Cryptic Diversity in the Neotropical Gecko Genus *Phyllopezus* Peters, 1878 (Reptilia: Squamata: Phyllodactylidae): A New Species from Paraguay

Pier Cacciali  
*Senckenberg Forschungsinstitut und Naturmuseum Frankfurt*

Sebastian Lotzkat  
*Senckenberg Forschungsinstitut und Naturmuseum Frankfurt*

Tony Gamble  
*Marquette University, anthony.gamble@marquette.edu*

Gunther Köhler  
*Senckenberg Forschungsinstitut und Naturmuseum Frankfurt*

Published version. *International Journal of Zoology*, Vol. 2018 (2018): Article ID 3958327. DOI © 2018 Pier Cacciali et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Research Article

Cryptic Diversity in the Neotropical Gecko Genus *Phyllopezus* Peters, 1878 (Reptilia: Squamata: Phyllodactylidae): A New Species from Paraguay

Pier Cacciali, Sebastian Lotzkat, Tony Gamble, and Gunther Köhler

1Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Senckenberganlage 25, 60325 Frankfurt am Main, Germany
2Johann Wolfgang Goethe University, Institute for Ecology, Evolution & Diversity, Biologicum, Building C, Max-von-Laue-Straße 13, 60438 Frankfurt am Main, Germany
3Instituto de Investigación Biológica del Paraguay, Del Escudo 1607, 425 Asunción, Paraguay
4Department of Biological Sciences, Marquette University, Milwaukee, WI 53201, USA
5Bell Museum of Natural History, University of Minnesota, St. Paul, MN 55108, USA

Correspondence should be addressed to Pier Cacciali; pier_cacciali@yahoo.com

Received 28 January 2018; Accepted 6 June 2018; Published 16 July 2018

Academic Editor: Hyniek Burda

Copyright © 2018 Pier Cacciali et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 are 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 lineages 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. periusus* Rodrigues, 1986 was described from northeastern Brazil [22], and more recently *P. maranonensis* Koch, Venegas & Böhme, 2006 from Peru [23]. Furthermore, a detailed phylogenetic study, based on a multilocus genetic approach, showed a deep phylogenetic nesting of *Bogettia lutzei* 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 “Seasonally 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) (Figure 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 Paraguari [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 Phylopus with 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 Phylopus from “Cordillera de Los Altos” constitute a genetic lineage differentiated from the remaining Paraguayan populations. Next, we incorporated additional mitochondrial genes (Cyb and ND2) to our analyses in order to assess phylogenetic relationships of Phylopus with previously published congeneric data (cluster arrangement according to Werneck et al. [11]).

2. Materials and Methods

We sampled Phylopus from three localities of Paraguay (Figure 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 -27°C using the standard glass fiber plate protocol of 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 about 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′-CGCCCTGTATTTACAAAAACAT-3′; also referred to as L2510) and 16sbr-H (reverse: 5′-CCGGTCTGAATCTCAGATCATG-3′; also referred to as H3056) [29], in an Eppendorf Mastercycler® pro. The PCR conditions were as follows: initial denaturation 2 min (94°C), denaturation 35 sec (94°C)x40, hybridization 35 sec (48.5°C), elongation 60 sec (72°C), and final elongation 10 min (72°C). Cycle-sequencing and sequencing (BigDye Terminator) were performed with the same forward and reverse primers mentioned above. Partial sequences of mtDNA from cytochrome b (Cyb) 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 0.90.9 [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 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 Phylodactylus 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 (UPBoot) 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 Phylopus, 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 eye 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 eye 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 suboculairs, infralabials (IL) from the first scale contacting the mental to the last in contact with the mouth border, and scales between 4th and 9th digital pads from the first lateral scale of the 5th 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 4th toe (Figure S1). When variation in the bilateral symmetry of morphological characters is present, a slash mark separates the respective values for left/right side. Measurements were always taken by the same person (PC). 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 the Appendix.

We also explored the value of diagnostic characters traditionally used to differentiate between Phyllopterus pollicaris and P. przewalskii [20], which are postcloacal 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 euthanizing and collecting specimens, through the permits SEAM N° 04/II, SEAM N° 009/2014, and SEAM N° 133/2015.

3. Results

The final alignments of 16S, Cytb, and ND2 were of 498, 907, and 886 nucleotide positions, respectively, for 64 Phyllopterus samples plus Phyllobdactylus unctus as an outgroup (Appendix S2). Partition schemes were recorded as follows: 16S (GTR+G); Cytb (1st pos KY+1 | 2nd pos K81UF+I | 3rd pos GTR+I); ND2 (GTR+I | KY+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 (Figures S2–S7). The clade from “Cordillera de Los Altos” always clustered as the sister taxon of P. przewalskii (Figures S2–S7). Based on the concatenated genes, P. maranjonensis was the sister taxon to the remaining Phyllopterus taxa, followed by P. lutzae, and P. periosus as the sister clade to the remaining species within the genus. The Bayesian analysis (Figures 2, S8) generally showed higher support values than ML (Figure 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 (Figure 2). Both hypotheses (BI and ML) suggest a branch with the southwestern taxa (Los Altos population + P. przewalskii + Phyllopterus pollicaris Clade IV) differentiated from the remaining P. pollicaris clades. The P. przewalskii clade shows 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 N° 22091) available at https://treebase.org/treebase-web/home.html.

The highest intraclide pairwise distance reaches 6.65% in Phyllopterus 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 (71%), 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 smallest 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. maranjonensis and P. lutzae clustered together and sister to the remaining Phyllopterus (Figure 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 + Phyllopterus sp. (Figure 3).

Results from ABGD suggest the presence of a higher diversity within Phyllopterus than currently recognized (Figure 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 were recognized by Cytb (Figure 4). Full results of ABGD are found in Appendix S4. The mitochondrial Cytb gene suggests a higher diversity especially in Clade VII. This gene, as well as ND2, distinguishes 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 (Figure 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 Phyllopterus. 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 Phyllopozos 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: urn:lsid:zoobank.org:act:9E9B8965-B517-49A0-84D9-F7BC57A85E33, and therefore they are available under the International Code of Zoological Nomenclature. The Life Science Identifier (LSID) for this publication is as follows: urn:lsid:zoobank.org:pub:IAE503A1-8CE0-460F-B29C-90B722DF905D. The LSID registration and any associated information can be viewed in a web browser by adding the LSID to the prefix "http://zoobank.org/".

Phyllopozos heuteri sp. nov.

Holotype. SMF 100494 (original field number GK 3559), adult female (Figure 5), collected on 12 September 2016 by G. Köhler, at the Cerro de Tobati (25.2797° S, 57.0925° W,
428 masl), in the Cordillera de Los Altos mountain range, Cordillera Department, Paraguay (Figure 6).


Diagnosis. A medium-sized species of Phylopeus 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. lutzei by the irregular reticulated or banded coloration (versus dotted pattern in P. lutzei) and by the presence of tubercles on the body (versus absent) (Figure S10). Phylopeus heuteri is distinguished from P. marajonicensis by the smaller size (max. SVL 88 mm versus 115 mm in P. marajonicensis) and presence of tubercles on the lateral surfaces of the body as well as between eyes and ear opening (versus absent from these regions) (Figure S11) and spiny scales surrounding the ear opening (versus cycloid) (Figure S12). Phylopeus heuteri is differentiated from P. periosus by irregularly shaped elements of color pattern (versus well defined transversal bands in P. periosus), by spiny scales surrounding the ear opening (versus cycloid scales) (Figure S12), and by the contact between the two greatly enlarged postmentals (versus enlarged lateral postmentals separated by small median postmentals) (Figure S13). Phylopeus heuteri is distinguished from P. pollicaris by the presence of two to three larger scales (tubercle-shaped) at the mouth commissure (versus small homogeneous scales at the mouth commissure in P. pollicaris), lateral body tubercles reaching further downwards (lowermost tubercle at 6–8 lateral scales from ventrals versus 13–15), and presence of more tubercles (five to eight) between eye and ear opening (versus up to three) (Figure S14). Phylopeus heuteri can be distinguished from P. przewalskii by the presence of tubercles on the prescapular region and sides of the neck (versus homogeneous sculation in P. przewalskii) (Figure S15), the presence of 36 to 39 scales between 4th and 5th toes (versus 33 to 36), and its large postmentals usually contacting only the first IL (versus contacting usually 1st and 2nd IL).

Description of the Holotype. SVL 74 mm, tail incomplete, HL 191 mm, HW 141.1, NED 6.9 mm, ED 4.6 mm, EO 1.6 mm, DBN 2.4 mm, and 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/II; 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 follows: 6/5 - 10/9 - 11/11 - 10/12 - 8/9; claws enclosed by a sheath of six rows of scales; a single slightly developed postdiscal 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. (Figure 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. Ventril surface of the head Cream White (52) with Hair Brown (227) stipple concentrated in the infralabial
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 (MNHN 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; postcloacal tubercles vary from two to three per side.

The most remarkable aspects of variation in color pattern are visible on the dorsum. The largest examined specimen (MNHN 12111) has no pattern other than the vertebral stripe present in all specimens. The remaining specimens show a pattern composed of bands similar to that of the holotype, but the bands are formed by a mottling in MNHN 11975, and two specimens exhibit only very diffuse bands. One specimen (MNHN 12001) has a paler ground color, and another (SMF 100696) exhibits a darker ground color.

**Distribution.** *Phyllopus heuteri* is known from rocky outcrops at three localities along the “Cordillera de Los Altos” formation (Figure 6) in the Paraguayan departments Cordillera and Paraguari, 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 *Phyllopus 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 of thorny or thick plants such as *Polycarpaea hassleriana*, *Cereus* sp., and *Bromelia* sp. Nothing is known about its feeding or reproductive habits, or any other aspects of its behavior.
Table 1: Morphological variation among Phylopecus heuteri, P. pollicaris, and P. przewalskii.

<table>
<thead>
<tr>
<th></th>
<th>Hands</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Feet</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Vent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pprz</td>
<td>5–6</td>
<td>8–9</td>
<td>9–11</td>
<td>9–11</td>
<td>8–9</td>
<td>5–6</td>
<td>8–10</td>
<td>10–12</td>
<td>10–11</td>
<td>8–10</td>
<td>24–33</td>
<td></td>
</tr>
<tr>
<td>Pheu</td>
<td>3–6</td>
<td>7–10</td>
<td>9–11</td>
<td>9–11</td>
<td>7–9</td>
<td>5–6</td>
<td>9–10</td>
<td>10–12</td>
<td>9–11</td>
<td>8–9</td>
<td>27–33</td>
<td></td>
</tr>
</tbody>
</table>

Infradigital lamellar variation (including holotype) of Phylopecus 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).

Figure 6: Known records of Phylopecus heuteri showing the type locality (black star) and additional localities (red dots). Capitalized names refer to Paraguayan Departments.

Figure 7: Coloration in life of the holotype (SMF 100949) of Phylopecus heuteri.

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 Phylopecus 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 postcloacal 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 I). 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 (“sylvus interioris Bahiæ 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 (Figure 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 Phyllopus goyazensis (Peters 1878) also needs to be accounted for. The P. goyazensis type locality is stated as “Goyaz” in Brazil [60], which, at the time, was larger than the current boundaries of the 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 Phyllopus (P. pollicaris, P. przewalskii, and P. heuteri), rendering it a genus with remarkable morphological crypsis. Nevertheless, Phyllopus 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. Phyllopus heuteri and P. przewalskii are morphologically similar, 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 in the prescapular region (Figure SII). 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 (Figure 9).

Phyllopus heuteri shows a high degree of genetic differentiation according to p-distances, having the lowest distance to its sister clade P. przewalskii (Appendix S3). Phyllopus 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 Phyllopus 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. Phyllopus 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 resolving the taxonomy of the genus Phyllopus, and we hope that the unnamed clades can be morphologically diagnosed in further studies.

Appendix

Examined Specimens

Phyllopus lutzae (3). Brazil: Bahia: São Salvador (AMNH 65381, MCZ 46190 [Syntype], UMMZ 115644 [Syntype]).

Phyllopus maranjonensis (3). Peru: Amazonas: Quebrada Honda (ZFMK 84995–7 [Paratypes]).

Phyllopus periosus (3). Brazil: Paraíba: Cabo de São Félix (AMNH 131825, MCZ 172929–30).

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).
Phyllopus pollicaris (2). Brazil: Bahia (no more specific locality data) (ZSM 165/0/1–2 [Paralecotypes]).


Paraguay: (No additional data) MNHN 11174, 11176. Alto Paraguay: Bahía Negra (MNHN 10020, 11691); Colonia Potrerito (MNHN 3371); Parque Nacional Defensores del Chaco (MNHN 2850, 4298); Puerto Ramos (MNHN 3243–6, 3248–9). Amambay: Parque Nacional Cerro Corá (MNHN 6983, 7046, 7640–4, 11919). Boquerón: Establecimiento Koé Pyahu (MNHN II069); Estancia Ágropol S.A. (MNHN 8042); Estancia Amistad (SMF 100495–96); Estancia Jabali (MNHN 8043–4, 8071); Estancia Mbutú Retá (MNHN 3818); Filadelfia (MNHN 2851); Parque Nacional Teniente Enciso (MNHN 2853, 3253, 4300, 11797, 11847, 11857). Concepción: Vallemí (MACN 12860–6).

Data Availability

Sequences used for this study are stored in GenBank. See Appendix for specifications. Sequence matrices and trees are deposited in TreeBASE ID N° 22091.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors’ thanks go to F. Bauer, N. Martinez, J. Méndez, M. Ruiz Díaz, E. Buongermini, and D. Vázquez for help during field work; also to M. Mote (MNHN), L. Avila (IJAMM-CNP), J. Faivovich (MACN), B. Álvarez (UNNEC), C. Spencer (MVZ), D. Rödder (ZFMK), F. Glaw (ZSM), R. Nussbaum (UMMZ), and D. Kizirian (AMNH) for letting them analyze specimens under their care; to C. Pérez (IJAMM-CNP), A. Marín (IJAMM-CNP), S. Nenda (MACN), S. Palomas (UNNEC), C. Koch (ZFMK), M. Fransen (ZSM), G. Schneider (UMMZ), L. Mogk (SMF), C. Zimmer (SMF), and E. Buongermini (Subtropica, Paraguay) for help and assistance during specimens’ revision; and to H. Kappes (Grunelius-Mölgaard Labor, SMF) and B. J. Pinto (MU) for assistance with laboratory work. The Consejo Nacional de Ciencia y Tecnología (CONACYT) from Paraguay, through the “Programa Nacional de Incentivo a los Investigadores” (PRONII) program, funded Pier Cacciali for fieldwork. Also, AMERISUR Paraguay provided financial support for revision of scientific collections. Pier Cacciali also is grateful to “Iki” (S. Saldivar) and “Chori” (V. Martinez) for delivering fieldwork equipment supplied by Idea Wild. Also thanks are due to N. C. Haase for the revision of an early draft of the manuscript. This paper arose six years ago from an early idea, strongly encouraged by A. Yanosky (Asociación Guayra Paraguaya, Paraguay), so Pier Cacciali gives him a special acknowledgment. This work is part of an ongoing project of Barcoding of Paraguayan Herpetofauna, as part of the Ph.D. work of Pier Cacciali, funded by the Deutscher Akademischer Austauschdienst (DAAD, Germany).

Supplementary Materials

Appendix S1: environmental traits of Phyllopus 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 4th and 5th 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 sculation. Figure S11: head and lateral body sculation. Figure S12: ear opening. Figure S13: mental sculation. Figure S14: lateral sculation of the head. Figure S15: prescapular sculation. (Supplementary Materials)

References


