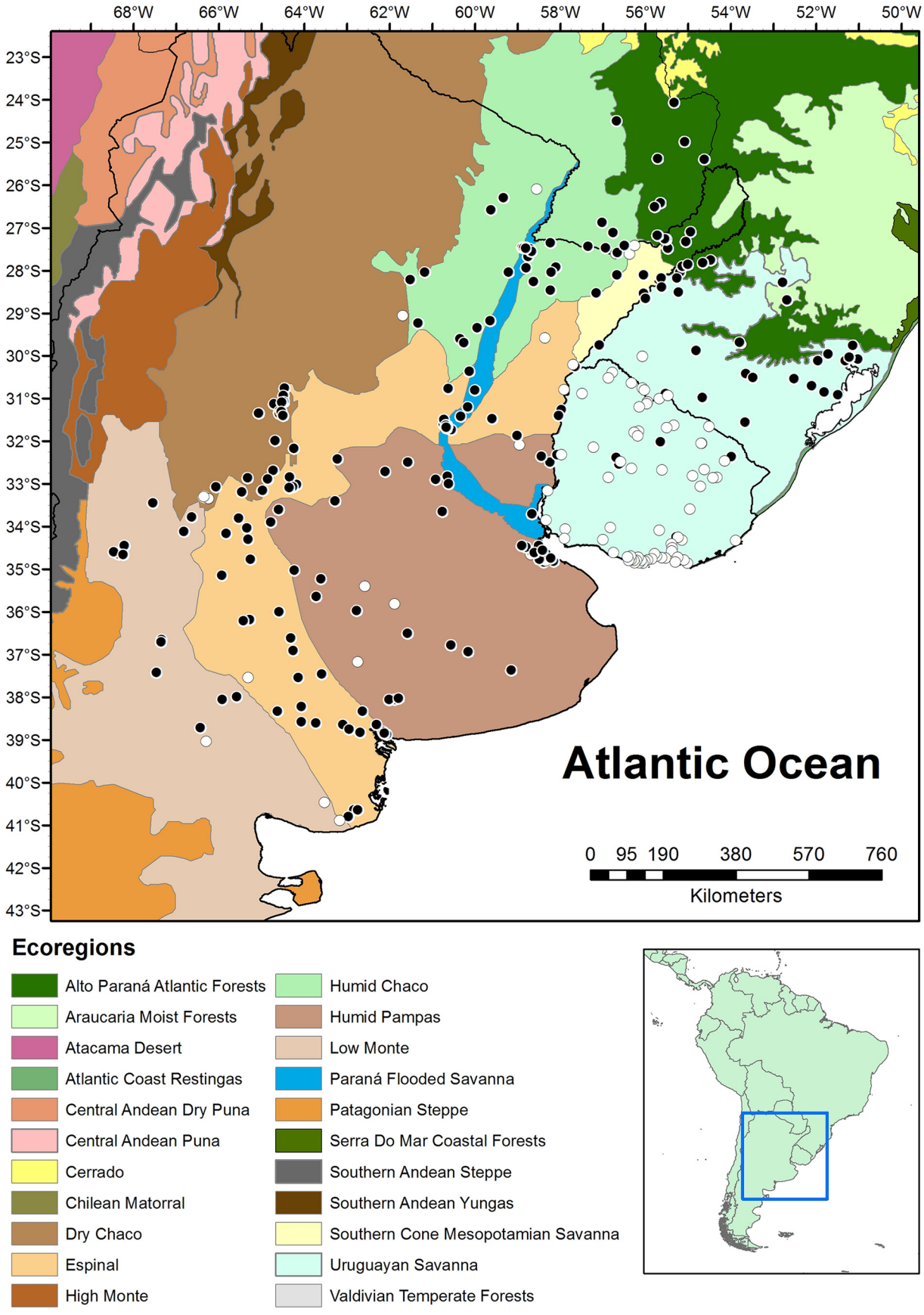

FIGURE 3. Ecoregional map for *Teius oculatus*. Black symbols: collection records, white symbols: literature records.

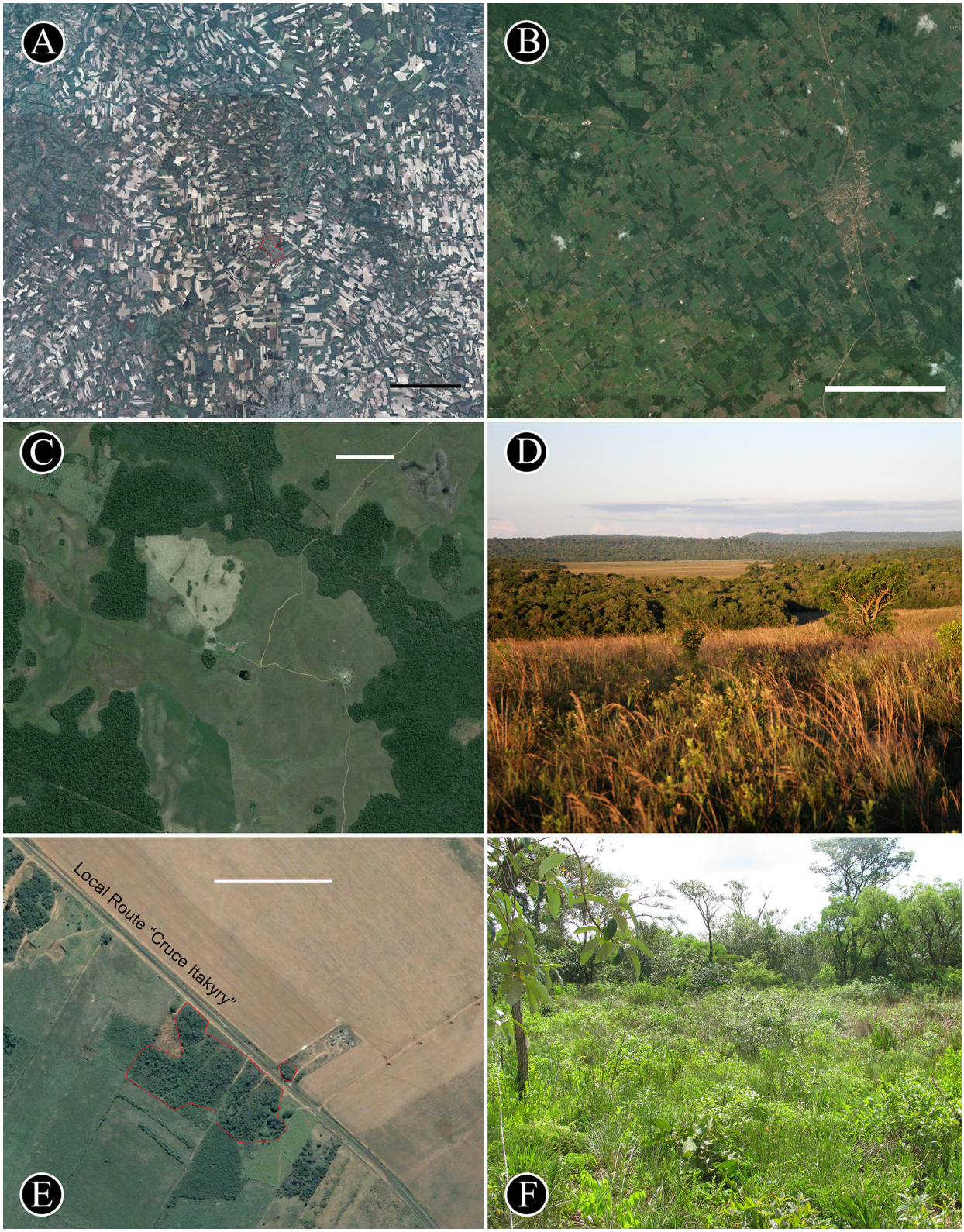

FIGURE 4. Satellite images of: A—Tuparendi, Rio Grande do Sul, Brazil (city marked with a red polygon), black bar 5 km (Date of image 6-XI-2013). B—Campo Viera, Misiones Argentina, white bar 4 km (Date of image 9-III-2014). C—Kangüery at San Rafael National Park, Itapúa, Paraguay, white bar 500 m (Date of image 29-VIII-2006); two major biomes are visible: forest (dark green) and grasslands (light green). D—Landscape showing the typical habitat for *Teius oculatus* in Kangüery (natural grasslands), mixed with patches of forest. E—Vicinities of Itakyry, Alto Paraná, Paraguay, white bar 250 m (Date of image 1-VIII-2006); areas where *Teius oculatus* was found during fieldwork delineated in a red polygon. F—Degraded habitat at Itakyry with extensive growth of shrubs, typical environment of *T. oculatus* (no presence was recorded in culture fields or around houses).

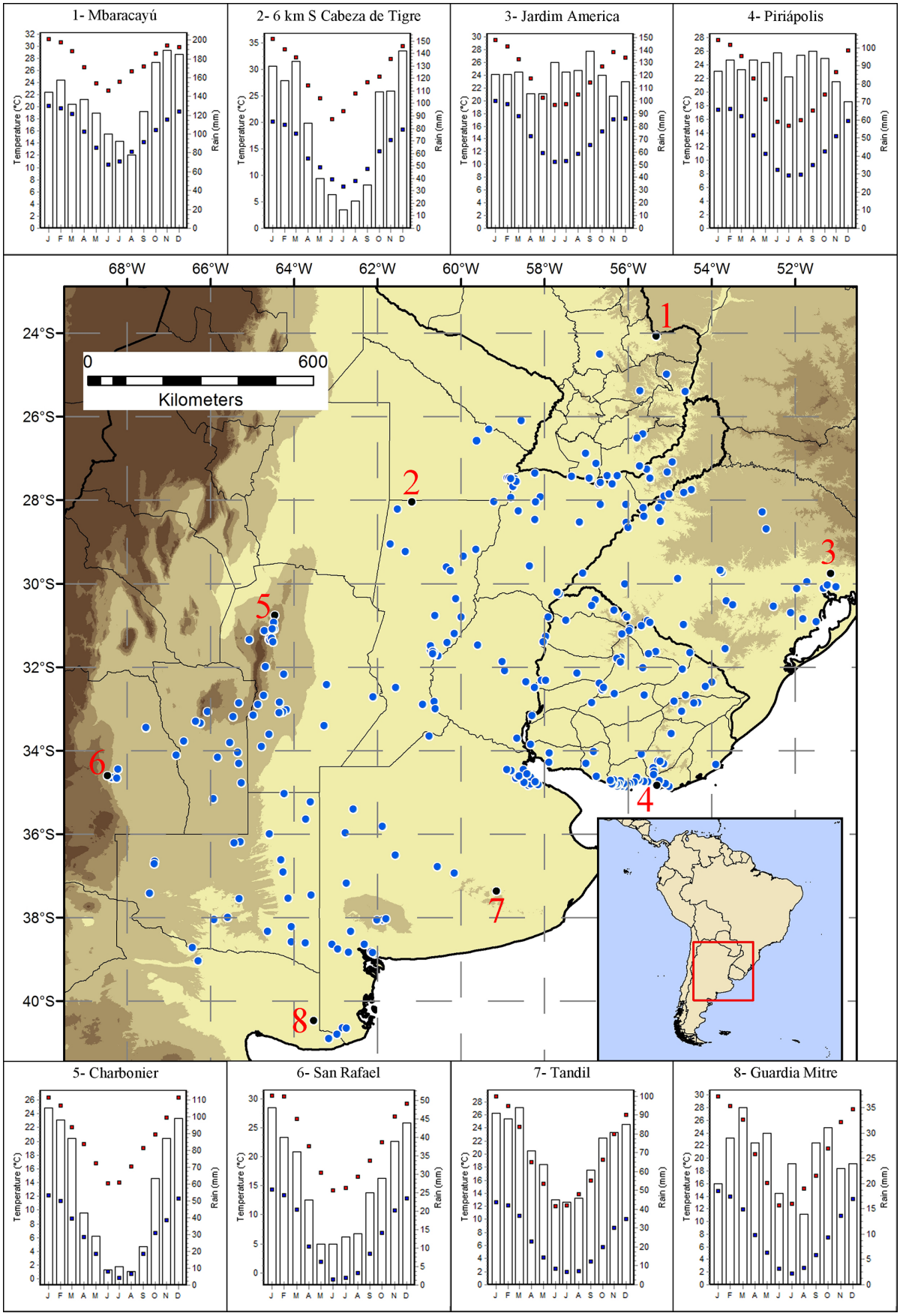

FIGURE 5. Climatic conditions (temperature and precipitation) in some localities of *Teius oculatus*. Temperature graphed with squares (minimum blue, maximum red) and rainfalls with vertical bars.

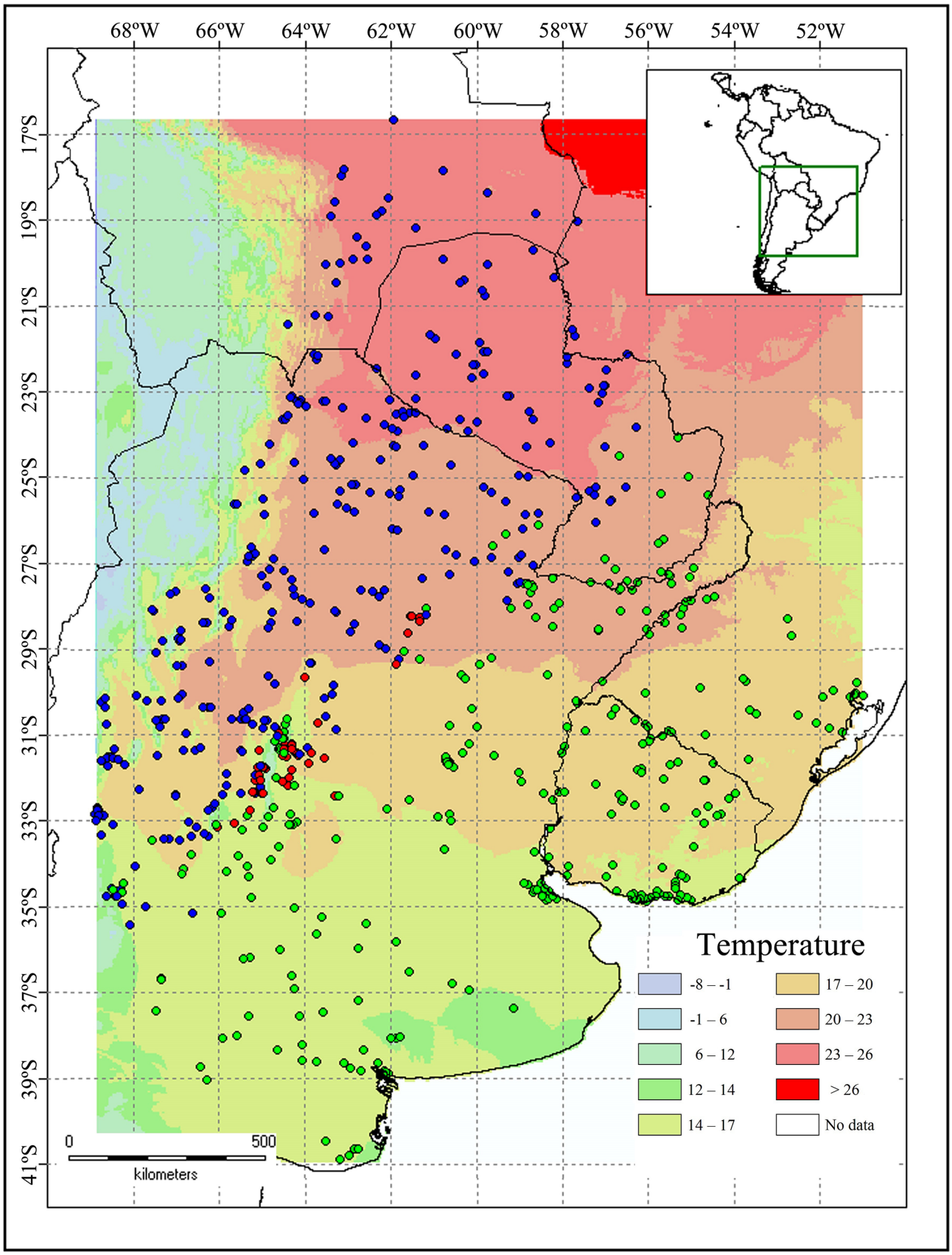

FIGURE 6. Annual mean temperature in the distribution range of *Teius* (blue: *T. teyou*, red: *T. suquiensis*, and green: *T. oculatus*). Temperature in ºC.

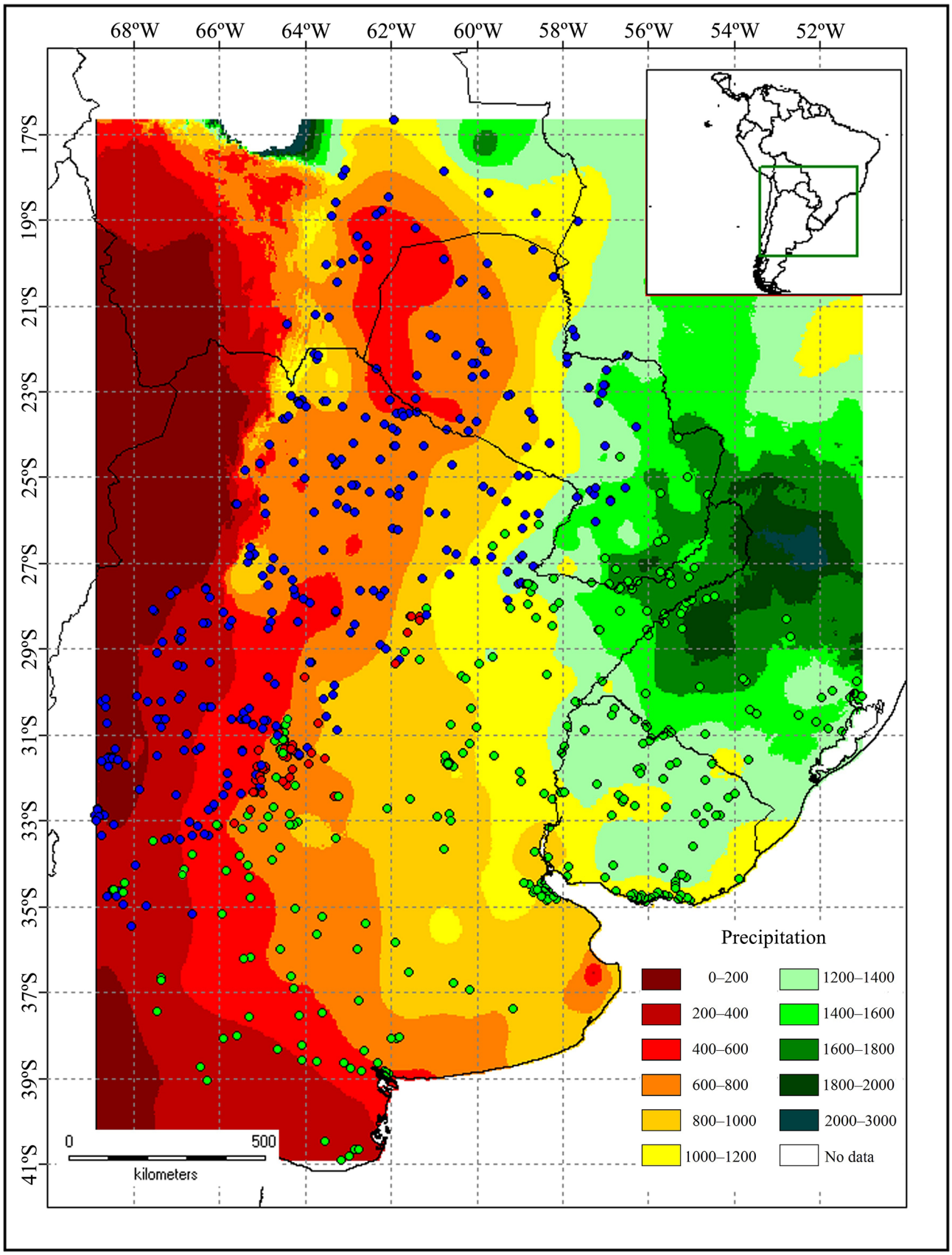

FIGURE 7. Annual precipitation in the distribution range of *Teius* (blue: *T. teyou*, red: *T. suquiensis*, and green: *T. oculatus*). Precipitation in mm per year.

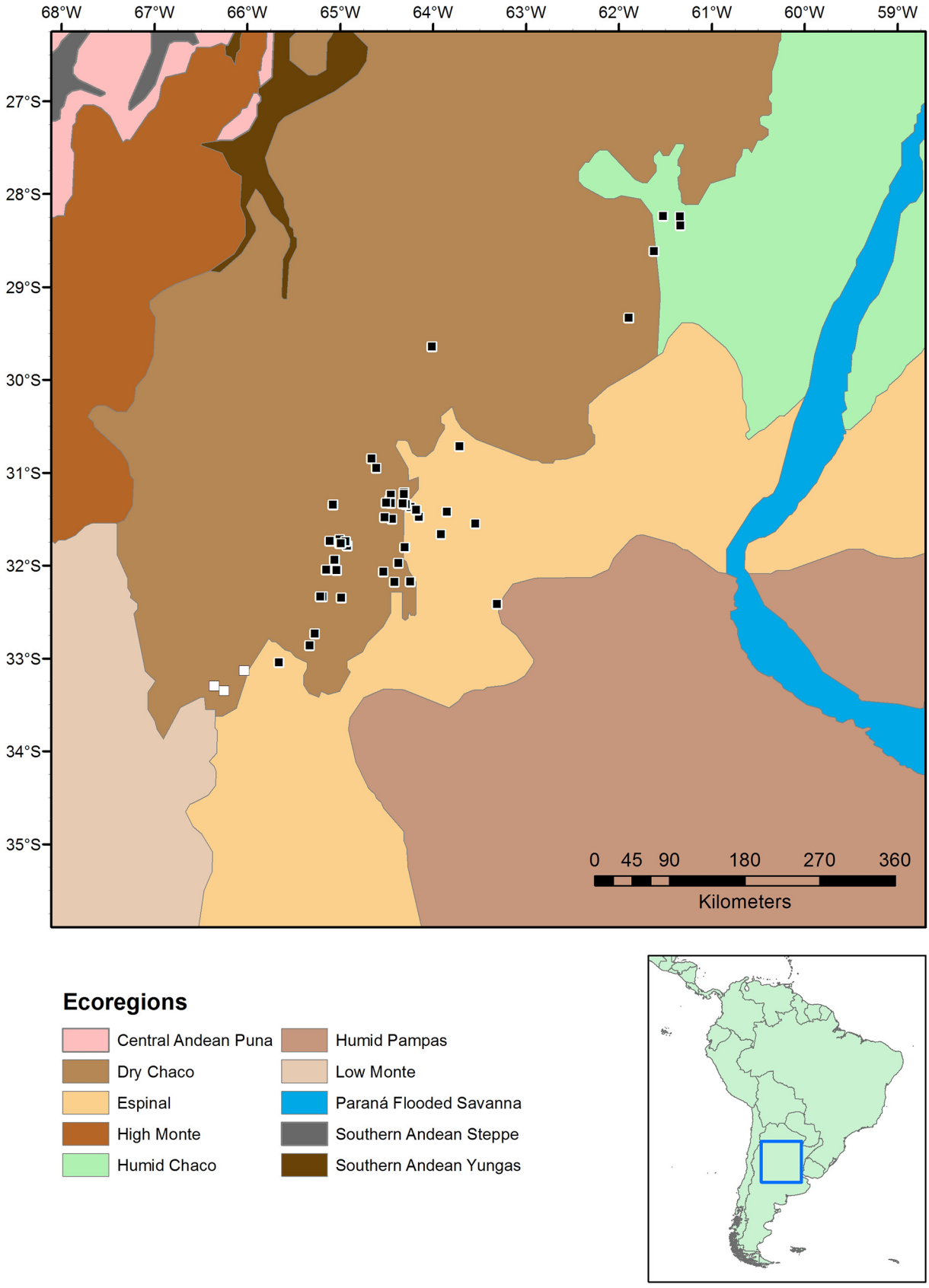

FIGURE 8. Ecoregional map for *Teius suquiensis*. Black symbols: collection records, white symbols: literature records.

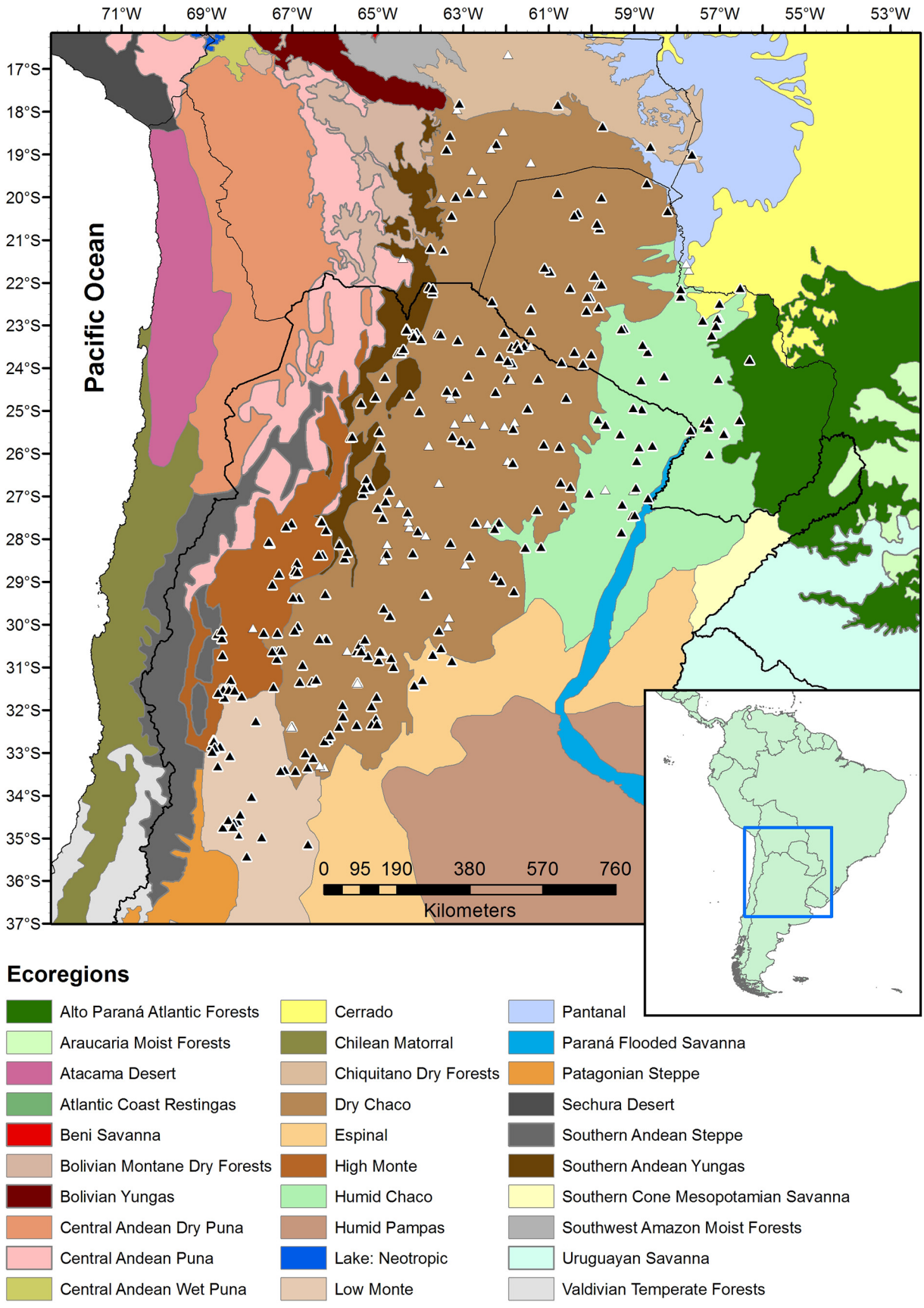

FIGURE 9. Ecoregional map for *Teius teyou*. Black symbols: collection records, white symbols: literature records.

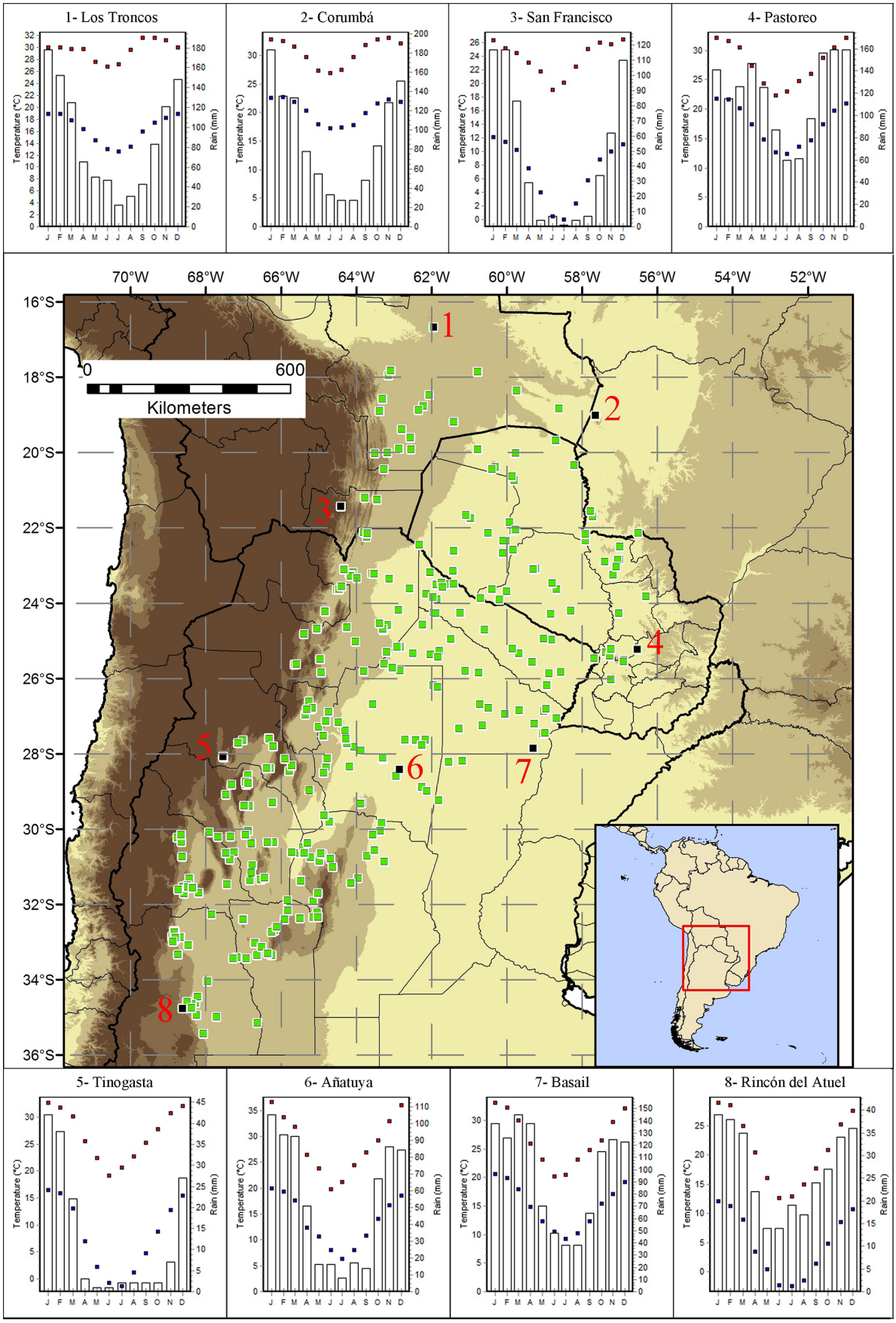

FIGURE 10. Climatic conditions (temperature and precipitation) in some localities of *Teius teyou*. Temperature graphed with squares (minimum blue, maximum red) and rainfalls with vertical bars.

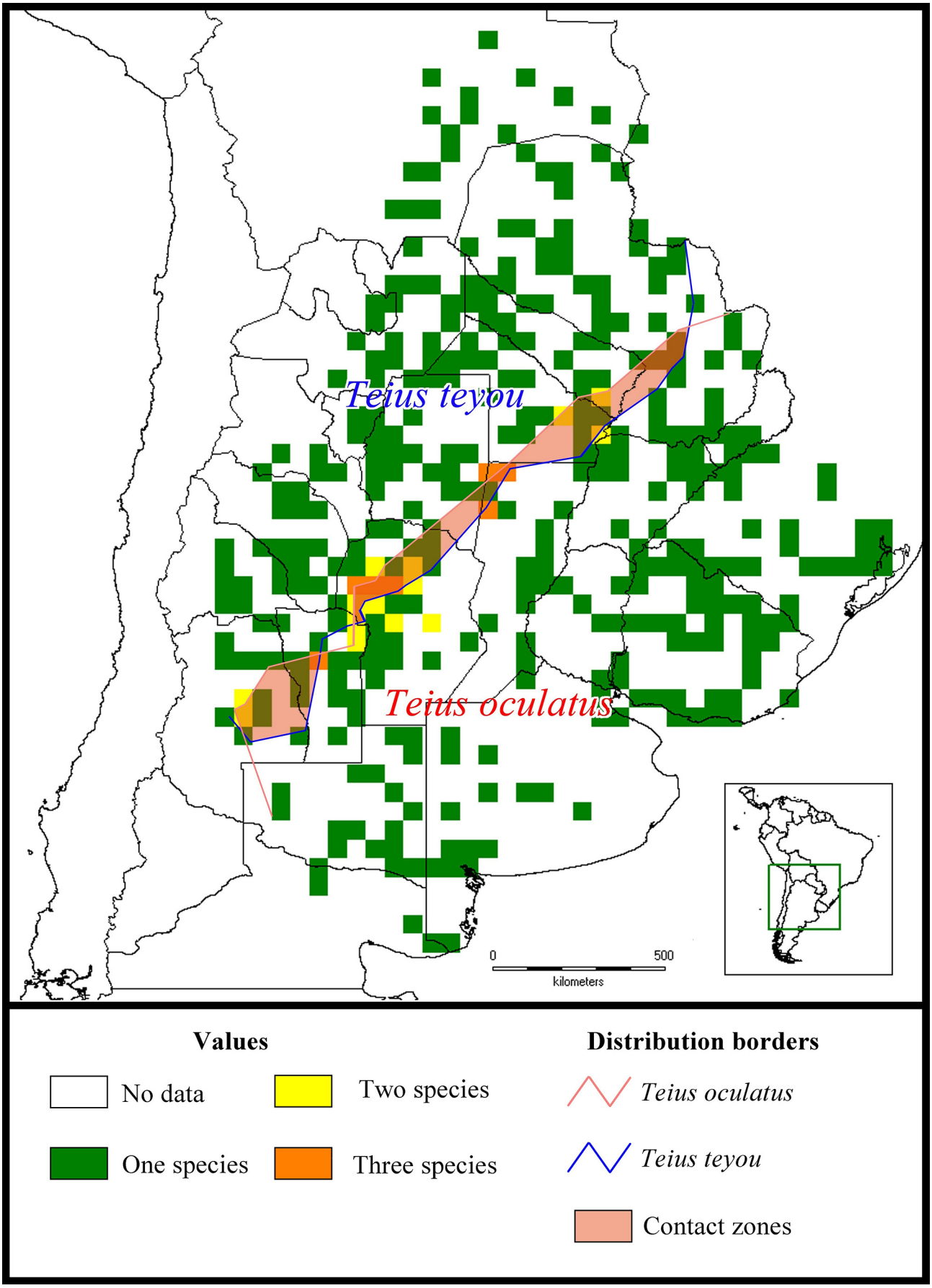

FIGURE 11. Richness map showing areas of sympatry among two or the three species of *Teius*.

FIGURE 12. Chronological change of the study site "Itakyry" (also shown in Fig. 3E). Dates of images from left to right: 13-IV-2003 / 1-VIII-2006 / 14-XI-2014. Note that suitable habitat for *Teius oculatus* is shrub lands (darker green).

FIGURE 13. Landscape at Laguna Blanca, being the only white sand environment where *Teius teyou* is present.

to 20ºC in the southern portion of its range (Fig. 6). The coldest record for the species was -2.6ºC (July) in Rincón del Atuel, Mendoza (Argentina), and the warmest was 36.6ºC (January) in Añatuya, Santiago del Estero (Argentina) (Fig. 10). The precipitation across its distribution fluctuates between less than 200 mm/yr to 1400-1600 mm/yr (Fig. 7). *Teius teyou* is distributed in areas with a very marked seasonality of dry and humid periods; the lowest precipitation rate is close to zero in the western portion of its distribution (near the Andes, Fig. 10).

Most of the distributions of the two sexual species show high levels of parapatry with some contact zones, where they are in sympatry (Fig. 11) but they were never found in syntopy. The three species of the genus are found in sympatry in some areas of northwestern Santa Fe, west of Córdoba, center of San Luis and central Mendoza in Argentina (Fig. 11). Contact areas between *T. oculatus* and *T. teyous* also include a fringe from Paraguay southwestwards to southeastern Chaco Province in Argentina (Fig. 11).

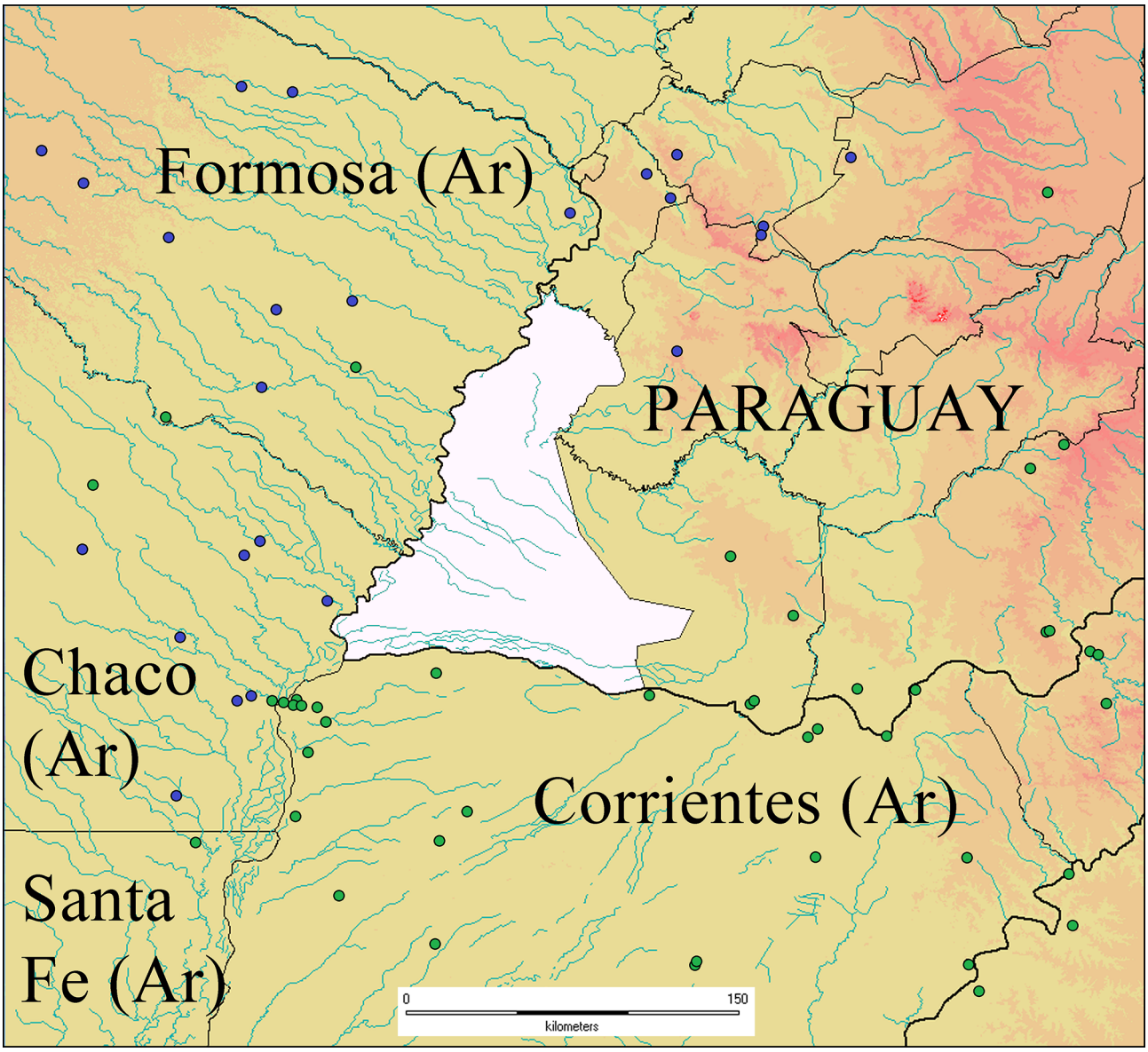

FIGURE 14. Map of southern Paraguay and northeastern Argentina (Ar), the Paraguayan Department of Ñeembucú (in blank) may be an area of potential sympatry according to known records of *Teius oculatus* (green dots) and *T. teyou* (blue dots), but there is no available information.

Discussion

In this review we present the northernmost locality record for *Teius oculatus* that corresponds to Canindeyú Department (Paraguay) (Fig. 1), with which we extend ca. 395 km northeast the previous known limit in Formosa, Argentina (Avila 2002). The southernmost record for this species is in Río Negro Province, being a record based on Scrocchi *et al*. (2010), but the records provided by these authors are based on probably unvouchered data from previous publications. Gallardo (1966) cited *T. teyou* for "northern Patagonia", but southern limits of this species are very distant from this area and probably his observations were based on specimens of *T. oculatus*. Avila (2002) considers the Río Negro River as the southern limit for the species, and indicates that previous records from Chubut Province most probably are mistakes.

Teius oculatus has a preference for open environments, with grasslands and shrubs its main habitat. Avila (2002) recorded the species in Pampas, Chaco, Monte, and Espinal. Even when *T. oculatus* is recorded in the Alto Paraná Atlantic Forest (also recorded by Avila 2002 as Paranaense province), it is important to note that the species

is not related to forests, but to grasslands or modified areas in the ecoregion (Fig. 4). Avila (2002) already stated that the species is well adapted to moderately disturbed environments, but its populations disappear when the anthropogenic impact is high (urban modifications, crop fields). Based on our field records, we present solid evidence that populations of *T. oculatus* are being affected by land modification (Fig. 11). Additionally, modifications in habitat structure are not the only factor affecting the persistence of the lizards. Habitat transformation into crop fields is usually followed by the use of chemicals, which inhibit the growth of herbs and also exterminate invertebrates' populations that are the main food source for *Teius*. In addition to this indirect effect on the lizard's survivorship (by absence of dietary source or by secondary poisoning), it is also known that some chemicals *per se* are harmful for lizards (Henle 1988; McIlroy 1992; Weir *et al*. 2010). More studies are needed in order to assess the specific effect of herbicides and pesticides on *Teius* populations.

The distribution of *Teius suquiensis* is limited to two spots (Avila 2002), the southern area is wider and associated with valleys between Sierras de San Luis and Sierras Grandes de Córdoba, and it extends eastern of the last one; the northern area is in the lowlands of Santa Fe Province (Fig. 1). Avila (2002) suggested that most probably populations from these two areas are connected. Later Cabrera & Monguillot (2007) registered this species in El Cercado (Córdoba), north of the Sierras' populations, but further sampling is needed in order to assess the level of connectivity between these areas. The southern population of *T. suquiensis* inhabits Espinal and Dry Chaco (Chaco Serrano according to Avila 2002), whereas the northern population is on a small area between both Humid and Dry Chaco. Although this species is more frequently found near the water (rivers or streams), Guerreiro *et al*. (1998) found it in a xeric environment.

For *Teius teyou* we extend the previous known distribution (Avila 2002) 60 km southwards in Mendoza Province (Argentina). The eastern limit is the record from Laguna Blanca (San Pedro, Paraguay, Fig. 2), not far from the previous records from Bella Vista (Amambay Department) and Piribebuy (Paraguarí Department) (Aquino *et al*. 1996). In Brazil, this species is marginal, being recorded only in Corumbá and Porto Murtinho (Mato Grosso do Sul State) (Gans 1960; Souza *et al*. 2010). Hellmich (1960) published the northernmost record for *T. teyou* in Bolivia, and whereas posterior works carried out in this country lack information about ecoregional affinities (Dirksen & De la Riva 1999; Padial *et al*. 2003), we found *T. teyou* associated with the Chiquitano forest. The presence of this species in Chaco and Monte was already mentioned by Avila (2002). One of the Paraguayan records came from Reserva Natural Laguna Blanca (San Pedro, Paraguay) (Fig. 9). The actual ecoregional affinities of Laguna Blanca is a mix of Cerrado with patches of Atlantic Forest and elements of Humid Chaco, with vegetation implanted in white sand soils (Smith *et al*. 2012; 2014) (Fig. 13). Avila (2002) stated that some populations of *T. teyou* in its western distribution range reach up to 2000 masl, but the highest record for this species is the record of Peracca (1897) mentioned also by Dirksen & De la Riva (1999) for San Francisco (Tarija, Bolivia) at 2595 masl.

Teius teyou has more distributional records than the other two species, which although may be indicative of higher densities, no extensive ecological studies have been performed on the three species of this genus. It is possible that an apparent less shy behavior of this species, is responsible for finding it more often on roads or uncovered areas, foraging longer distances away from shelters.

The two sexual species of the genus follow a parapatric NE-SW distribution (Avila 2002) with several areas where both species occur in sympatry. Similar patterns are also observed in other reptile taxa such as *Tropidurus spinulosus* with *T. torquatus*, *Boa constrictor amarali* with *B. c. occidentalis*, *Epicrates alvarezi* with *E. crassus*, *Chironius maculoventris* with *C. quadricarinatus*, and *Oxyrhopus rhombifer rhombifer* with *O. r. inaequifasciatus* among others. Avila (2002) defined three main areas of sympatry for Argentina,with the northeastern limit near the border of Paraguay. In Figure 14 it is possible to observe the absence of information from the Ñeembucu Department in Paraguay, which could be key to understand the sympatry pattern in that area. Also, it would be advisable to collect more information in other contact zones to understand the degree of sympatry between species and its relationship with the parthenogenetic species. According to Cano *et al*. (2015) the two sexual species occur in syntopy in the Chaco (Pilcomayo National Park) in Argentina. A genetic study must be carried out in order to study the origin of parthenogenesis and *T. suquiensis* in some areas of sympatry of the two sexual species in Central Argentina.

Teius lizards tolerate rather well environmental alterations and modifications when the impact is not too high (Avila 2002); Cabrera & Monguillot (2007) stated that the area where *T. suquiensis* occurs receives high anthropogenic pressure. In fact, the anthropogenic impact probably affects almost all of the distribution of the

genus. Eva *et al*. (2004) showed extreme modification in the ecoregions of Pampas and Espinal, and currently the deforestation is also threatening the Chaco (Caballero *et al*. 2014).

Climatic conditions of areas where either sexual species of *Teius* are distributed seem to be different. The northern and western distribution of the genus is occupied by *T. teyou*, where the climate is xeric, and lays in some Neotropical xerophytic areas where precipitation in the driest moths can be near zero (Fig. 10) near the Andes. On the other hand, some areas of the distribution of *T. oculatus* have no differentiation among dry and humid months. Thus it is not possible to discriminate between dry and humid seasons (Fig. 5). These areas mainly correspond to the Atlantic coast, having the greatest distance to the contact zones with *T. teyou*. Nevertheless, most of the climatic factors of the areas of both species have a marked seasonality in dry and humid months. Cappellari *et al.* (2007) analyzed the diet of *T. oculatus* along a period of three years but did not mention whether diet may be influenced by temperature or rainfall; but Acosta *et al*. (1991) did find differences in trophic ecology between different seasons.

Finally, the climate analyses of the distribution of *Teius* can illustrate some scenarios about its evolutionary history. It is well established that *Teius* and *Dicrodon* correspond to the oldest divergences within the phylogeny of Teiinae (Reeder *et al*. 2002; Giugliano *et al*. 2007; Harvey *et al*. 2012). Additionally, Harvey *et al*. (2012) showed morphological evidence to argue that *Dicrodon* may be intermediate between *Teius* and the rest of the Teiinae. *Dicrodon* inhabits xeric environments in Peru (Lehr *et al*. 2002; Venegas 2005), similar to *Teius teyou* (in most of its distributional range), whereas *T. oculatus* tends to inhabit moister areas along the Atlantic coast. It is possible that the common ancestor of *Dicrodon* and *Teius* was most related to xeric environments, and dispersal and further divergence leading to *Teius oculatus* could have been in response to an adaptive process for moist environments. Detailed genetic and past climate niche reconstruction analyses are needed to test this hypothesis.

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APPENDIX 1

Teius oculatus

ARGENTINA: Buenos Aires: Bahía Blanca (3) (LJAMM-CNP 447-8, MLP 215); Barreto (1) (MLP 424); Berazategui (1) (CENAI 813); Buenos Aires (3) (MACN 5266, SMF 11749-50); Burzaco (1) (MACN 1683); Capital Federal (5) (MLP 213, MACN 1663, 2085, 2604, MNHN 2656); Carmen de Patagones (1) (MACN 5391); 20 km N Carmen de Patagones (1) (MVZ 127352); Chasicó (2) (MLP 213, 791); Ezpeleta (2) (CENAI 853, 1425); General Lavalle (2) (MACN 3243, 3247); Laguna de Chasico (1) (LJAMM-CNP 6915); Los Talas (1) (MLP 217); Médanos (3) (MVZ 127349-51); Olivos (1) (MACN 1440); Pilar (1) (MACN 971); Pirovano (2) (MACN 2306, 3755); Punta Alta (1) (LJAMM-CNP 8520); Querandí (1) (MACN 2844); Saenz Peña (1) (MACN 3220); San Blas (1) (MLP 223); San Isidro (1) (MACN 3413); Sierra de la Ventana (4) (MACN 32565-6, 32833, 32840); Sierras Bayas (1) (MACN 5003); Sierras de Tandil (1) (MACN 2471); Trenquelauquen (1) (MLP 5592); Villa Ventana (1) (MACN 32810). Chaco: Barranqueras (1) (MACN 3058); Cierco Petizo (1) (FML 89); Colonia Las Mercedes (1) (CHC L 131); Resistencia (1) (CM 72486); 25 km S Santa Sylvina (1) (MACN 4735). Córdoba: Achiras (43) (LJAMM-CNP8489-93, FML 20405-9, UNRC-ZV 1204, 1206-7, 1211-2, 1266-9, 1270-1, 1273, 1275, 1376, 1658-60, 1664-6, 1891-5, 2216, 3432-4, 3987, 4006, 4223, 4478); Achiras, Balneario Municipal (2) (LJAMM-CNP 16506-7); Almafuerte (29) (UNRC-ZV 491-2, 495-6, 498-500, 575, 577-8, 634-5, 695-7, 700-3, 705, 707, 721-3, 725, 728, 730, 732, 1336); Alpa Corral (5) (FML 10461-3, 20453, UNRC-ZV 1361); Atos Pampa (2) (FML 20448-9); Baigorria (1) (FML 20437); 8 km E Charboniel (1) (FML 20447); Chucul (1) (FML 20458); Chucul, Arroyo (2) (FML 20433 UNRC-ZV 2213); Copacabana (2) (LJAMM-CNP 17043, 17056); Coronel Moldes (1) (FML 20457); Laboulaye (2) (CENAI 584-585); La Carlota (1) (MACN 4928); La Falda (5) (MHN SR H 41, 43, 58, 184, 280); Laguna Oscura (7) (FML 10467-71, 20455-6); La Puerta (1) (LACM 131313); Las Higueras (25) (FML 10436-7, 10293-304, 10427-35, 20435, UNRC-ZV 520); Leones (1) (MACN 2040); Mina Clavero (2) (MLP 1722-3); Piedras Grandes (2) (FML 20452, 20454); Río Cuarto (132) (LJAMM-CNP 5001, 8494, 8498, FML 10267-92, 10310-2, 10318-33, 10335-9, 10341-9, 10352-4, 20445, 20450-1, 20712, UNRC-ZV 89, 93, 96, 104, 109, 112-3, 117, 130, 135, 138-44, 436-7, 548-52, 791-2, 794, 796, 799, 801-4, 806, 810-20, 831, 833, 836, 922-4, 926-7, 962-4, 966-7, 2178, 2364, 2366, 2468); Tanti (18) (LJAMM-CNP 8499, FML 10440-1, 10446-52, 10454, 10458, 20436, 20687-8, UNRC-ZV 50, 2429, MVZ 127312-5); 2 km E Tuclame (1) (LACM 131321); Villa del Lago (1) (FML 10453); Villa El Chacay (8) (FML 10438-9, 10459-60, 10465-6, UNRC-ZV 2033-4); Villa María (2) (FML 20353, 20355); 10 km W Washington (5) (FML 20723, UNRC-ZV 2156-9). Corrientes: Colonia Galarza (1) (CHC L 71); Corrientes (4) (LJAMM-CNP 8488, MHN SR H 203, MLP 437, UNRC-ZV 1811); Concepción (1) (MACN 3379); El Sombrerito (1) (MACN 4858); Empedrado (2) (CHC L 180-1); Estación Biológica Corrientes (1) (FML 20434); Iberá (1) (MLP 5454); Isla Apipé Grande (1) (FML 222); Itá Ibaté (1) (CHC L 82); Ituzaingó (2) (CHC L 395, UNNEC 389); Laguna Brava (1) (CHC L 135); Las Marias (1) (CHC L 176); Manantiales (1) (MACN 3284); Mburucuyá (1) (CHC L 395); Monte Caseros (1) (MACN 3220); Ombú Chico (4) (UNNEC 547-50); General Belgrano (1) (CHC L 139); Saladas (4) (CHC L 307-9, MACN 3559); Santo Tomé (1) (CHC L 177); Santa Tecla (1) (UNNEC 887); Yacareí (2) (CHC L 151-2). Entre Ríos: Alcaraz (1) (CENAI 357); Ayui (2) (CENAI 918-9); Concepción del Uruguay (4) (MACN 4450, 4618, 25177-8); Concordia (1) (MACN 859); Paraná (1) (MACN 282); Paranacito (12) (CENAI 589, 642, 797, 640-1, 904, 1245-8, 4225, 5185); Villaguay (1) (MACN 933). Formosa: El Colorado (1) (CHC-L 293). La Pampa: Almacén El 52 (3) (RVP 72-4); Cerro Centinela (2) (RVP 110-1); Conelo (2) (MACN 914, 1048); Cuchillo Có (1) (RVP 20); Estancia Arco Iris (1) (RVP 43); General Campos (1) (IADIZA-CH 234); General Pico MACN (4) (1034, 1044, 1083, 32808); Gobernador Duval (1) (LJAMM-CNP 4493); Guatraché (1) (RVP 71); Intendente Alvear (1) (MACN 5309); La Adela, 45.5 km N (1) (LJAMM-CNP 6024); La Adela, 87.8 km N (1) (LJAMM-CNP 6026); Lihué Calel (2) (MACN 31376, 5212); Loventué (1)

(MACN 1310); Parque Luro (1) (RVP 63); 17 km SE Puelén (1) (LJAMM-CNP 8433); Realicó (7) (FML 10444-6, 10455-7, 10464); Ruta Provincial 107, 10.1 km N Ruta Nacional 152 (1) (LJAMM-CNP 14692); Santa Rosa (1) (RVP 62); Victorica (2) (USNM 60398, 63951). Mendoza: Cuadro Nacional (24) (FML 20438-40, MHN SR H 705, 709, 809, 814, UNRC-ZV 54-5, 86, 122, 124, 145-55); La Paz (1) (MACN 1587); Lavalle (1) (IADIZA-CH 5); Mendoza (2) (MNHN 334, 1997); Isla Diamante (3) (MHN SR H 865-6, UNRC-ZV 1908); Rincón del Atuel (2) (MHN SR H 15, 717); Las Horquetas, 10.6 km W (1) (LJAMM-CNP 5730); Las Horquetas, 22 km W (1) (LJAMM-CNP 17202); San Rafael (20) (LJAMM-CNP 8497, FML 20441, MHN SR H 247, 287-8, 398, 400, 491, 493, 524-5, 637-8, 641-2, 799, 982, 1040, 1101, 1284). Misiones: Azara (11) (MCP 3092-101, 4216); Bompland (2) (MLP 212, 214); Campo Vieira (1) (MLP 811); Cuña Pirú (1) (MLP 2199); Cuña Pirú, Arroyo (2) (MLP 2197-8); San Ignacio (2) (MACN 1582, 1614). San Luis: Beazley, 4 km SW (1) (LJAMM-CNP 5772); Buena Esperanza, 50 km N (2) (LJAMM-CNP 16508, 16532); Buena Esperanza, 82.5 km N (1) (LJAMM-CNP 3126); Buena Galia, 37.7 km N (1) (LJAMM-CNP 14806); Cerro Centinela (1) (LJAMM-CNP 4198); 7 km N Naschel (1) (FML 20442); El Morro (1) (MACN 2324); Estancia El Centenario (1) (MLP 5307, 5588); Las Isletas (1) (MACN 212); Unión (1) (IBAUNC 440). Santa Fe: Alto Verde (1) (MHN SR H 226); Cayasta (1) (FML 2677); Cerrito (1) (MHN SR H 77); Colastine Sur (3) (MHN SR H 35, 278-9); Colonia Mascias (1) (MACN 1643); Florencia (1) (FML 20714); Gregoria Perez de Denis (36) (CENAI 397, 403-4, 431, 439, 441, 443, 447, 450-1, 456, 461, 474-5, 477, 479, 481, 488-9, 492-3, 500, 503-4, 506-7, 509, 513, 517, 526, 532, 536-7, 544, 563, 567); La Brava (1) (MACN 4676); Las Rosas (1) (MACN 4745); Piquete (1) (MHN SR H 185); 5 km de Reconquista (1) (IBAUNC 260); Reserva Ecológica El Pozo (1) (MHN SR H 236); Roldán (1) (MACN 4911); Rosario (2) (FML 860, UNRC-ZV 2926); San Justo (1) (MHN SR H 221); Santa Fe (6) (MHN SR H 3, 8-9, 74, 127, 185); Santa Rosa de Calchines (1) (CENAI 799); Santo Tomé (1) (MHN SR H 181); Tostado (1) (MACN 1845). BRAZIL: Rio Grande do Sul: Arambare (9) (MCP 4494, 4559-61, 4617, 4664, 5037-9); Butia (1) (MCP 6931); Caçapava do Sul (1) (MCP 10022); Cacequi (1) (CM 928); Camaqua (1) (MCP 5040); Candiota (3) (MCP 11398, 11863, 19148); Carazinho (3) (MCP 3510-2); Dom Feliciano (170) (MCP 7840, 12508-14, 12606-12, 12823-51, 13191-203, 13320-7, 13485-96, 13822-33, 14353-68, 14370-83, 14604-17, 15896-922, 18319-27, 18391); Dom Pedrito (4) (MCP 18592-4, 18702); Encruzilhada do Sul (7) (MCP 2661, 7864, 7880, 7964-6, 8973); Garruchos (1) (MCP 3054); Guaiba (3) (MCP 1257, 6928-9); Mormaço (1) (MCP 17718); Pirapo (1) (MCP 3060); Porto Alegre (19) (CAS 87096, MCP 289-90, 352-8, 1052, 1231, 1553, 4871, 5361, 12078, 12083, 14842, TNHC 20991); Porto Lucena (2) (MCP 11689-90); Porto Xavier (1) (MCP 3313); Santana do Livramento (1) (MCP 3733); Santa Maria (17) (MCZ 126774-6, 126778, 126780, 43351-9, 126773, 126777, 126779); Santo Antonio das Missoes (1) (MCP 3085); Santo Cristo (1) (MCP 11667); São Borja (3) (MCP 13273-5); São Francisco de Assis (2) (MCP 18887-8); São Jeronimo (23) (MCP 14459, 15335, 15649-51, 15780-3, 15874-5, 15968, 16203-5, 17753-5, 17886-9, 18658); São Leopoldo (1) (MCP 2368); São Nicolau (1) (MCP3546); Turarendi (2) (MCP 11595, 11603); Uruguaiana (8) (8760-1, 11892-7); Viamão (2) (MCP 4657, 6930). PARAGUAY: Alto Paraná: Itaipú Reserves (1) (Mai 52); Itakyry (5) (MNHNP 11802-6). Caaguazú: J.E. Estigarribia (11) (PCn 32-42). Canindeyú: Reserva Natural del Bosque Mbaracayú (1) (MNHNP 10860). Itapúa: El Tirol (1) (USNM 253543); Isla Talavera (2) (UNNEC 871-2); Parque Nacional San Rafael (4) (MNHNP 9221, 11822, 11836, 11838). Misiones: San Ignacio (1) (MNHNP 2761); Santiago (1) (MNHNP 2762). San Pedro: Colonia Primavera (3) (NHMUK 1956.1.3.21, 1960.1.2.64-5). URUGUAY: Artigas: Arroyo de la Invernada (1) (MCP 14870). Canelones: Carrasco (3) (CM 57073-5); Solymar (1) (IBAUNC 136). Cerro Largo: Melo, 20 km E (2) (Field number: SC 407-8). Lavalleja: Cerro Arequita (1) (CM 58332). Río Negro: Tres Árboles (1) (SC s/n, tissue sample at SMF). San José: Sierra Mahoma (1) (IBAUNC 162). Montevideo: Montevideo (5) (MNHN 567, 2655, 8387, USNM 60413, 65575). Tacuarembó: Estación "Francia" (1) (CM 57076); Estancia La Loma (1) (CM 55379).

Teius suquiensis

ARGENTINA: Córdoba: Arroyo Los Algarrobos, 1 km E (1) (LJAMM-CNP 8238); Almafuerte (25) (UNRC-ZV 493-4, 497, 573, 581, 631-3, 636, 693-4, 698-9, 704, 706, 724, 726-7, 729, 731, 1332-35, 1337); Bialet Massé (71) (FML 02537, LACM 131299-309, UNRC-ZV 38-41, 44-49, 57, 119-124, 126-128, 345-366, 480-489, 534-5, 557, 570-2, 574); Cabana (1) (MACN 4658); Cañada de Luque (1) (LACM 131312); Capilla del Monte (2) (UNRC-ZV 2085-6); Cienaga de Allende (1) (CENAI 284); Córdoba (3) (LACM 131324, MVZ 127329, UNRC-ZV 590); Cosquín (1) (UNRC-ZV 579); Despeñaderos (12) (UNRC-ZV 708-715, 717-720); Embalse Rio Tercero (1) (UNRC-ZV 2018); Estancia El Cercado, 12 km E of Pozo Nuevo (3) (UNRC-ZV 484-5, 488); Falda del Carmen (1) (UNRC-ZV 716); La Carolina, 2 km SE of Villa Warcalde (14) (MVZ 127353, 127355-7, 127361-3, 127373, 127381, 127383, 127388, 127393, 127910, 128175); Mina Clavero (11) (UNRC-ZV 606-611, 613-617); Montecristo (1) (UNRC-ZV 1646); Niña Paula (2) (UNRC-ZV 1754-5); Nono (2) (UNRC-ZV 3201-2); Nono, 2 km W (6) (LJAMM-CNP 13982-4, 12995-7); Pintos Arriba (1) (UNRC-ZV 1364); Rio Segundo (1) (LACM 131310); San Agustín (2) (UNRC-ZV 782-3); San Antonio de Arredondo (11) (UNRC-ZV 505, 516-524, 526); San Roque (2) (MLP 1186, UNRC-ZV 788); Santa Rosa de Calamuchita (1) (UNRC-ZV 4128); Taninga (1) (UNRC-ZV 1632); Tanti (47) (UNRC-ZV 371, 374, 376, 378-81, 387-90, 414, 425, 444, 453-56, 462, 475, 477, 529, 540-1, 563-4, 591-94, 599, 601-2, 776, 984, 986-9, 991-999); Unquillo (1) (MACN 2299); Villa del Rosario (2) (UNRC-ZV 1363, 1756); Villa Las Rosas (4) (UNRC-ZV 1422, 1443, 1453, 2730); Villa Maria (1) (UNRC-ZV 4258); Villa Warcalde (17) (MVZ 127316-7, 127319-21, 127330-2, 127338-41, 127345, UNRC-ZV 586-9); Yacanto (5) (UNRC-ZV 1434-7, 2733). San Luis: Ayacucho (1) (UNRC-ZV 2729); Chacabuco (3) (UNRC-ZV 664-6); Coronel Pringles (1) (LJAMM-CNP 5729); Junin (2) (UNRC-ZV 1431, 1433). Santa Fe: Gregoria Perez de Denis, 20 km E (2) (UNRC-ZV 3546-7); Villa Minetti (1) (UNRC-ZV 4257); Villa Minetti, 45 km (1) (UNRC-ZV 4176); Gregoria Perez de Denis (Estancia El Nochero) (35) (CENAI 342, 399, 433-4, 438, 445, 448, 454, 458, 460, 462, 466, 469, 472-3, 480, 482-5, 490, 497, 527, 529, 538, 551-2, 554, 557, 560-1, 565, 572-4); Tostado (1) (MACN 1845).

Teius teyou

ARGENTINA: Catamarca: Andalgalá (3) (CM 70192, 70204, UNRC-ZV 2145); Andalgalá, 19 km S (1) (CM 70203); Belen (2) (FMNH 10684, 10873); Campo del Breal (1) (FML 1627); Catamarca, 1 km N (1) (MVZ 127298); Crossing National Route 60 and Local Route 46, Km 35 (1) (UNRC-ZV 2123); Icaño, 6.3 km W (1) (LJAMM-CNP 3166); Pomancillo (1) (MACN 1713); Puesto Rio Blanco (1) (FML 1614); Rio Poman (12) (CM 70190-1, 70195, 70197-8, 70200-2, 70205, 70209-10, 70213); Londres (1) (LJAMM-CNP 4246); Tinogasta (1) (MACN 3211). Chaco: Avia Terai (2) (CHC L 182-3); Basail (2) (UNRC-ZV 1150-1); Colonia Las Mercedes (9) (CHC L 25-33); Comandante Frías (3) (CHC L 2100, 103, FML 12014); Florida Grande (3) (CHC L 80-1, FML 2015); General Pinedo (1) (MACN 1923); Las Palmas (1) (CHC L 312); Loro Hablador (4) (MLP 2224, 2547, 5154-5); Machagai (2) (CHC L 164-5); Makallé (3) (MLP 1812, CHC L 178-9); Nueva Pompeya (6) (CHC L 14-6, 58-60); Presidencia Roque Saenz Peña (3) (LJAMM-CNP 186-7, UNRC-ZV 1503); Resistencia (6) (IBAUNC 259, MACN 5394, 5481, 29820-1, 29824); Selvas del Rio de Oro (3) (CHC L 281, 310-1); Taco Pozo (3) (CHC L 212-4). Córdoba: Cañada de Luque (2) (LACM 131311, 131325); Chancaní (3) (UNRC-ZV 1767, 3504-5); Colonia Tirolesa (1) (MVZ 127382); El Brete (3) (UNRC-ZV 1192-4); Guanaco Muerto (8) (UNRC-ZV 1679-86, 2017); La Paz (1) (MACN 1659); La Posta (16) (UNRC-ZV 1859-61, 1863-76); La Batea (3) (MLP 271-3); La Puerta (3) (LACM 131314-6); Lucio V. Mansilla (4) (FML 2679, 2681, UNRC-ZV 2091, 3021); Media Naranja (2) (UNRC-ZV 1608-9); Piedra Pintada (1) (UNRC-ZV 1142); San Marcos Sierra (4) (UNRC-ZV 2081-3, 2719); San Jose de Las Salinas (1) (MVZ 127270); Sebastián Elcano (4) (CHC L 154-7); Serrezuela (1) (UNRC-ZV 1191); Serrezuela, 11 km W (2) (LACM 131328-9); Serrezuela, 2 km E (1) (LACM 131327); Totoralejos (1) (MVZ 127311); Tristan Narvaja (1) (MVZ 127368); Tuclame, 2 km E (6) (LACM 131312-3, 131317-20); Villa Cura Brochero (1) (MLP 1265); Villa de Soto (4) (LACM 131330-2, 134448). Formosa: Comandante Fontana (3) (MACN 712, 2298, CHC L 467); El Bagual Ecological Reserve (3) (TCWC 69344, 70229, 70250); El Mistolar (1) (TCWC 70318); El Quebracho (2) (UNRC-ZV 477-8); El Tayí (1) (CHC L 269); Estancia Don Theo (1) (CHC L 372); Gran Guardia (2) (MACN 2828, IBAUNC 77); Ibarreta (3) (CHC L 161-3); Ingeniero Guillermo Juarez (1) (CHC L 466); Ingeniero Guillermo Juarez, 13.2 km W (2) (LJAMM-CNP 12098-9); La Rinconada (3) (CHC L 483, 486-7); Laguna Yema (2) (CHC L 477, FML 1716); Las Lomitas (6) (CHC L 17, 19, 132-3, 174, FML 2181); Los Pocitos (1) (CHC L 455); Misión Franciscana Tacaaglé (1) (FML 1717); Palmar Largo (9) (LJAMM-CNP 8486-7, MHN SR H 202, UNRC-ZV 807-10, CHC L 433-4); Palo Santo (1) (CHC L 469); Paraje Urbana Vieja General Belgrano (11) (CHC L 20-4, 76, 85-9); Pozo de Maza (1) (CHC L 412); Teniente General Fraga (2) (CHC L 420-1). Jujuy: Estancia Yuto (1) (IBAUNC 80); Ingenio La Esperanza (1) (FML 789); Yuto (8) (FML 255, 443, 473, MVZ 127277-81). La Rioja: Aimogasta (2) (MACN 1488, 2233); Anillaco (6) (CM 147859, 147896, LJAMM-CNP 700, 736-7, 1143); Anillaco, 6 km E (1) (LJAMM-CNP 501); Antinaco (2) (FML 1735-6); Chamical, 11 km E (2) (CM 70206-7); Chamical, 19 km E (3) (CM 70193, 70196, 70212); Chamical, 5 km W (1) (CM 70198); Chamical, 5 km W (7) (CM 70214-20); Chamical, 6 km E (3) (CM 70194, 70208, 70211); Chepes, 18 km E (4) (LJAMM-CNP 5733-6); Chepes, 23 km W (1) (LJAMM-CNP 13985); Chepes, 5 km E (1) (MVZ 127297); Chilecito, 12 km N (1) (LJAMM-CNP 698); Ilisca, 4.2 km N (1) (LJAMM-CNP 735); La Rioja (1) (IBAUNC 477); Los Molinos (16) (LJAMM-CNP 1978, 2012-5, 2025-6, 2039-40, 2060-1, 2103, 2193, 2234, 2252, 8578); Los Sauces (1) (MACN 2554); Paganzo, 7 km W (1) (LJAMM-CNP 2257); Patquía (4) (MACN 1181, 1293, 4663, 9510); Patquia, 33 km S (1) (LJAMM-CNP 17143); San Antonio (1) (LJAMM-CNP 17203); Villa Union (1) (LJAMM-CNP 4167). Mendoza: Carrizal (1) (MVZ 180775); Chacras de Coria (2) (IBAUNC 1098, 1137); Cuadro Nacional (1) (MHN SR H 709); Desaguadero, 0.5 km W (3) (MVZ 127299-301); El Challao (2) (IBAUNC 147, 849); Estacion Capdevila, 2 km S (1) (MVZ 127269); Estacion Capdevila, 4 km NW (5) (MVZ 127226, 127261-2, 127265, 128176); Estacion Capdevila, 7 km NW (1) (MVZ 127259); Estacion Pampita (1) (IBAUNC 1039); Finca El Sauce (1) (IBAUNC 1331); General Alvear (1) (MLP 826); General San Martín (1) (MLP 825); Godoy Cruz (2) (IBAUNC 325, 481); Guaymallen (1) (IBAUNC 243); Hornito del Grinco (1) (FML 2260); La Mora, 18 km E (1) (LJAMM-CNP 3144); Lavalle (1) (IADIZA-CH 52); Medanos de Picardo (1) (MVZ 127348); Mendoza (6) (IADIZA-CH 16, 79, 152, MCZ 14916, MLP 432, MVZ 127268); Reserva Ñacuñan (7) (IADIZA-CH 53-4, 61, 78, 92, IBAUNC 1114, 1170); Rincón del Atuel (16) (LJAMM-CNP 8495-6, MHN SR H 97-9, UNRC-ZV 1820-5, 1906-7, 2175, 2177, 2493); Route 179, 9.2 km N from crossroad of Routes 179 and 190 (1) (LJAMM-CNP 5036); San Rafael (13) (LJAMM-CNP 8500, MHN SR H 399, 490, 562, 1085, 1269, UNRC-ZV 56, 1820-5). Salta: Aguaray (1) (MACN 6075); Alemania, 3 km S (3) (LJAMM-CNP 17119-21); Cierva Muerta (1) (FML 92); El Carmen, 10 km W (3) (LJAMM-CNP 12057-8, 12060); El Duraznito (1) (MACN 35520); Embarcacion (9) (FML 518, 2470, LACM 73993-5, 73997-4000); Hickmann (4) (FML 34, 251, 290, 1718); La Población (1) (FML 311); La Quena (2) (FML 02471-2); Los Blancos (1) (MLP MLP 5420); Los Colorados (1) (FML 2710); Metán (1) (MACN 2892); Morillo (3) (MLP 1528-9, 2170); Obraje Salta Forestal (1) (FML 1111); Pluma de Pato, 3 km W (1) (LJAMM-CNP 12100); Puesto Vialidad Salta, 7.1 km W (2) (LJAMM-CNP 12043-4); Quebrada de Acambuco (1) (FML 874); Quebrada de las Conchas (1) (LJAMM-CNP 11807); Rio Bermejo (1) (MACN 4335); Rio Blanco (1) (FML 590); Rio del Valle (1) (FML 1119); San Ramón de la Nueva Orán (1) (MACN 8506); Talapampa, 8.6 km SW (1) (LJAMM-CNP 11806); Tobantirendá (1) (FML 205); Urundel (36) (MACN 2202, 9925-59). San Juan: Aguango (2) (IMCN-UNSJ 63, 164); Astio, 20 km N (1) (LJAMM-CNP 8585); Baños del Salado (1) (IMCN-UNSJ 230); Estacion ferroviaria Adan Quiroga (1) (LJAMM-CNP 3142); La Rinconada (1) (MACN 4349); Los Baldecitos (1) (LJAMM-CNP 12488); Marayes, 15 km W (1) (IBAUNC 1293); Medanos río San antonio (1) (IMCN-UNSJ 64); Pie de Palo, 5 km SE (1) (MVZ 127271); Punta del Agua, 32.8 Km W (1) (LJAMM-CNP 4060); San Agustin del Valle Fertil (1) (IBAUNC 1147); San Jose de Jachal, 3 km NW (3) (MVZ 127273-5); San Juan (6) (IMCN-UNSJ 70, 85, 249-50, 268, 462); Valle Maradona (1) (IMCN-UNSJ 281); Villa los Olivos (1) (IMCN-UNSJ 442); Villa Mercedes (1) (MACN 1449). San Luis: Alto Pencoso (8) (MLP 435-6, 406, 408, 429, 431, 433-4); Balde, 1 km W (4) (MVZ 127302-5); El Caldén (2) (UNRC-ZV 72-3); La Aguada (1) (MACN 396); La Chañarienta (1) (MACN 34225); La Higuerita (1) (UNRC-ZV 1877);

Merlo (2) (UNRC-ZV 1430, 1432); Quines (4) (LJAMM-CNP 1990-3); Rio Amieva (1) (JMC-DC 60); San Francisco del Monte de Oro (1) (MACN 396); San Francisco del Monte de Oro, 11 km S (1) (MVZ 127306); San Gerónimo (1) (MACN 30315); Santa Rosa del Conlara (1) (UNRC-ZV 1505); Sierra de las Quijadas (2) (4152-5). Santa Fe: Estancia El Nochero (49) (CENAI 364, 396, 401, 416, 418, 420-2, 430, 432, 436-7, 442, 444, 449, 452, 455, 457, 463-4, 467-8, 470-1, 478, 486, 491, 494-6, 498, 502, 5, 8, 511, 514, 518, 528, 530-1, 534, 539, 541, 543, 546-7, 549, 553, 558); Santa Sylvina, 40 km S (1) (MACN 4735); Tostado (1) (MACN 287). Santiago del Estero: Añatuya (2) (FML 2676, MLP 1108); Bandera (4) (CENAI 333-4, 365, 520); Beltrán (4) (MLP410, 439-41); Caspi Corral (1) (FML 02261); Dique El Frontal (1) (FML 1209); Girardet (1) (MLP 816); Guardia Escolta (1) (CENAI 327); Huyamampa (1) (FML 294); Matará (1) (MACN 4999); Monte Quemado (3) (CHC L 184, FML 1751, 1896); Pampa de los Guanacos (1) (MACN 4999); San Juan, 3.5 km W (1) (LJAMM-CNP 12128); Santiago del Estero (1) (MHN SR H 50); Tintina (1) (MHN SR H 190); Urutaú (3) (CHC L 198-200); Villa La Punta (1) (FML 2632); Villa Ojo de Agua (1) (LJAMM-CNP 12127); Villa San Martín, 5 km S (1) (MACN 5083). Tucumán: Laguna Carimayo (1) (FML 165); Las Cejas (1) (FML 7091); Los Puestos (1) (FML 504); Rio Sali (13) (FML 461, MVZ 127282-92, IBAUNC 79); Rio Tapia (1) (FML 2606); San Miguel de Tucumán (3) (FML 1058, IBAUNC 78, MLP 428); Tacanas (3) (MACN 2675, MCZ 66990-1); Yerba Buena (1) (FML 1237). BOLIVIA: Santa Cruz de la Sierra: Boyuibi (2) (LACM 37678-9); Carandaiti, 30 km SE (1) (LACM 37680); Curuyuqui (2) (USNM 336159,336202); El Carmen (2) (CM 35887-8); Abapó Norte (4) (MACN 19102-5); Rio Seco (7) (MACN 19005-11); Robore (1) (CM 35889); San Antonio de Parapetí (1) (MACN 33045); San Jose de Chiquitos (4) (MCZ 39981-2, SMF 11749–50); Santa Cruz de la Sierra (1) (MACN 9270); Turenda (1) (MACN 4495). Tarija:Capirenda (1) (LACM 37677); Villamontes (46) (KU 136375-95, 136397-8, 136400, 136402-7, 136409, SMF 26551-65).. BRAZIL: Mato Grosso do Sul: Corumbá: (6) (CM 35879-84). PARAGUAY: Alto Paraguay: Agua Dulce (1) (MNHNP 2739); Cerro León (8) (CM 109160-1, MNHNP 2755-60); Colonia Potrerito (1) (MNHNP 9282); Estancia Punto Alto (1) (MNHNP 10165); Madrejón, 45 km S (1) (USNM 342486); Mayor Pablo Lagerenza (1) (MNHNP 7231); Parque Nacional Defensores del Chaco (1) (MNHNP 7232); Puerto Casado (1) (UMMZ 94089). Amambay: Bella Vista, 2 km W (1) (MNHNP 2743). Boquerón: Campo Loro (1) (MNHNP 10654); Comunidad Ayoreo Jesudi (2) (MNHNP 10727, 11112); Comunidad Ayoreo Tunucojai (1) (MNHNP 10655); Estación Experimental Chaco Central (1) (MNHNP 9994); Estancia Casilda (1) (MACN 1795); Estancia Guayhú (1) (MACN 1769); Estancia Jabalí (1) (MNHNP 8068); Estancia La Gama (2) (MNHNP 8075, 11031); Filadelfia (11) (CM 94219, 94224, MNHNP 2734-8, 2740-1, USNM 341970-1); Neuland (4) (MNHNP 8047, 8069, 8072-3); Pedro P. Peña (6) (MNHNP 4021, 4030, 4022-3, 4029, 8077); Route IX Km 517 (3) (MNHNP 7082, 7301, 7662); Teniente Ochoa (5) (UCS 5729-31, USNM 341974-5); Villa Hayes (2) (USNM 341974-5). Central: Lago Ypacaraí (1) (USNM 341972). Concepción: Asentamiento San Ramón (4) (MNHNP 11816-9); Estancia Kumaré (3) (MNHNP 11815, 11820-1); Loreto (1) (MNHNP 9848); Río Apa (1) (MZUT 958); Paso Barreto (2) (MNHNP 8373, 9847); Rancho Z (7) (CM 142497-9, 142535, MNHNP 7648-9, 7653); San Lázaro (1) (MNHNP 2742). Cordillera: Piraretá (1) (USNM 341973); Quinta Las Andreas (1) (Field number: PCS 215). Paraguarí: Caapucú, 22 km N (1) (MNHNP 10018). Presidente Hayes: Agroganadera Solito (8) (MNHNP 11807-14); Estancia Juan de Salazar (27) (MNHNP 3403, 3608-15, 4204, 4222, 4318-9, UCS 5727-8, 6976-86, USNM 341976); Estancia La Golondrina (2) (CM 109252, MNHNP 328); Estancia La Victoria (1) (MNHNP 4321); Estancia Santa María (4) (CM 142617-20); Estancia Tinfunqué (4) (CM 94018, 94150-1, 94158); Estancia Toro Mocho (1) (MNHNP 10628); Fortín Teniente Coronel Miguel A. Ramos (1) (MNHNP 10770); Pozo Colorado (2) (MNHNP 9725, MVZ 110970). San Pedro: Laguna Blanca (8) (CZPLT 86, 105, 288, 289, 319, 429, 436, 469).

Affidavit

I herewith declare in lieu of an oath that I have produced the present dissertation:
“Lizards of Paraguay: an integrative approach to solve taxonomic problems in central South America”
autonomously and in doing so did not avail myself of resources which are not specified therein. In particular, all borrowings taken from other writings are marked with references to the respective writings.
I assure that I have adhered to the principles of good scientific practice and did not make use of the services of any commercial doctorate agencies or consultants.

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorgelegte Dissertation
“Lizards of Paraguay: an integrative approach to solve taxonomic problems in central South America”
selbständig angefertigt und mich anderer Hilfsmittel als der in ihr angegebenen nicht bedient habe, insbesondere, dass alle Entlehnungen aus anderen Schriften mit Angabe der betreffenden Schrift gekennzeichnet sind.
Ich versichere, die Grundsätze der guten wissenschaftlichen Praxis beachtet, und nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Frankfurt am Main, den .....

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	Geometrics Morphometrics: Applications to mammalogy Sociedad Argentina para el Estudio de los Mamíferos. Buenos Aires, Argentina. Instructor: Dr. Andrea Cardini.	2012
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	VIII Taller de Genética para la Conservación Red de Genética para la Conservación *Chillán, Chile* Instructors: Several instructors	2012
	Natural History Collections Management Training Program for Latin American and Caribbean Professionals. *National Museum of Natural History, Smithsonian Institution. Washington D. C., U.S.A.* Instructors: Several instructors.	2008
	Curso teórico práctico: “Manejo de animales para bioterios no tradicionales”. Segundo Módulo. *Bioterio-Serpentario (Instituto de Higiene) Hospital de Clínicas/ Facultad de Ciencias. Montevideo, Uruguay.* Instructors: Tec. Santiago Carreira y Mag. Melitta Meneghel.	2005
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Publications

Cacciali, P. & G. Köhler. 2018. Diversity of *Tropidurus* (Squamata: Tropiduridae) in Paraguay—an integrative taxonomic approach based on morphological and molecular genetic evidence. *Zootaxa*, 4375(4): 511–536.

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Smith, P., **P. Cacciali**, K. Atkinson, A. Källberg & H. Pheasey. 2011. Nuevos registros de Gymnophthalmidae (Reptilia: Sauria) en la Reserva Natural Laguna Blanca, Departamento San Pedro, Paraguay y una clave para las especies paraguayas. *Nótulas Faunísticas*, 81: 1-6.

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Scott, N. J. & **P. Cacciali**. 2011. Reptilia, Squamata, Teiidae, *Dracaena paraguayensis* Amaral, 1950: In Paraguay, *Dracaena* sí, *Crocodilurus* no. *Check List*, 7(1): 52.

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