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A new species of *Homonota* (Squamata: Gekkota: Phyllodactylidae) from Paraguay

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Abstract.

The 12 recognized species of the genus *Homonota* are geographically restricted to central and southern South America in Argentina, Brazil, Uruguay, Bolivia, and Paraguay. The phylogenetic relationships within the genus are well known where three groups are identified, each of them containing four species: the *whitii* group (*Homonota darwinii*, *H. williamsii*, *H. whitii*, and *H. andicola*), the *borellii* group (*H. borellii*, *H. taragui*, *H. rupicola*, and *H. uruguayensis*) and the *horrida* group (*H. horrida*, *H. underwoodi*, *H. septentrionalis*, and *H. marthae*). During fieldwork in Paraguay, we found a specimen of *Homonota* with unique morphological and geographical traits. Thus, we performed a comparative morphological analysis against specimens housed in different Paraguayan collections. Additionally, we performed a DNA analysis of our specimen to assess its relationships within the genus. Results suggest that this specimen represents a new species of the *Homonota horrida* group based on molecular and morphological evidence. The new species is characterized by a robust body, 10 longitudinal rows of strongly keeled scales separated by one or two granular scales, square-shaped mental scales, small postmental scales, one or two rows of rectangular scales in the upper eye scales, rostral scale divided, edge of auditory meatus serrated posteriorly, and black and brown background coloration with one white vertebral line and seven white transverse bands. The new species is restricted to Paraguarí Department, characterized by herbaceous grasslands with rocky outcrops.

KEYWORDS: GECKOS, HOMONOTA, GRASSLANDS, PHYLOGENY, TAXONOMY, SOUTH AMERICA

Introduction

Geckos of the genus *Homonota* Gray 1845 are known from central and southern South America (Kluge 1964; Cajade et al. 2013; Morando et al. 2014) in Argentina (Avila et al. 2012; Cajade et al. 2013; Morando et al. 2014), Brazil (Vaz-Ferreira & Sierra de Soriano 1961), Uruguay (Carreira et al. 2005), Bolivia (Langstroth 2005), and Paraguay (Cacciali et al. 2007; 2017), and currently 12 species are recognized (Avila et al. 2012; Cajade et al. 2013; Morando et al. 2014; Cacciali et al. 2018). The genus is in the Phyllodactylidae family with the genera *Garthia* Donoso-Barros & Vanzolini, 1965, *Gymnodactylus* Spix, 1825, *Phyllodactylus* Gray, 1928, *Phyllopezus* Peters, 1978, and *Thecadactylus* Goldfuss, 1820 (Gamble et al. 2011).

The phylogenetic relationships within the genus are well known (Morando et al. 2014) with three groups recognized, the whitii group with four species (Homonota darwinii Boulenger, 1885, H. williamsii Avila, Perez, Minoli & Morando, 2012, H. whitii Boulenger, 1885 and *H. andicola* Cei, 1978) with the most austral distribution in the genus (Morando et al. 2014), the borellii group (H. borellii (Peracca, 1897), H. taragui Cajade, Etchepare, Falcione, Barraso & Alvarez, 2013, H. rupicola Cacciali, Ávila & Bauer, 2007, and H. uruguayensis (Vaz-Ferreira & Sierra de Soriano, 1961)) and the horrida group (H. horrida (Burmeister, 1861), H. underwoodi Kluge, 1964, H. septentrionalis Cacciali, Morando, Medina, Kölher, Motte & Avila, 2017 and H. marthae Cacciali, Morando, Avila & Kölher, 2018) (Morando et al. 2014; Cacciali et al. 2018). Cacciali et al. (2017) consider Homonota *fasciata* a species inquirenda and until new works appear, we would agree with this taxonomic arrangement.

Between geckos in the genus Homonota there is a great diversity and degree of speciation; this is demonstrated by later works describing new species using molecular and morphological data (Cacciali et al. 2007; 2017; 2018; Avila et al. 2012; Cajade et al. 2013). In Paraguay, three species of Homonota are recorded: H. rupicola in the department of Cordillera and apparently endemic to the Cerro Pedregal (Cacciali et al. 2007), H. septentrionalis in the western part of the Dry Chaco ecoregion (Cacciali et al. 2017), and *H. marthae* distributed in the central area of the Dry Chaco ecoregion (Cacciali et al. 2018). In December 2016, we carried out an expedition in the Department of Paraguarí, near the city of Caapucu where an unusual specimen of *Homonota* was collected, and after further examination using morphological and molecular analyses we found the specimen to belong to an undescribed species which we describe below as a new species from Paraguay.

Material and Methods

We analysed specimens from the following collections: Museo Nacional de Historia Natural del Paraguay (MNHNP) and Instituto de Investigación Biológica del Paraguay (IIBP), and data from a recent publication (Cacciali et al. 2017; 2018) of the genus in Paraguay were compared to have a more precise description. When paired structures exist, data are presented in left/right orientation. We evaluated characters normally used in the taxonomy of the genus following recent works (Avila et al. 2012; Cacciali et al. 2017; 2018).

Morphological analysis

We include the following meristic data: SAM (scales around midbody), DS (dorsal scales), VS (ventral scales), 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 infralabials scales), 4TL (number of lamellae under the fourth toe), 3FL (number of lamellae under the third finger).

We also include the following metric characters: snout-vent length (SVL) from tip of the 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, measure at parietal level area, the eve-nostril distance (END) from anterior edge to the posterior edge of nostril, eye-snout distance (ESD) from the anterior edge of the eye to the tip of the snout, eyemeatus distance (EMD) from the posterior edge of the eye to the anterior border of the ear opening, interorbital distance (ID), and internostril distance (IND).

DNA analysis

Based on morphological examination, we allocated the new taxon in the *Homonota horrida* group, sensu Cacciali et al. (2017; 2018). We then carried out a genetic analysis using sequences of the mtDNA 16S gene as it has proved to be useful for taxonomic identification (Batista et al. 2014; Kölher et al. 2014). Accession number and localities are presented in Table 1. We used three sequences of *H. septentrionalis*, one of *H. marthae* from Paraguay and three of *H. horrida* from Argentina, using *Phyllopezus przewalskii* as the outgroup, and since no 16S sequence of *H. underwoodi* were available, we did not include it in this analysis.

Alignment was performed using MAFFT 7 (Katoh & Standley 2013) to automatically align sequences through its webserver. We included the Q-INS-I search strategy for corrections with the secondary structure for 16S, then we used Gblocks 0.91b (Castresana 2000) to eliminate poorly aligned positions and divergent regions of DNA through its online server. MSA viewer (Yachdav et al. 2016) were used to visualize and export the alignment to fasta format. The best substitution model for the gene was perform with jModelTest 2.1.10 (Darriba et al. 2012), lower value of delta Akaike Information Criterion (Δ AIC) were used to select the best model.

We preformed two phylogenetic analyses, first a Maximum Likelihood (ML) approach, and then a Bayesian inference (BI). We estimated the uncorrected genetic pairwise distance of our dataset in MEGA10.0.5, for 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). For BI we convert the alignment to be used in MrBayes v3.2 in Mesquite 3.6 (Maddison & Maddison 2017). For this we ran the analysis in independent duplicates, with 40,000,000 generations for MCMC with a sampling frequency of 1,000 generations. Trees were visualized and exported then using FigTree v1.4.4 (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 Potential Scale Reduction Factor approached 1.0 (Gelman & Rubin 1992).

The degree of intraspecific divergence within the alignment (removing the outgroup) were performed with the species delimitation test ABCD (Puillandre et al. 2012) through its webserver (https://bioinfo.mnhn.fr/abi/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).

Results

Phylogenetic inference

Our final alignment of 16S consisted of 271 bp, and the best substitution model was HKY+I. Sequence divergence between the new species and the other species in our analysis range between 4.1% and 4.8% for 16S (Table 2). The ML and BI tree (Figure 1) using 16S recognized 4 clusters, both showing the new species as a sister clade to specimens of *H*. *horrida* from southern Argentina with strong support (bootstrap value = 82%, and posterior probability = 0.89). Both trees (ML and BI) have the same topology, with *H. septentrionalis* as a sister clade of *H. marthae*, *H. horrida* and the new species. Analysis of intraspecific divergence with ABGD found two groups (Table 3), with H. horrida, H. marthae and H. septentrionalis in group one, and the new species in group 2, this is congruent with our previous ML and BI trees, providing strong evidence for the recognition of a new taxon. In addition to the morphological support, the molecular analysis also provided strong support for the hypothesis that our sample represents an undescribed taxon, which we here formerly describe and name.

Homonota itambere sp. nov.

Etymology: the epithet is combination of two Guarani words, ita = rock, ambere = lizard, a lizard who lives in the rocks. The species name is an allusion of the habitat where the species was found, as a lizard that lives in the rocks. The name is used here as a noun in apposition.

Holotype: IIBP 4437, an adult female from Estancia Guajho, Departamento of Paraguari (26°14'55.81"S, 57°17'48.03"W, 143 m) (Figure 2), collected by Hugo Cabral, 14 December 2016. Collecting permits were provided by the Ministerio del Ambiente y Desarrollo Sostenible, MADES 126/2016 and 258/2016.

Diagnosis

A species of *Homonota* assigned to the *horrida* group (sensu Cacciali et al. 2018) based on molecular evidence and by the color pattern composed of a white vertebral line and five to seven transverse white lines appearing as banded, similar to *H. horrida*, *H. septentrionalis* and *H. marthae*. A large species of *Homonota* (SVL= 56 mm) with robust body, with 10 longitudinal rows of strongly keeled scales separated by one or two granular scales, a square shape mental scale, small postmental scales





Figure 2. Photograph of the holotype of *Homonota itambere* **sp. nov.** minutes after euthanasia. A) Dorsal coloration, and B) lateral view of the head, note the enlarged white tubercle at the edge of the auditory meatus.



Figure 1.

Species tree of Homonota horrida group with related species showing the positions of the new specie A) Maximum likelihood inference B) Bayesian inference. Bars represent substitution per site. Support values are shown at the beginning of each clade.

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Figure 3.

Lateral, ventral, dorsal and auditory meatus view of the holotype of Homonota itambere sp. nov. (A, D, G, J) and comparisons with *H. marthae* (B, E, H, K) and *H. septentrionalis* (C, F, I, L). A-C) white arrow indicating the position of the auditory meatus in relation with to the commissure of the mouth, D-F) Detail of the mental region, showing the size and shape of mental and postmental scales, G-I) Dorsal view of the head showing the coloration pattern of *H. itambere* **sp. nov.**, *H. marthae* and *H.* septentrionalis, J-L) The white arrow indicates the sizes and shape of the enlarged tubercle and the edge of the auditory meatus of *H. itambere* **sp. nov.**, *H. marthae* and *H. septentrionalis*.

(Figure 3D), one or two rows of rectangular scales Adult female, SVL= 56 mm, TrL= 26.2 mm, tail in the upper eye scales, rostral scale divided, edge of broken near the base, FL= 10 mm, TL= 9.3, AL= 24 auditory meatus serrated posteriorly with an enlarged mm, HL= 13.1 mm, HW=11.3, HH= 8.5 mm, END= 5.6 tubercle, and black and brown background coloration mm, EMD= 4.2 mm, ESD= 6.1 mm, ID= 5.79 mm, IND= with one white vertebral line with seven transverse 2.39 mm. Rostral wider with a groove complete in the white bands (Figure 3A). It can be distinguished from middle of the scales, nares surrounded and contacting H. andinicola, H. whitii and H. underwoodi, H. darwinii the rostral, supranasal and first SL contacting the by the presence of longitudinal rows of dorsal keeled rostral and nares, two postnasal contacting the nares, scales (smooth dorsal scales in H. andinicola, H. not the rostral; (left first) SL 8/8, IL 5/5. whitii and H. underwoodi, keeled scales restricted to the posterior part of the dorsum in *H. darwinii*), Three tubercular scales on the mouth commissure; also from *H. underwoodi* by the low number of 4TL upper region of the muzzle and head slightly convex (17) (vs 20-25 4TL in *H. underwoodi*), also from *H.* covered by large and small heterogeneous juxtaposed and granular scales; superciliary scales imbricated *rupicola* by the high number of 4TL (17) (vs 14-15 in *H. rupicola*), differs from *H. rupicola*, *H. taragui*, *H.* forming a serrated edge, associated to spiny-like williamsi, H. borelli, H. andinicola and H. whitii by the scales on the posterior half of the orbit; lateral sides higher number of 3FL (15) (vs 10-12 in H. rupicola, of the head covered with large, keeled tubercles and 11-13 in *H. taragui*, 11-14 in *H. williamsi*, 10-13 in *H.* small, granular scales (Figure 3A, G); auditory meatus borelli, 13-14 in H. andinicola and 12-14 in H. whitii), oblique with serrated edge posteriorly (Figure 3]), from *H. borelli* and *H. rupicola* by the oblique shape one large scale in the upper border; IL 5/5; mental of the auditory meatus (rounded in *H. borelli* and *H.* quadrangular, postmental circular and small, almost rupicola), from H. rupicola, H. taragui, H. borelli and H. the same size of the following posterior scales, first IL *uruguayensis* by serrated edge of the auditory meatus contacting the mental twice as large than postmental (smooth in H. rupicola, H. taragui, H. borelli and H. scales; scales under the head small with black dots, uruguayensis) from H. septentrionalis, H. marthae becoming less notable reaching the commissure of and *H. horrida* by the number of keeled longitudinal the mouth; dorsolateral of the neck with granular, dorsal scales (10) (vs. 4-8 in H. septentrionalis and H. juxtaposed scales mixed with keeled scales; throat marthae and 16 in H. horrida) from H. horrida and H. region covered by imbricate cycloid scales; dorsum covered with 10 longitudinal strongly keeled scales *rupicola* by the relative high position of the auditory meatus to the mouth commissure (lower position in separated by one or two granular scales, except on H. horrida and H. rupicola). Differs from H. rupicola, H. the vertebral, where the keeled scales are separated taragui, H. williamsi by the well-developed tubercules, by three or four granular ones; ventral scales cycloid some with keeled scales on the side of the head, from and imbricate with 17 longitudinal rows at midbody; the eve to the neck (vs. less developed tubercules suprascapular and axillary region surrounded by or moderate keeled in H. rupicola, H. taragui, H. small, imbricate scales, inguinal region and cloacal williamsi), from H. rupicola, H. taragui, H. williamsi, opening surrounded by smooth scales, anterior and *H. uruguayensis* by the dorsal coloration of the head dorsal surfaces of forelimbs covered by large imbricate and body consisting of transverse clear bands on a and keeled scales; posterior region and ventral darker dorsum (vs. reticulate pattern, resembling a surface of forelimbs covered by small, juxtaposed salt and pepper pattern), also differs from H. marthae, granules; ventral surface of hind limbs covered by *H. septentrionalis* and *H. horrida* by the tail's poorly large, imbricate scales; subdigital lamellae of hands, developed keeled scales, (strongly keeled scales counting from the pollex first are: (left first) 7/7, especially at the base of the tail in *H. marthae*, *H.* 11/11, 15/13, 13/14, 11/9; and subdigital lamellae *septentrionalis* and *H. horrida*), *H. marthae* by the of feet, starting from hallux were recorded as follows: presence of a thin white occipital band (lack of a white 14/14, 17/15, 15/15, 13/11, 8/7; scales at the base band in H. marthae), also from H. septentrionalis and of the tail (regenerated stump) imbricate and weekly H. horrida by the thin white occipital band (vs. wider to moderately keeled on dorsal region of the tail. white occipital band), from H. septentrionalis by the shape of the mental scales, with a more quadrangular Coloration in life (minutes after euthanasia). shape (vs. mental scales with a triangular shape, longer then wider in *H. septentrionalis* and finally Dorsal surface of the head with small white and from *H. horrida* by the small size of the postmental brown blotches irregularly mixed with black marks scales, about the same size of posterior scales and a thin occipital white band from eye to eye, this (moderately enlarged postmental scales, about twice occipital band is followed posteriorly by a dark brown the size of corresponding scales in *H. horrida*). coloration, dorsal surface of the neck with a black

Description of the holotype

Table 1. Specimen vouchers and GenBank accession numbers of samples used in this study.

Species	Voucher	16S	
Homonota septentrionalis	MNHNP 11873	MF278833	
	MNHNP 12238	MF278832	
	SMF 101984	MF278831	
Homonota horrida	LJAMM-CNP 10576	MF278830	
	LJAMM-CNP 10495	MF278829	
	BYU 47941	MF278828	
Homonota marthae	SMF 101438	MG947388	
Homonota sp. nov	IIBP 4437	MZ098621	
Phyllopezus przewalskii	LG 1093	JN935567	

Table 2. Uncorrected pairwise genetic distance (in percentages) based on 16S mtDNA among H. septentrionalis, H. horrida, H. marthae and the new taxon (grey cells). Minimum and maximum values between species in bold. Accession and voucher numbers are provided in Table 1.

	MNHNP 11873	MNHNP 12238	SMF 101984	LJAMM-CNP 10576	LJAMM-CNP 10495	BYU 47941	SMF 101438	IIBP 4437
Homonota septentrionalis	-							
Homonota septentrionalis	0.0	-						
Homonota septentrionalis	0.0	0.0	-					
Homonota horrida	1.9	1.9	1.9	-				
Homonota horrida	1.1	1.1	1.1	0.7	-			
Homonota horrida	1.1	1.1	1.1	0.7	0.0	-		
Homonota marthae	0.4	0.4	0.4	1.9	1.1	1.1	-	
Homonota sp.nov.	4.4	4.4	4.4	4.8	4.1	4.1	4.5	-

Table 3. Intraspecific divergence.

	Prior maximal distance
Partition 1 found 2 groups	P= 0.001000
Partition 2 found 2 groups	P= 0.001668
Partition 3 found 2 groups	P= 0.002783
Partition 4 found 2 groups	P= 0.004642
Partition 5 found 2 groups	P= 0.007743
Partition 6 found 1 groups	P= 0.012915

band separated with a white transverse band (Figure 2). Supralabials, infralabials and rostral dark brown with white on the suture of scales, upper lateral view of the head dark brown from nostril to eyes, and from eyes to auditory meatus, covered by thin light brown line and white scales from the commissure of the mouth to the suprascapular region. Dorsal coloration is black and brown bands separated by a transvers white band and a vertebral white stripe from the neck to the first four scales of the tail. Dorsal surface of the limb, forelimb, fingers and toes with a salt and pepper coloration with brown, black and very conspicuous white marks, dorsal coloration of the tail with brown and black incomplete and diffused stripes. Ventral coloration is immaculate white from mental scale to cloaca region, with the presence of black chromatophores in all the ventral scales, not visible with the naked eye, tail with grey coloration. Only the ventral region of the arms and finger lamellae have a brown coloration.

Color in preservative

Dorsal coloration of the head with white and dark brown small blotches irregularly mixed, and a brown and a thin occipital white band from eye to eye, this occipital band is followed posteriorly by a dark brown coloration, dorsal surface of the neck with a brown band separated with a white transverse band. Supralabials, infralabials and rostral brown with white on the suture of scales, upper lateral view of the head brown from nostril to eyes, and from eyes to auditory meatus, covered by a thin light brown and white scales from the commissure of the mouth to the suprascapular region. Dorsal coloration with dark brown bands separated by transverse white lines and a vertebral white stripe from the neck to the first four scales of the tail. Dorsal surface of the limb, forelimb, fingers and toes with a salt and pepper coloration of brown, and very conspicuous white marks, dorsal coloration of the tail with brown and black incomplete and diffused stripes. Ventral coloration immaculate white from mental scale to cloaca region, with the presence of black chromatophores in all the ventral scales, not visible with the naked eye, tail with grey coloration. Only the ventral region of the arms and finger lamellae have a brown coloration.

Distribution and habitat

Homonota itambere sp. nov. is restricted to grasslands of the Paraguarí Department (Figure 4), the environment inhabited by *H. itambere* **sp. nov** is characterized by grasslands with rocky outcrops (Cartes et al. 2016), hydromorphic soil and a slight wavy topography (Fretes et al. 1970). The locality is a transition area between the Humid Chaco and Atlantic Forest and it is known for the presence of the Caacupe Craton

(Fúlfaro 1996), with a complex geological history (Nuñez 1999; Cubas et al. 1998) (Figure 5). This species does not appear to be abundant as we carried out six additional expeditions in the area to locate more specimens with unsuccessful results.

Discussion

The description of this new species elevated to five the number of taxa in the Homonota horrida group, being the most diverse species group of the genus so far. As stated by Cacciali et al. (2018), the taxonomy of the H. horrida group was ignored for many years, and the latest described new species demonstrates that this group had been overlooked for quite some time. The phylogenetic relations of the genus were studied in detail by Morando et al. (2014) and then updated by Cacciali et al. (2018) with all the new species described until that date. We are aware that describing a new species based on a single specimen, is not the best practice for taxonomy, but after six unsuccessful trips aimed to find additional specimens, and given that our data are supported by molecular, morphological, and chorological analyses, we decided to describe the species to provide a name to the new taxon, with the intention of carrying out additional expeditions to the type locality and surrounding areas in order to have a better understanding of the population and conservation status of Homonota itambere sp. nov. It is important to note that Homonota species are usually easy to find, however, this is not the case for the new species, and we believe that this is a new cryptic species with a presumably restricted distribution, like other related species such *H. rupicola*, and *H. taragui* (Cacciali et al. 2007; Cajade 2013).

Morphologically, the new species belongs to the H. *horrida* group due to the presence of keeled dorsal scales, a white vertebral stripe, and other similar coloration features. According to our molecular data, *Homonota itambere* **sp. nov.** is most closely related to specimens of H. horrida (fasciata) from Mendoza, Argentina. The distance between these two species is more than 1,000 km. This may also be due to the lack of available samples within those 1,000 km. However, it is surprising that the species is more closely related to southern populations, more than H. septentrionalis and *H. marthae* due to the proximity in the distribution of both species on the other side of the Paraguayan River. Also, this represents the first species of the horrida group on the other side of the Paraguayan River, and the locality where the species was found is part of the Caacupe Craton (Fúlfaro 1996) and the Alto Asunción (Hernandez et al. 2005), which may act as a barrier for the isolation of the new species. All these questions could be tested as new data become available and test the diversification





Figure 4.

Distribution map of *Homonota itambere* **sp. nov.** showing the type locality (black star) and *H. septentrionalis* (circle), *H. marthae* (triangles) and *H. horrida* (squares). Black symbols represent molecular material used in or study. Figure 5. General view of the habitat of *Homonota itambere* **sp. nov**., showing the rocky formations and grasslands habitats. hypothesis made by Morando et al. (2014). The new species can be easily differentiated from the congeners in the *H. horrida* group by their robust body, with 10 longitudinal rows of strongly keeled scales separated by one or two granular scales, a square-shaped mental scale, small postmental scales, one or two rows of rectangular scales in the upper eye scales, rostral scale divided, edge of auditory meatus serrated posteriorly and black and brown background coloration with a white vertebral line with seven transverse white bands.

The locality where the new species is found presents a mix of open grassland vegetation and rocky outcrops. We believe that this species may well be found in other similar rocky outcrops in southern Paraguay and additional expeditions are needed to understand better the distribution and habitat use of this cryptic species. Nevertheless, we propose that this new species can be considered as Data Deficient until new populations or specimens are found.

According to Morando et al. (2014) the ancestor of the *horrida* group (referred as *fasciata* group in Morando et al. 2014) was isolated in emergent areas of southern Bolivia and northern Argentina and dispersed later to the south and east. While our new species is far from these areas, the ancestor of *horrida* may have reached the other side of the Paraguay River before the river formerly appeared or reached the other side of the river by crossing on debris or aquatic vegetation during flood events. Or, perhaps it occurred another way; the ancestor of the *horrida* group originated in central Paraguay, and from there the dispersion to the south and west began. This may explain why the new species is more related to the Mendoza clade of *H. horrida*, and not to those in the Chaco of Paraguay. However, future studies and analyses of the phylogeographics of the genus are necessary to elucidate these theories.

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Front cover image General view of the habitat of *Homonota itambere* **sp. nov.**, showing the rocky formations and grasslands habitats.

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