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

Pier Cacciali [Corresp., 1, 2, 3] , Mariana Morando [4] , Cintia D. Medina [4] , Gunther Köhler [1] , Martha Motte [5] , Luciano J. Avila [4]

[1] Herpetology Section, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt (M), Hesse, Germany

[2] Institute for Ecology, Evolution & Diversity, Biologicum, Johann Wolfgang Goethe Universität Frankfurt am Main, Frankfurt (M), Hesse, Germany

[3] Instituto de Investigación Biológica del Paraguay, Asuncion, Paraguay

[4] Grupo de Herpetología Patagónica. IPEEC-CENPAT-CONICET, Puerto Madryn, Chubut, Argentina

[5] Sección de Herpetologia, Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Central, Paraguay

Corresponding Author: Pier Cacciali
Email address: pcacciali@senckenberg.de

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 (2 mitochondrial and 9 nuclear genes), with concatenated and species tree approaches.

1 **Taxonomic analysis of Paraguayan samples of *Homonota fasciata* Duméril & Bibron (1836)**

2 **with the revalidation of *Homonota horrida* Burmeister (1861) (Reptilia: Squamata:**

3 **Phyllodactylidae) and the description of a new species**

5 Pier Cacciali[1,2,3,*], Mariana Morando[4], Cintia D. Medina[4], Gunther Köhler[1], Martha Motte[5],

6 Luciano J. Avila[4].

8 *[1] Herpetology Section, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt (M),*

9 *Hesse, Germany*

10 *[2] Institute for Ecology, Evolution & Diversity, Biologicum, Johann Wolfgang Goethe-Universität*

11 *Frankfurt am Main, Frankfurt (M), Hesse, Germany*

12 *[3] Instituto de Investigación Biológica del Paraguay, Asunción, Paraguay*

13 *[4] Grupo de Herpetología Patagónica. IPEEC-CENPAT-CONICET. Puerto Madryn, Chubut,*

14 *Argentina*

15 *[5] Sección de Herpetología, Museo Nacional de Historia Natural del Paraguay, San Lorenzo,*

16 *Central, Paraguay*

19 *Correspondence: E-mail: pier_cacciali@yahoo.com

21 **Abstract**

22 *Homonota* is a Neotropical genus of nocturnal lizards characterized by the following

23 combination of characters: absence of femoral pores, infradigital lamellae not dilated, claws

24 without sheath, inferior lamellae laterally not denticulate, and presence of a ceratobranchial

25 groove. Currently the genus is composed of 10 species assembled in three groups: two groups

26 with four species, and the *fasciata* group with only two species. Here, we analyzed genetic and

27 morphologic data of samples of *Homonota fasciata* from Paraguay. According to Maximum

28 Likelihood and Bayesian inference methods applied to species delimitation and phylogenetic

29 analyses, the Paraguay population represents an undescribed species. Additionally,

30 morphological analysis of the holotype of *H. fasciata* (MNHN 6756) shows that it is

31 morphologically different from the banded, large-scaled *Homonota* commonly referred to as "*H.*

32   *fasciata*". Given the inconsistency between morphological characters of the name-bearing type

33   of *H. fasciata* and the species commonly referred to as *H. fasciata*, we consider them as different

34   taxa. Thus, *H. fasciata* is a *species inquirenda* which needs further studies, and we resurrect the

35   name *H. horrida* for the banded, large-scaled *Homonota*. The undescribed species from Paraguay

36   is similar to *H. horrida*, but can be differentiated by the high position of the auditory meatus

37   relative to the mouth commissure (vs. low position in *H. horrida*); and less developed tubercles

38   on the sides of the head, including a narrow area between the orbit and the auditory meatus

39   covered with small granular scales with or without few tubercles (vs. several big tubercles on the

40   sides of the head even in the area between the orbit and the auditory meatus). The new species is

41   distributed in the Dry Chaco in South America. With the formal description of this species and

42   the revalidation of *H. horrida*, the actual diversity of the genus *Homonota* is increased to 12

43   species. Furthermore, we infer phylogenetic relationships for 11 of the 12 described species of

44   the genus, based on 11 molecular markers (2 mitochondrial and 9 nuclear genes), with

45   concatenated and species tree approaches.

47   **INTRODUCTION**

48   The genus *Homonota* is a gecko of Gondwanan origin, distributed in South America, being

49   present in southern Bolivia, northern to southern Argentina, western Paraguay, Uruguay, and the

50   Brazilian state of Rio Grande do Sul (Gamble et al., 2008a; Morando et al., 2014). Along its

51   distribution it inhabits dry environments like Monte, Chaco, Espinal, Patagonian, Andean, and

52   Pampas (Morando et al., 2014). Regardless of the ecoregion, the genus is terrestrial and with the

53   exception of *Homonota fasciata*, all species have a reticulated coloration pattern that imitates

54   lichens on rocky backgrounds (Avila et al., 2012: Fig. 1). Unlike other geckos in South America,

55   *Homonota* is adapted to a terrestrial life-style being only infrequently found in trees (Cei, 1986).

56   All species in the genus are nocturnal, oviparous –laying one or two eggs–, insectivorous

57   lizards that can be found frequently in human dwellings feeding on a wide range of arthropods

58   (Cei, 1986; Cei, 1993; Abdala, 1997; Carreira et al., 2005; Ibargüengoytía & Casalinas, 2007;

59   Kun et al., 2010). Members of this genus are characterized by the following combination of

60   characters: absence of femoral pores, infradigital lamellae not dilated, claws without sheath,

61   inferior lamellae laterally not denticulate, and presence of a ceratobranchial groove (Peters &

62   Donoso-Barros, 1970; Cei, 1986; Carreira et al., 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 50º 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* (as a different species of *H. fasciata*), 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 Scaled Gecko" [sic] *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 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.

94 greatly enlarged in *H. fasciata*), and size and shape of gular scales (large and plate-like in *H.*

95 *horrida* vs. small and granular in *H. fasciata*). According to this author, *H. horrida* is present in

96 southern Bolivia and Brazil, Paraguay, and northwestern Argentina, whereas the distribution of

97 *H. fasciata* is unknown because its type locality "Martinique" is apparently based on a mistake,

98 and no more additional locality records were available. Abdala & Lavilla (1993) suggested that

99 diagnostic characters between *H. horrida* and *H. fasciata* as proposed by Kluge (1964) were

100 intraspecific variation, and they synonymized *H. horrida* with *H. fasciata*. Since then the name

101 *H. fasciata* was applied to the banded, large-scaled *Homonota* distributed from northern

102 Paraguay and southern Bolivia, to Río Negro Province (central Argentina).

103 An almost complete molecular phylogenetic analysis was performed by Morando et al.

104 (2014) including topotypes of all the recognized species. For *H. fasciata* the authors used

105 specimens from Mendoza, since the original type locality (Martinique) is a mistake, and Abdala

106 & Lavilla (1993) restricted the type locality of *H. fasciata* to Mendoza (in den Schluchten der

107 Sierra bei Challao), which is actually the type locality for *Homonota horrida*.

108 In Paraguay, *Homonota fasciata* is distributed mainly in the Dry Chaco, with only one

109 record in a transition zone of Dry Chaco with Humid Chaco (Cacciali et al., 2016). Given that *H.*

110 *fasciata* has a complex taxonomic history, is one of the widest distributed members of the genus,

111 and the almost complete absence of samples from Paraguay in previous publications, here we

112 follow an integrative approach to assess the taxonomic status of samples from this country. First,

113 within the framework of a barcoding project of Paraguayan herpetofauna, we generated

114 molecular data and inferred a first round of hypotheses. Second, based on 11 genes, we inferred

115 the taxonomic position of the Paraguayan populations in a phylogenetic tree that includes all the

116 described species. Lastly, we analyzed detailed morphological data and also examined the

117 holotype of *H. fasciata*.

118

119 **MATERIALS AND METHODS**

120 **Genetic analyses**

121 We carried out a first genetic inspection of the taxonomic status of Paraguayan populations

122 currently referred to as *Homonota fasciata* using sequences of the mtDNA 16S gene as it was

123 proved to be a useful tool for taxonomic identification (Jansen & Schulze, 2012; Batista et al.,

124 2014; Köhler et al., 2014) with a desirable relation of cost/benefit. The Paraguayan samples

125 (N=3, GenBank accession numbers presented in Appendix S1, Supplementary Information

126 online) from two localities were compared with available samples of the species from Mendoza,

127 Argentina (used by Morando et al., 2014) located ~1.400 km in straight line (N=3). Localities of

128 vouchers used for genetic analyses are shown in Appendix S2. Paraguayan samples were

129 collected with collecting permits SEAM Nº 04/11 and SEAM Nº 133/2015 issued by the

130 Secretaría del Ambiente in Paraguay. Specimens were euthanized using anesthetic injections of

131 barbituric acids (Tiopental Sódico® 1g).

132   Tissue samples were first washed for 15 h with 50 µl Phosphate-buffered saline (PBS)

133 (diluted of 1:9 PBS: H_2O). They were digested in a solution of Vertebrate lysis buffer (60 µl per

134 sample) and proteinase K (6 µl per sample) at 56°C for 15 h. Protocol for DNA extraction

135 followed Ivanova et al. (2006). After extraction, DNA was eluted in 50 µL Tris-EDTA (TE)

136 buffer. Amplification of mtDNA 16S gene fragments was made using the eurofins MWG Operon

137 primers L2510 (forward: 5'–CGCCTGTTTATCAAAAACAT–3') and H3056 (reverse: 5'–

138 CCGGTCTGAACTCAGATCACGT–3') in an Eppendorf Mastercycler® pro. PCR conditions

139 were: 94ºC–2 min, 40× [94ºC–35 s, 48.5ºC–35 s, 72ºC–1 min], 72ºC–10 min. Sequencing was

140 performed using a BigDye® Terminator with the following cycling conditions: 95ºC–1 min, 30×

141 [95ºC–10 s, 50ºC–10 s, 60ºC–2 min], with 10 µl of reaction volume.

142   The examination of chromatograms and generation of consensus sequences was performed

143 using SeqTrace 0.9.0 (Stucky, 2012). Sequences were aligned first automatically with Clustal W

144 (Larkin et al., 2007) followed by a visual inspection and edition if necessary, with the freeware

145 MEGA 6 (Tamura et al., 2013). The alignment and the tree are available at TreeBase (ID:

146 20987). The substitution model for our dataset was identified according to the corrected (for

147 finite sample size) Akaike Information Criterion (AICc) (Burnham & Anderson, 2002) and

148 computed in MEGA 6.

149   We estimated the uncorrected genetic pairwise distances for our dataset, and ran Maximum

150 Likelihood (ML) analysis with 30,000 bootstrap replicates in MEGA 6. We used *Phyllopezus*

151 *przewalskii* as outgroup (SMF 100495, GenBank accession number pending), due to availability

152 of relevant genetic information.

153   We used a species delimitation methods to assess the degree of intraspecific divergences

154 and, to support the cluster arrangement suggested by the ML approach. This exploration was

155 performed separately for the alignment and for the tree. The alignment was analyzed with ABGD

156   (Puillandre et al., 2012) using simple distances to compare with the uncorrected genetic distance.

157   For the tree based on 16S analysis, we applied the Poisson tree process (PTP) (Zhang et al.,

158   2013) conducted through the bPTP web Server (http://species.h-its.org/), using default

159   parameters and the outgroup removed. This algorithm does not require an ultrametric tree as

160   input (Zhang et al., 2013), and it is a robust tool to estimate species delimitation from ML

161   phylogenetic reconstructions (Tang et al., 2014). To assess the phylogenetic position of the

162   Paraguayan samples within the genus, we used data from the recently published phylogenetic

163   inference by Morando et al. (2014) and generated new sequences for all markers for samples

164   from Paraguay (Appendix S3). We followed Morando et al. (2014) for amplification of the same

165   two mitochondrial and nine nuclear genes, alignment protocols and gene and species trees

166   approaches. Primers are specified in Appendix S4.

167   Consensus sequences for each sample was generated with Sequencher v4.8 (^{TM}Gene Codes

168   Corporation Inc. 2007, Ann Arbor, MI, USA), and aligned with Mafft (Katoh & Standley, 2013).

169   Confirmation of open reading frames for protein-coding genes was made by translation into

170   amino acids.

171   The best evolutionary substitution model for each gene was selected using the AICc

172   (Burnham & Anderson, 2002) and ran in jModelTest v2.1.10 (Darriba et al., 2012).

173   Recombination was tested and excluded for nuclear genes using RDP: Recombination Detection

174   Program v3.44 (Martin & Rybicki, 2000; Heath et al., 2006). We conducted Separate Bayesian

175   analyses (BI) for each gene using MrBayes v3.2.2 (Ronquist & Huelsenbeck, 2003). Four heated

176   Markov chains (with default heating values) and run for five million generations were used for

177   each analysis. The equilibrium samples (after 25% of burn-in) were used to generate a 50%

178   majority-rule consensus tree, and posterior probabilities (PP) were considered significant when

179   ≥0.95 (Huelsenbeck & Ronquist, 2001). Maximum Likelihood (ML) analyses for each gene were

180   performed with RAxML v7.0.4 (Stamatakis, 2006), based on 1000 rapid bootstrap analyses for

181   the best ML tree.

182   We performed concatenated analyses with ML and BI for the following datasets: (1) two

183   mitochondrial genes combined, (2) nine nuclear genes combined, (3) all genes combined.

184   Likelihood analyses were performed using RAxML v7.0.4, based on 1000 rapid bootstrap

185   analyses. Bayesian analyses were conducted using MrBayes v3.2.2, with four heated Markov

186   chains (using default heating values) and run for 50 million generations, with Markov chains

187 sampled at intervals of 1000 generations. Equilibrium samples (after 25% of burn-in) were used

188 to generate a 50% majority-rule consensus tree, and posterior probabilities (PP) were considered

189 significant when ≥0.95 (Huelsenbeck & Ronquist, 2001).

190   For construction of a species tree incorporating the multispecies coalescent approach, we

191 used the hierarchical Bayesian model integrated in *Beast v1.8.0 (Drummond & Rambaut,

192 2007). For all genes were run two separate analyses for 100 million generations (sampled every

193 1000 generations). Clades with PP > 0.95 were considered strongly supported.

194   To ensure that convergence was reached before default program burn-in values, we

195 evaluated convergence of Bayesian MCMC phylogenetic analyses (MrBayes and *Beast) by

196 examining likelihood and parameter estimates over time in Tracer v1.6 (Rambaut et al., 2009).

197 All parameters were between 157 and 23400 effective sample sizes (ESS).

198   All alignments and trees were stored in TreeBase (ID: 20987); phylip files produced by

199 RAxML were converted to nexus with ALTER (Glez-Peña et al., 2010), and trees merged with

200 matrices in Mesquite v3.2 (Madison & Madison, 2017).

201

202 **Morphological Approach**

203   Voucher specimens are listed in Appendix S5. Coordinates are presented in decimal degrees

204 and WGS 84 datum, and all the elevations are in meters above sea level (masl). Institution codes

205 follow Sabaj Pérez (2014).

206   Metric characters were taken following Avila et al. (2012), and include snout–vent length

207 (SVL) from tip of snout to vent; trunk length (TrL) distance from axilla to groin from posterior

208 edge of forelimb insertion to anterior edge of hindlimb insertion; foot length (FL) from tip of

209 claws of the 4th toe to heel; tibial length (TL) greatest length of tibia, from knee to heel; arm

210 length (AL) from tip of claws of the 3rd finger to elbow; head length (HL) distance between

211 anterior edge of auditory meatus and snout tip; head width (HW) taken at level of the temporal

212 region; head height (HH) maximum height of head, at level of parietal area; eye–nostril distance

213 (END) from the anterior edge of the eye to the posterior edge of the nostril; eye–snout distance

214 (ESD) from the anterior edge of the eye to the tip of the snout; eye–meatus distance (EMD) from

215 the posterior edge of the eye to the anterior border of the ear opening; interorbital distance (ID)

216 interorbital shortest distance; internostril distance (IND). Meristic data consist of: number of

217 keeled dorsal tubercles (DT) from occipital area to cloaca level; number of transversal rows of

218 ventral scales (TVS), counted longitudinally at midline from the chest (shoulder level) to

219 inguinal level; number of longitudinal rows of ventral scales (LVS), counted transversally at

220 midbody; number of supralabial scales (SL); number of infralabial scales (IL); number of fourth

221 toe lamellae (4TL); and number of third finger lamellae (3FL). Paired structures are presented in

222 left/right order. In the color descriptions, the capitalized colors and the color codes (in

223 parentheses) are those of Köhler (2012).

224   Based on the genetic clusters recognized by the barcoding analysis, we performed a

225 discriminant function analysis (DA). As a first step we tested normality with Shapiro-Wilk (*W*)

226 test (Shapiro et al., 1968; Zar, 1999). Then we performed the DA including variables with

227 normal distribution, analyzing continuous characters (metrics) that are sensitive to ontogeny,

228 separated from discrete (non-sensitive to body growth) characters. All statistical procedures were

229 performed with Past 3.14 (Hammer et al., 2001).

230

231   **RESULTS**

232 **Phylogenetic inference**

233   Following we present the size of each aligned gene (in brackets) and the best substitution

234 model identified: 16S [527 bp]: GTR+G; 12S [951 bp]: GTR+G; cyt-b [794 bp]: TRN+I+G;

235 MXRA5 [961 bp]: TPM1lf+G, NKTR [1074 bp]: TRN+G, SINCAIP [449 bp]: TPM2 lf+G,

236 RBMX [600 bp]: HKY+G, DMXL1 [959 bp]: HKY+G, ACA4 [1218 bp]: HKY+G, PRLR [543

237 bp]: TRN+G, Homo_30b [664 bp]: TRN+I, Homo_19b [642 bp]: F81+G.

238   The ML tree based on an initial exploration with 16S mtDNA gene sequences shows two

239 separate clades of geckos, formerly referred to as *Homonota fasciata* (Fig. 1), with uncorrected

240 16S p-distances ranging between 1.8 and 2.5% (Table 1). In the alignment we identified 11 fixed

241 different sites between these clades (Table 2). We interpret the documented genetic differences

242 as evidence for heterospecificity of these two clades. Thus, we recognize two potential species of

243 geckos formerly referred to as *H. fasciata*: Species A (sampled in Low Monte ecoregion) and

244 Species B (sampled in Dry Chaco, Paraguay).

245   The ABGD analysis for the 16S dataset resulted in the recognition of three groups (1-

246 Species A, 2- Species B, 3- Outgroup) with a range of intraspecific genetic variation from 0.1 to

247 0.77%; and two groups (1- *Homonota*, 2- Outgroup) with an intraspecific variation of 1.29%

248 (Appendix S6). This is only slightly higher than the higher intraspecific distance between two of

249   our samples (p-distance=1.0% between LJAMM-CNP 10495 and LJAMM-CNP 10576; Table 1)

250   of Species A, whereas the intraspecific distance among specimens of Species B (<0.01%). The

251   PTP also proposed two different clades (both with ML and Bayesian algorithms) grouping

252   separately Argentinean samples (Species A) and Paraguayan samples (Species B) (Appendix

253   S7).Species A was inferred as the sister taxon of Species B in nine of the 11 independent gene

254   trees obtained with both BI and ML (Appendix S8). Exceptions include: 1-the gene Homo_30b

255   (both with BI and ML), which infer Species B as sister of the clade Species A +*H. underwoodi*;

256   2-DMXL1 inferred the *borelli* group as sister to Species A+Species B (both with BI and ML); 3-

257   the gene SINCAIP (ML only) showed the groups *fasciata* and *whitii* nested together; 4- the gene

258   NKTR with ML inferred *H. underwoodi* as a member of a different group (Appendix S8).

259   All phylogenies inferred from concatenated datasets of (1) two mitochondrial genes

260   combined, (2) nine nuclear genes combined, (3) all genes combined with both BI and ML

261   showed high support in recognizing Species B from Paraguay as a sister to Species B from

262   Argentina, with *Homonota underwoodi* as sister to these two within the *fasciata* group

263   (Appendix S9). The species tree inferred with *Beast presents the same arrangement within the

264   *fasciata* group as those inferred by BI and ML using concatenated datasets (Fig. 2).

265

266   **Morphological analyses**

267   All the continuous variables had normal distributions, but two discrete variables (SL and IL)

268   did not (Table 3), thus, they were excluded from further morphological analysis. Convex hulls

269   for metric variables show a significant discrimination between Species A and Species B, which

270   support the cluster differentiation inferred from molecular data (Fig. 3). The most contributing

271   variables were SVL and TrL for Axis 1 (Appendix S10). Sexual dimorphism was not recorded

272   for Species A, whereas an evident sexual dimorphism in Species B was documented (Fig. 3).

273   Nevertheless, the probability ellipse (confidence=95%) propose a high overlap, and females of

274   Species B is the most different group (Fig. 3).

275   Regarding meristic data, sexual dimorphism is more pronounced in *H. fasciata* than in

276   *Homonota* sp. "Paraguay" (Fig. 4). Raw data are available in Appendices S11 (metric variables)

277   and S12 (meristic variables).

278

279   **Taxonomic implications**

280   We take the significant level of genetic differentiation between these two clusters of

281   *Homonota* as evidence for the recognition of two different taxa. In order to correctly assign

282   names to these two species, we examined the relevant primary types of the nominal taxa in this

283   species complex. The holotype of *H. fasciata* is MNHN 6756 (LSID:

284   urn:lsid:zoobank.org:act:14CDAB98-810F-43B3-8F16-B29C830AB80C). As mentioned above,

285   the original type locality of *H. fasciata* was given as "Martinique" and is without doubt

286   erroneous. A detailed analysis of MNHN 6756 (Fig. 5) revealed that it differs in pholidosis in

287   several significant characters from the biological species currently referred to as *H. fasciata*

288   (Table 4), such as margin of auditory meatus (Fig. 6), size of first infralabial scale (Fig. 7), and

289   the arrangement of dorsal scales (Fig. 8). Given these differences in several taxonomically

290   important scalation traits, there is no doubt that MNHN 6756 is not conspecific with the

291   biological species currently referred to as *H. fasciata*. The scalation traits of MNHN 6756

292   presented above resemble the external morphology of *Homonota uruguayensis* (Vaz-Ferreira &

293   Sierra de Soriano, 1961). However, *H. uruguayensis* does not have transversal bands on the

294   dorsum, and in the original description of *H. fasciata* transversal bands on the dorsum of the type

295   specimen are mentioned. In its current state, the holotype of *H. fasciata* is completely bleached

296   and does not show any trace of banding (Fig. 5). In conclusion, we cannot link the holotype of *H.*

297   *fasciata* to any of the known populations of *Homonota* which renders this name a *species*

298   *inquirenda* which needs further studies and cannot be linked to either Species A or Species B.

299   Our examination of the lectotype of *H. horrida* (IZH-R 1) revealed that it is conspecific with our

300   Species A which is supported by the fact that the Argentinian specimens used in our genetic

301   analysis are from the general area of the type locality of *H. horrida*.. We therefore resurrect it

302   from synonymy with *H. fasciata* and apply it to our Species A. As mentioned above, the original

303   description of *H. mattogrossensis* is very brief, does not provide a precise type locality (and no

304   representative of the genus *Honomota* is known to occur in Brazil) and no type material or other

305   voucher specimen is known. Therefore this name cannot be applied to any of the known

306   populations of this genus and we consider *Homonota mattogrossensis* to constitute a *nomen*

307   *dubium*.

308   No name is available for our Species B and we therefore describe it as a new species below,

309   presenting also a species account and a redescription of *H. horrida*. The electronic version of this

310   article in Portable Document Format (PDF) will represent a published work according to the

311 International Commission on Zoological Nomenclature (ICZN), and hence the new names

312 contained in the electronic version are effectively published under that Code from the electronic

313 edition alone. This published work and the nomenclatural acts it contains have been registered in

314 ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science

315 Identifiers) can be resolved and the associated information viewed through any standard web

316 browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication

317 is: [Pending]. The online version of this work is archived and available from the following digital

318 repositories: PeerJ, PubMed Central and CLOCKSS.

319

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

321   - *Gymnodactylus horridus* Burmeister 1861

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

323 *Types*: Original description based on three syntypes. Lectotype (IZH-R 1, Fig. 9) and

324 paralectotype (IZH-R 2) designation according to Müller (1941).

325   - *Wallsaurus horridus* comb. nov. Underwood 1954

326   - *Gymnodactylus pasteuri* nom. nov. Wermuth 1965

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

328

329   *Diagnosis*: It is a large species of *Homonota* with a dark dorsal color (grey or brown) with a

330 pattern of clear transversal bands connected with a vertebral stripe. Additionally, it is

331 differentiated from any other *Homonota* by the large size and development of the keeled scales

332 on the head (including laterals) and dorsum.

333

334   *Redescription of the lectotype (Fig. 9)*: Adult male, SVL 44 mm, TrL 19 mm, tail 49 mm,

335 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,

336 ESD 4.6 mm, EMD 4.1 mm, ID 4.3 mm, IND 1.4 mm; rostral wider than high; nares surrounded

337 by rostral, supranasal, two postnasals, and first SL; SL 9/9; one elongated tubercular scale on the

338 mouth commissure; upper region of the muzzle covered by big homogeneous juxtaposed scales;

339 upper surface of the head covered with medium-sized (smaller than those on the muzzle)

340 homogeneous juxtaposed scales intermixed with small granules; superciliary scales imbricated,

341 associated to spiny-like scales on the posterior half of the orbit; lateral sides of the head

342   heterogeneously covered profusely with large keeled tubercles and small granular (sometimes

343   elongated) scales; auditory meatus oblique and with serrated edge, and one big scale on the upper

344   border; IL 6/6; mental triangular; postmentals big (about twice the size of the following posterior

345   scales) contacting the mental, the first IL, and a row of six posterior scales (the two centrals

346   smaller); scales under the head reducing in size posteriorly; dorsolateral parts of the neck with

347   granular juxtaposed scales mixed with tubercles; throat region covered by imbricated cycloid

348   scales; dorsum covered with 16 strongly keeled scales separated by one or two small granular

349   scales; ventral scales cycloid and imbricated arranged in 18 longitudinal rows at midbody;

350   suprascapular, axillar, and inguinal regions surrounded by small imbricated granules; sides of

351   cloacal opening with two to three conical tubercular scales; anterior and dorsal surfaces of limbs

352   covered by imbricated scales, slightly keeled on the dorsal surface; posterior region of limbs

353   covered by small juxtaposed granules; ventral surface of forelimbs with juxtaposed granules, and

354   ventral surface of hind limbs with large imbricated scales; subdigital lamellae of hands starting

355   from pollex were recorded as follows: 8/8 - 12/12 - 14/14 - 16/16 - 8/11; subdigital lamellae of

356   feet starting from hallux were recorded as follow: 17/17 - 21/18 - 17/17 - 13/13 - 7/8; large

357   imbricated keeled scales around the tail disposed in rings, separated by two to three series of

358   small scales.

359

360   *Coloration in preservative of the lectotype*: The specimen is at least 147 years old, and

361   coloration is faded in most parts of the animal. The whole body is basically Cream White (52)

362   with vestiges of blotches on the scapular region, pre and postocular lines, and rings around the

363   tail of Salmon Color (58).

364

365   *Variation*: (Based on specimens referred in Appendix S5) SVL 42–64 mm; TrL 16–29 mm

366   (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

367   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

368   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

369   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

370   (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

371   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

372   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

373   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

374   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%

375   in males); SL 7–9; one or two elongated tubercular scales on the mouth commissure; upper

376   region of the muzzle usually flattened, rarely slightly convex (LJAMM-CNP 6520); auditory

377   meatus with one large scale on the upper border; IL 6–8; 13–20 longitudinal rows of ventral

378   scales at midbody.

379   The coloration pattern (lost in the type series) consist of a dark and clear reticulation on the

380   dorsal surface of the head, a dark longitudinal stripe from the tip of the snout across the temporal

381   region extending posteriorly and upwards reaching the nuchal region. Dorsal background color

382   usually dark with whitish transversal bands connected with a vertebral stripe of the same color.

383   Limbs with an irregular reticulation. Ventral region of head and body always immaculate clear.

384   Tail with dark and clear rings that can be present only on the dorsal and lateral areas of the

385   organ, or continued to the ventral surface. Some melanic specimens (LJAM-CNP 6532, 6968)

386   lack the vertebral stripe, and the clear transversal bands are inconspicuous.

387

388   *Distribution*: As mentioned before, this is a species complex which needs further analyses.

389   As currently recognized, this clade is distributed from the Argentinean Province of Rio Negro in

390   southern Argentina, to the center of Paraguayan Chaco, according to Morando et al. (2014). Our

391   analyzed samples came from Low Monte ecoregion in southern Argentina.

392

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

394   LSID: Registration pending

395

396   *Holotype*: MNHNP 12238 (original field number PCS 200), adult female (Fig. 10), collected

397   on 10 December 2014 by P. Cacciali, at Fortín Mayor Infante Rivarola (21.679º S, 62.401º W,

398   277 masl), Boquerón Department, Paraguay.

399

400   *Paratypes*: MNHNP 2821, 9037–8, 9131, 11406*, 11409*, 11410, 11419, 11421, 11423

401   (Parque Nacional Teniente Enciso, Boquerón Department, Paraguay; 21.209ºS, 61.655ºW, 253

402   masl); MNHNP 11850, 11855, 11860, 11872, 11873* (Cruce San Miguel, in front of Parque

403   Nacional Teniente Enciso, Boquerón Department, Paraguay; 21.203ºS, 61.662ºW, 254 masl);

404   SMF 101984* (topotype); SMF 29277 (Villamontes, Tarija Department, Bolivia; 21.266ºS,

405   63.451ºW, 398 masl). Holotype and specimens marked with an asterisk (*) were used for

406   molecular analyses.

407

408   *Etymology*: The specific name *septentrionalis* is Latin, meaning "northern" and refers to the

409   fact that this species has the northernmost distribution of all the *Homonota* species.

410

411   *Diagnosis*: This is the largest species of the genus (max. 65 mm SVL) with robust body,

412   prominent keeled tubercles disposed in four to eight longitudinal rows, and coloration pattern of

413   dark background with one vertebral and six or seven transversal clear bands. It can be

414   distinguished from *H. andicola*, *H. whitii*, and *H. underwoodi* by the presence of strongly keeled

415   dorsal scales (vs. smooth dorsal scales in *H. andicola*, *H. whitii*, and *H. underwoodi*), transversal

416   clear bands on a darker dorsum (vs. reticulated pattern), and from *H. underwoodi* also by a lower

417   number of 4TL (16–20) and 3FL (11–15) (vs. 20–25 and 15–17 respectively in *H. underwoodi*).

418   From *H. borellii* and *H. rupicola* by the oblique shape of the auditory meatus (vs. round in *H.*

419   *borellii* and *H. rupicola*), transversal clear bands on a darker dorsum (vs. reticulated pattern), and

420   also from *H. borelli* by the presence of strongly keeled dorsal scales (vs. moderately keeled), and

421   from *H. rupicola* by a higher number of 4TL (16–20) (vs. 14–15). From *H. darwinii* by the

422   presence of strongly keeled dorsal scales (vs. smooth at least on the anterior part of the dorsum in

423   *H. darwinii*), and by transversal clear bands on a darker dorsum (vs. reticulated pattern). From *H.*

424   *rupicola* and *H. taragui* by the presence of enlarged keeled tubercles on the sides of the head

425   behind the orbits (vs. homogeneous granular scales). From *H. uruguayensis* by a higher number

426   of IL scales (6–7, vs. 4–5 in *H. uruguayensis*), by the coloration, and by the serrated edge of the

427   auditory meatus (vs. smooth granular edge in *H. uruguayensis*). From *H. williamsii* by the

428   presence of strongly keeled dorsal scales (vs. moderately keeled) and by transversal clear bands

429   on a darker dorsum (vs. reticulated pattern). From *H. horrida* (the most similar species) by the

430   high position of the auditory meatus relative to the mouth commissure (vs. lower position in *H.*

431   *horrida*) (Fig. 11); less developed tubercles on the sides of the head, including a narrow area

432   between the orbit and the auditory meatus covered with small granular scales with without or

433   with few tubercles (vs. several big tubercles on the sides of the head even in the area between the

434   orbit and the auditory meatus) (Fig. 11).

435

436   *Description of the holotype*: Adult female, SVL 60 mm, TrL 26 mm, tail broken near the

437   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

438   4.6 mm, ESD 6.6 mm, EMD 5.1 mm, ID 5.5 mm, IND 2.5 mm; rostral wide with a median

439   groove at the upper half; nares surrounded by rostral (slight contact), supranasal, two postnasals,

440   and first SL (slight contact); SL 9/8; two elongated tubercular scales on the mouth commissure;

441   upper region of the muzzle slightly convex covered by big homogeneous juxtaposed scales;

442   upper surface of the head covered with big homogeneous juxtaposed scales intermixed with

443   small granules; superciliary scales imbricated forming a serrated edge, associated to spiny-like

444   scales on the posterior half of the orbit; lateral sides of the head heterogeneously covered with

445   large keeled tubercles and small granular (sometimes elongated) scales; auditory meatus oblique

446   and with serrated edge, and two big scales on the upper border; IL 6/6; mental triangular;

447   postmentals big (less than twice the size of the following posterior scales) contacting the mental,

448   the first IL, and a row of six posterior scales (the two centrals smaller); scales under the head

449   reducing in size posteriorly; dorsolateral parts of the neck with granular juxtaposed scales mixed

450   with tubercles; throat region covered by imbricated cycloid scales; dorsum covered with eight

451   strongly keeled scales separated by one or two small granular scales, except on the vertebral area

452   where keeled scales are separated by four granules; ventral scales cycloid and imbricated

453   arranged in 20 longitudinal rows at midbody; suprascapular, axillar, and inguinal regions and

454   cloacal opening surrounded by small imbricated granules; anterior and dorsal surfaces of limbs

455   covered by large imbricated scales, keeled on the dorsal surface; posterior region of limbs

456   covered by small juxtaposed granules; ventral surface of forelimbs with juxtaposed granules, and

457   ventral surface of hind limbs with large imbricated scales; subdigital lamellae of hands starting

458   from pollex were recorded as follows: 7/8 - 12/10 - 13/14 - 13/13 - 12/10; subdigital lamellae of

459   feet starting from hallux were recorded as follow: 13/13 - 18/18 - 15/14 - 12/12 - 10/10; large

460   imbricated scales around the tail (stump) with the eight uppermost strongly keeled.

461

462   *Coloration in life*: Dorsal surface of head Grayish Horn Color (268) with groups of Dusky

463   Brown (285) scales, irregularly mixed with Hair Brown (277) scales; posterior surface of the

464   head with a curved Hair Brown (277) line interrupted by five groups of Dusky Brown (285)

465   scales; upper lateral view of the head Grayish Horn Color (268), edged below by a thick Dusky

466   Brown (285) stripe from the muzzle (interrupted by the orbit) to the temporal region; supralabial

467   and infralabial regions Smoky White (261) with irregular Raw Umber (280) suffusions on the 1st

468   and 2nd SL and 1st to 5th IL; region between mouth commissure and shoulder Smoky White (261)

469   with irregular Dusky Brown (285) speckles, edged above (bordering the upper edge of the ear

470   opening) by an irregular Cream Yellow (82) stripe; ventral surface of the head Smoky White

471   (261); dorsal ground color Dusky Brown (285), with a Light Straw Yellow (95) vertebral stripe,

472   and five transversal Light Sulphur Yellow (93) lines; lateral parts of the body Cream Yellow (82)

473   with irregular Dusky Brown (285) speckles; venter Smoky White (261); dorsal surface of limbs

474   Cream Color (12) with irregular Dusky Brown (285) speckles on the forelimbs, and groups of

475   Dusky Brown (285) scales (eventually forming short stripes) on the hind limbs; ventral surface

476   of limbs Smoky White (261).

477

478   *Coloration in preservative*: Dorsal surface of head Drab (19) with groups of Vandyke

479   Brown (282) scales; posterior surface of the head with a curved Vandyke Brown (282) line;

480   upper lateral view of the head Smoke Gray (266), edged below by a thick Raw Umber (260)

481   stripe from the muzzle (interrupted by the orbit) to the temporal region; supralabial and

482   infralabial regions Cream White (52) with irregular Raw Umber (260) suffusions on the 1st and

483   2nd SL and 1st to 5th IL; region between mouth commissure and shoulder Cream White (52) with

484   irregular Raw Umber (260) speckles; ventral surface of the head Cream White (52); dorsal

485   ground color Raw Umber (260), with a Beige (254) vertebral stripe, and five transversal Cream

486   White (52) lines; lateral parts of the body Cream White (52) with irregular Raw Umber (260)

487   speckles; venter Cream White (52); dorsal surface of limbs Beige (254) with irregular Sepia

488   (279) speckles on the forelimbs, and groups of Sepia (279) scales (eventually forming short

489   stripes) on the hind limbs; ventral surface of limbs Cream White (52).

490

491   *Variation*: SVL 37–65 mm; TrL 15–28 mm (43.3–48.2% of SVL in females, 38.3–48.8% in

492   males); Tail length 47–63 mm (ratio SVL:Tail - 1:1 in one female, 1:1.18–1:1.22 in two males,

493   and 1:1.17 in a juvenile of unknown sex); FL 8–9 mm (8.8±0.37) in males, 10–12 mm

494   (11.2±0.83) in females; TL 7.2–9.8 mm (8.7±0.36) in males, 9.4–11.3 mm (10.5±0.81) in

495   females; AL 10.2–13.1 mm (11.7±0.91) in males, 13.1–15.0 mm (14.1±0.76) in females; HL

496   10.7–13.3 mm (11.8±0.38) in males, 12.9–17.3 mm (14.6±1.66) in females; HW 8.1–13.3 mm

497 (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

498 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

499 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

500 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

501 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%

502 in males); SL 6–9; one or two elongated tubercular scales on the mouth commissure; upper

503 region of the muzzle slightly convex or flattened; auditory meatus with one or two big scales on

504 the upper border; IL 6–7; 12–20 longitudinal rows of ventral scales at midbody.

505   The coloration variation follows the same pattern observed for the holotype. Smaller animals

506 (MNHNP 11419, 11423) are clearer and the clear transversal bands are reduced to the

507 paravertebral area; vertebral stripe reduced in MNHNP 11855; three paratypes (MNHNP 2821,

508 9037, 9131) have a darker pattern being reddish dorsal background color, and in two of them

509 (MNHNP 2821, 9131) the transversal bands are almost faded; the original tail (MNHNP 9131,

510 11419, 11421, 11850, 11860, 11872, SMF 29277) has transversal dark and clear bands dorsally,

511 and clear or reddish hue ventrally.

512

513   *Distribution*: *Homonota septentrionalis* is distributed in the northernmost range of the genus.

514 The examined specimens come from the Dry Chaco, at the westernmost part of the Paraguayan

515 Chaco and southeast of Bolivia (Fig. 12).

516

517   *Habitat*: The environment inhabited by *H. septentrionalis* is a xerophytic (precipitation

518 varies between 300 and 400 mm per year) and thorny dry forest, with null or scarce herbaceous

519 stratum (Fig. 13). This species is a nocturnal ground dweller, being abundant in natural areas,

520 and also present in anthropogenically modified areas.

521

522   **DISCUSSION**

523   The analysis of genetic barcodes of the mtDNA gene 16S provided the first evidence for the

524 existence of an undescribed species of *Homonota* in Paraguay, which was posteriorly tested with

525 additional data. The uncorrected genetic distance of the 16S fragment between *H. horrida* and *H.*

526 *septentrionalis* is rather low (1.8–2.5%) compared to distances between species of other genera

527 of geckos such as *Diplodactylus* (4–12%; Pepper et al., 2006), *Phyllopezus* (6–15%; Gamble et

528   al., 2012), and *Lepidoblepharis* (12–23%; Batista et al., 2015). Using the species delimitation

529   program ABGD, we estimated the intraspecific variation since this program explores the

530   pairwise differences in barcode datasets, providing limits for intraspecific divergence (Puillandre

531   et al., 2012). The expected intraspecific variation for *Homonota* Species A and Species B,

532   matches with the variation in uncorrected pairwise distance (Table 1), with a clear difference

533   between the two taxa. The tree-based PTP analysis provides speciation models based on number

534   of substitution in a phylogenetic hypothesis, for which the branch length of a tree represents the

535   number of substitutions (Zhang et al., 2013). This algorithm also suggested two putative species,

536   one from Argentina (Species A) and the other from Paraguay (Species B).

537   The topology of the species tree (Fig. 2) shows *Phyllodactylus* as the sister genus of

538   *Homonota*, congruent with Gamble et al. (2008b, 2011) and Morando et al. (2014). The

539   arrangement among groups of *Homonota* inferred the *fasciata* group as the most basal clade, a

540   hypothesis contrary to that proposed by Morando et al. (2014) where the *whitii* group was the

541   most basal clade within *Homonota*. The majority of the topological arrangements among the

542   concatenated trees are identical, with the exception of the position of *H. taragui* which was

543   closely related to *H. rupicola* using mitochondrial genes, and related to *H. borellii* using nuclear

544   genes (Appendix S9); a conflict that was already reported by Morando et al. (2014). In our

545   phylogeny *H. horrida* and *H. septentrionalis* were inferred as sister taxa with high statistical

546   support (PP=1, Fig. 2). Given the taxonomic modifications proposed here, we suggest referring

547   to the group that contains *H. underwoodi*, *H. horrida*, and *H. septentrionalis* as the *H. horrida*

548   species group.

549   The holotype of *Homonota fasciata* was sent to Paris by Auguste Plée who was a botanist

550   who collected several samples of plants and animals in the Antilles, and some of his collections

551   are valid records for Martinique (i.e., type locality of *H. fasciata*) such as *Monstera adansonii*

552   (Alismatales: Araceae), *Auxis thazard* (Actinopterygii: Scombridae), *Eleutherodactylus*

553   *martinicensis* (Amphibia: Eleutherodactylidae), *Mabuya mabouya* (Reptilia: Scincidae),

554   *Megalomys desmarestii* (Mammalia: Cricetidae), whereas some others were recorded but

555   currently extinct as *Leptodactylus fallax* (Amphibia: Leptodactylidae) and *Leiocephalus*

556   *herminieri* (Reptilia: Leiocephalidae) (Madison, 1977; Collette & Aadland, 1996; Borroto-Páez

557   & Ramos García, 2012; Hedges & Conn, 2012; Breuil, 2015). Thus, although some locality

558   records provided by Plée are trustable, the name *H. fasciata* based on specimen MNHN 6756,

559   remains has to be considered as a *species inquirenda*. More historical analyses could shine some

560   light on the real origin of this specimen.

561   Abdala & Lavilla (1993) stated that differences between *Homonota horrida* and the type of

562   *H. fasciata* were due to variation, which is true for some meristic characters. Nevertheless, the

563   small size of postmental scales and serrated edge of auditory meatus are common morphological

564   traits of *H. horrida*. These authors suggested that some specimens of *H. horrida* could have big

565   postmentals and smooth auditory meatus (referring to specimens FML 35 and FML 114) which

566   is rare for the species. Another common trait for *H. horrida* is the presence of a tubercular scale

567   on the upper edge of the auditory meatus, which is absent in the type of *H. fasciata*. Further

568   genetic and morphological analyses of Argentinean populations of *H. horrida* are required for a

569   better understanding of variation within the species.

570   *Homonota septentrionalis* is a large species of *Homonota*, with a marked sexual dimorphism

571   in measurable characters according to the DA analysis (Fig. 3), where SVL and TrL are the

572   variables that contribute more to the differentiation (Appendix S10). This differs from what is

573   known for *Homonota darwinii* where Ibargüengoytía & Casalins (2007) found no sexual

574   dimorphism, although Fitch (1981) reported differences in SVL between males and females in

575   Gekkonidae with females usually larger than males. More analyses are needed in order to

576   explore the extent of this pattern in other species of the genus.

577   Genetic analyses were key for the recognition of the new species, since the morphological

578   differences between *H. septentrionalis* and *H. horrida* are subtle and they could be considered

579   cryptic species. High degree of genetic differentiation and low degree of morphological

580   distinction is a common phenomenon for lizards, leading to situations in which authors designate

581   candidate species without formal descriptions (Gamble et al., 2012; Werneck et al., 2012), or

582   cases in which authors base the entire diagnosis upon genetic clustering (Leaché & Fujita, 2010).

583   Currently, *Homonota septentrionalis* is known from the type locality (Fig. 11), in plain areas

584   and xerophytic environments. Given the similarity in external morphology between *H.*

585   *septentrionalis* and *H. horrida* it is difficult to elaborate a cresonymy list of the previous records

586   for these species. Records published by Mendoza et al. (2015) as *H. fasciata* from Bolivia,

587   probably are *H. septentrionalis*, but further morphological and genetic analyses are required for a

588   better understanding of the distribution pattern of *H. septentrionalis*.

589   Based on these results, the actual diversity of the genus *Homonota* is as follows: *borellii*

590   group: *H. borellii, H. uruguayensis, H. rupicola*, and *H. taragui*; *horrida* group: *H. horrida, H.*

591   *underwoodi*, and *H. septentrionalis* sp. nov; *whitii* group: *H. whitii, H. darwinii, H. andicola*, and

592   *H. williamsii*; *Incertae sedis*: *H. fasciata*.

593   Currently, the conservation status of *Homonota septentrionalis* is totally unknown.

594   *Homonota fasciata* was categorized as Least Concern (LC) by Motte et al. (2009) given its big

595   range, but since we actually do not know the range of *H. septentrionalis*, the conservation status

596   might be different. This species is related to the Dry Chaco, which for a long time was a

597   sanctuary for wildlife because of the lack of anthropogenic impacts; but unfortunately in the last

598   decade the deforestation is severely threatening many areas of the Dry Chaco (Eva et al., 2004;

599   Caballero et al., 2014). An assessment of the status of this new taxon is required.

600

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610

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Figure 1

Fig. 1. Maximum Likelihood tree

Maximum Likelihood clusters of *Homonota fasciata* from Argentina (blue polygon) and from Paraguay (red rectangle), 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*.

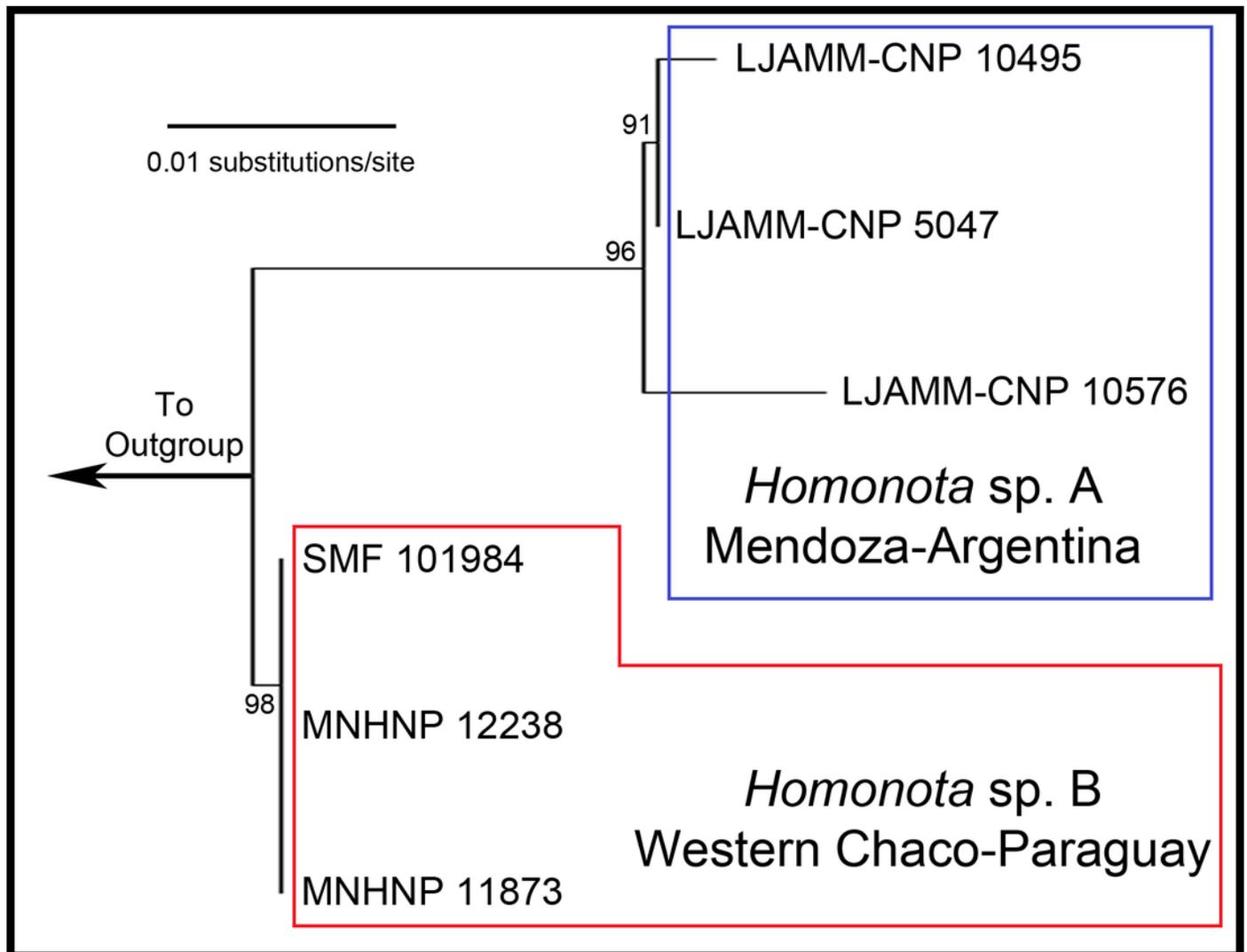



Figure 2

Fig. 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.

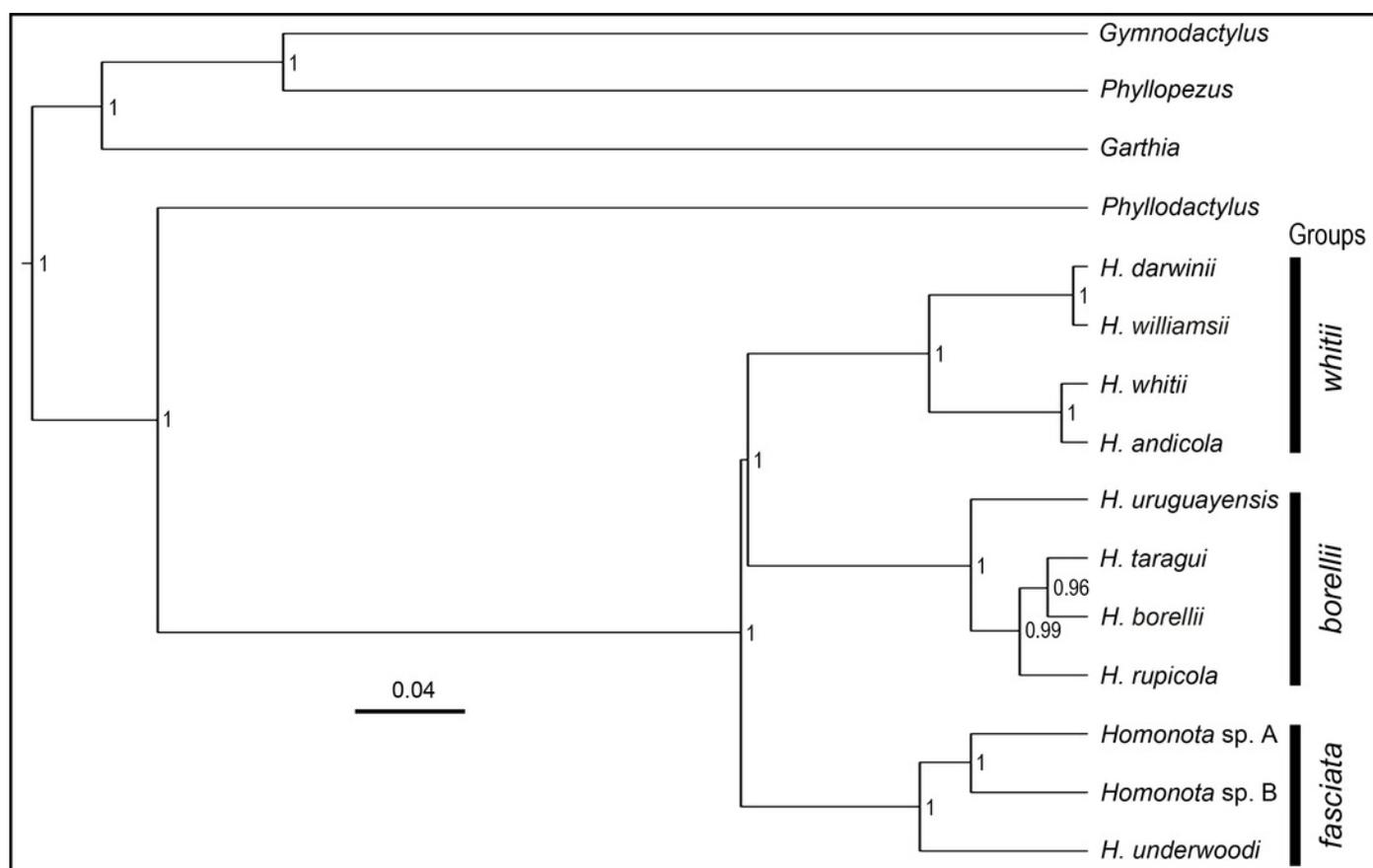



Figure 3

Fig. 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.

Axis 1: Eigval 1.149 (Percent 59.32)
Axis 2: Eigval 2.458 (Percent 35.15)
Axis 3: Eigval 0.387 (Percent 05.53)

Axis 2

Axis 1

H. fasciata
  M +
  F +

Homonota sp.
  M ●
  F ●

Correctly classified 88.24%

	H_fas_ssF	H_fas_ssM	H_aff_fasF	H_aff_fasM
H_fas_ssF	10	2	0	0
H_fas_ssM	2	8	0	0
H_aff_fasF	0	0	5	0
H_aff_fasM	0	0	0	7

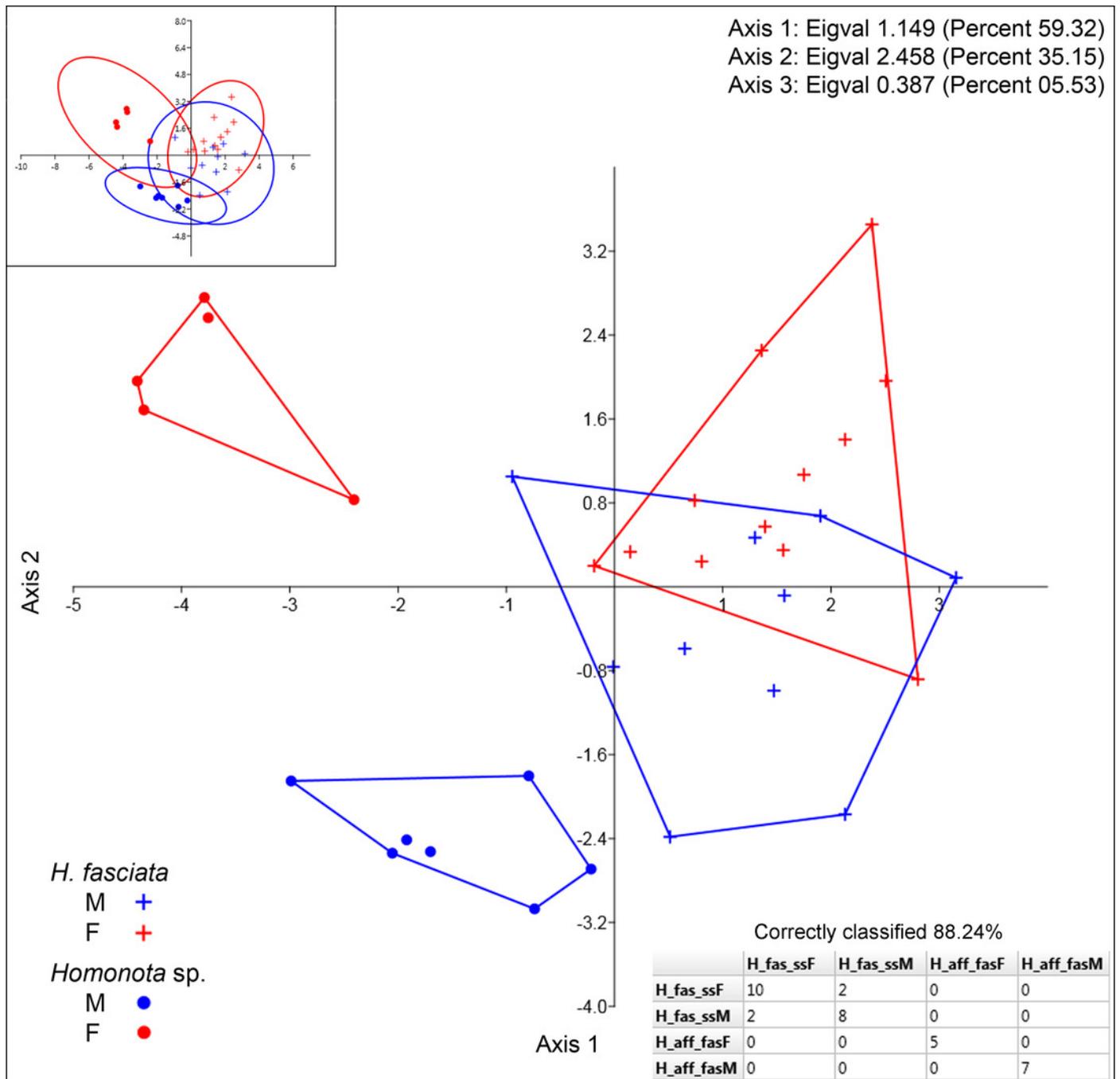



Figure 4

Fig. 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.

Axis 1: Eigval 1.448 (Percent 79.01)
Axis 2: Eigval 0.342 (Percent 18.67)
Axis 3: Eigval 0.042 (Percent 02.32)

H. fasciata
M   +
F   +

Homonota sp.
M   ●
F   ●

Axis 2

Axis 1

Correctly classified 70.59%

	H_fas_ssF	H_fas_ssM	H_aff_fasF	H_aff_fasM
H_fas_ssF	9	2	0	1
H_fas_ssM	3	7	0	0
H_aff_fasF	1	0	3	1
H_aff_fasM	0	0	2	5

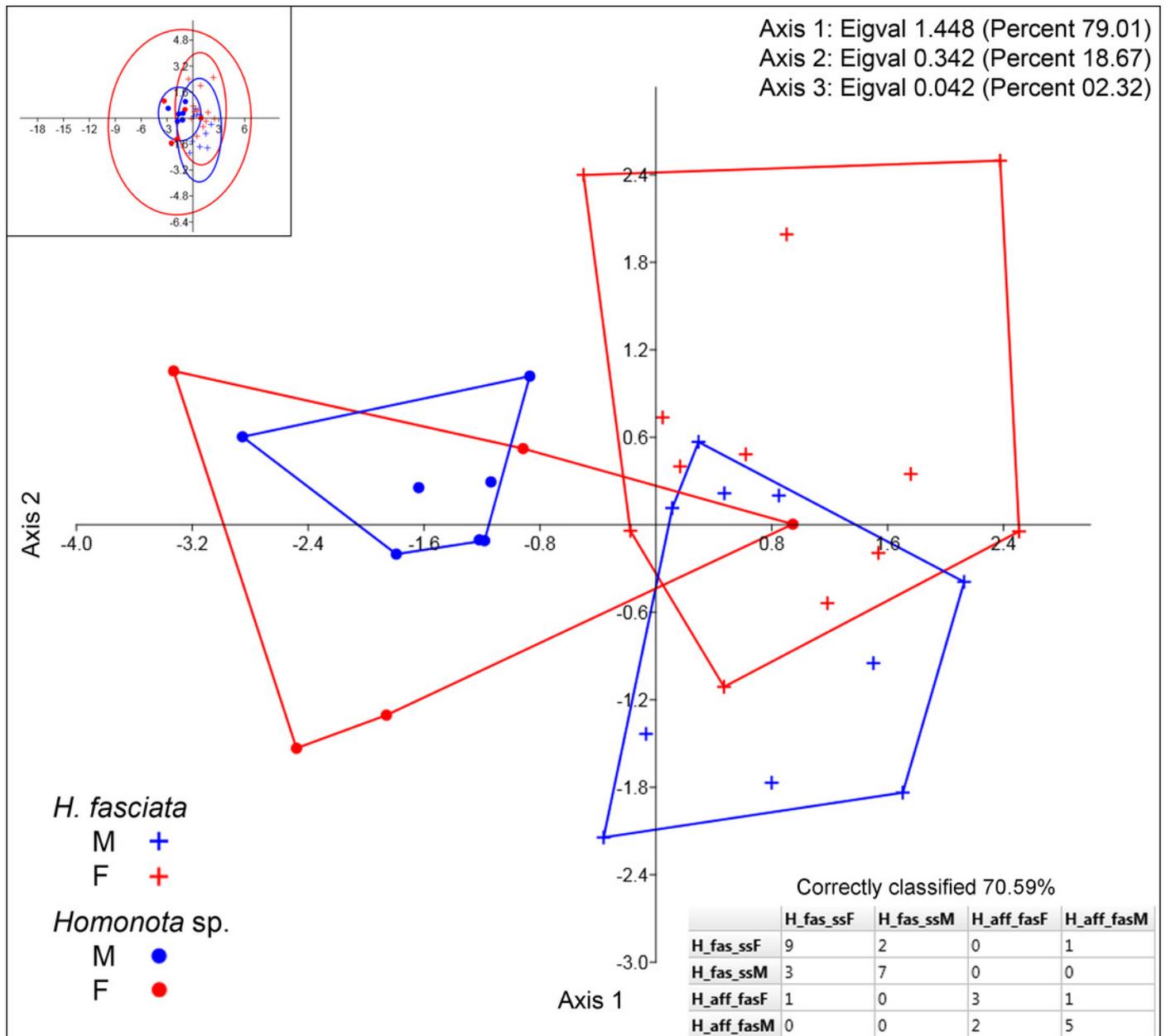



Figure 5

Fig. 5. Image of holotype of *Homonota fasciata*

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

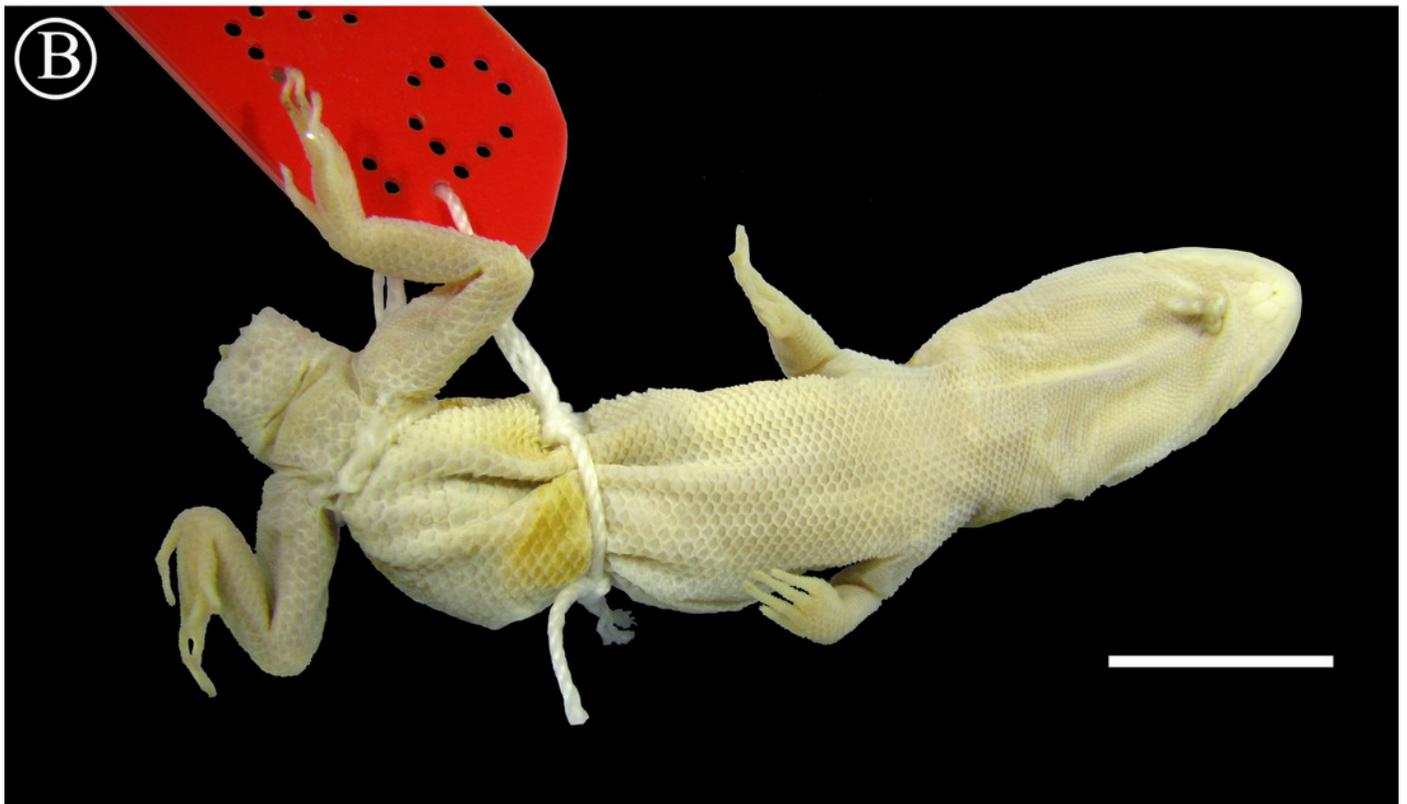
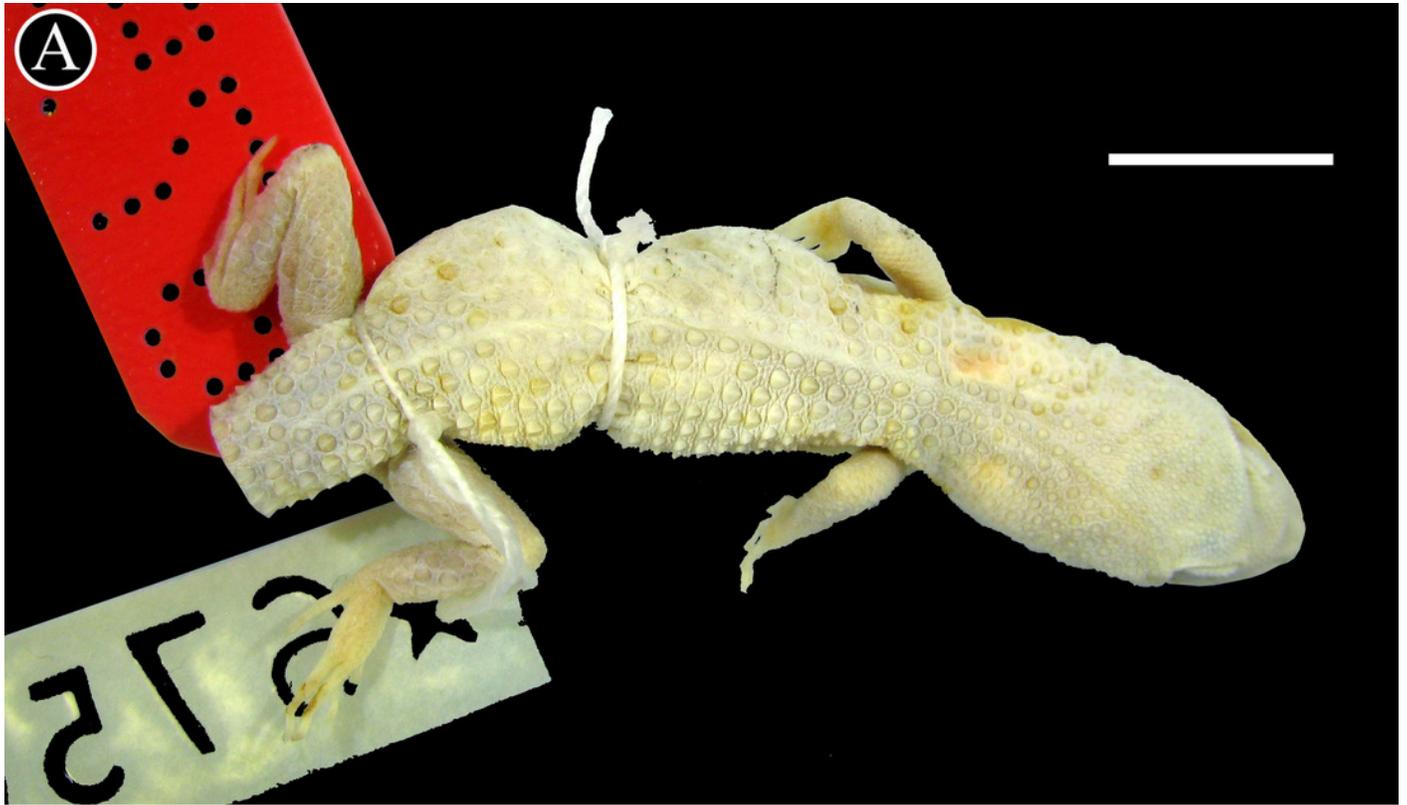



Figure 6

Fig. 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.

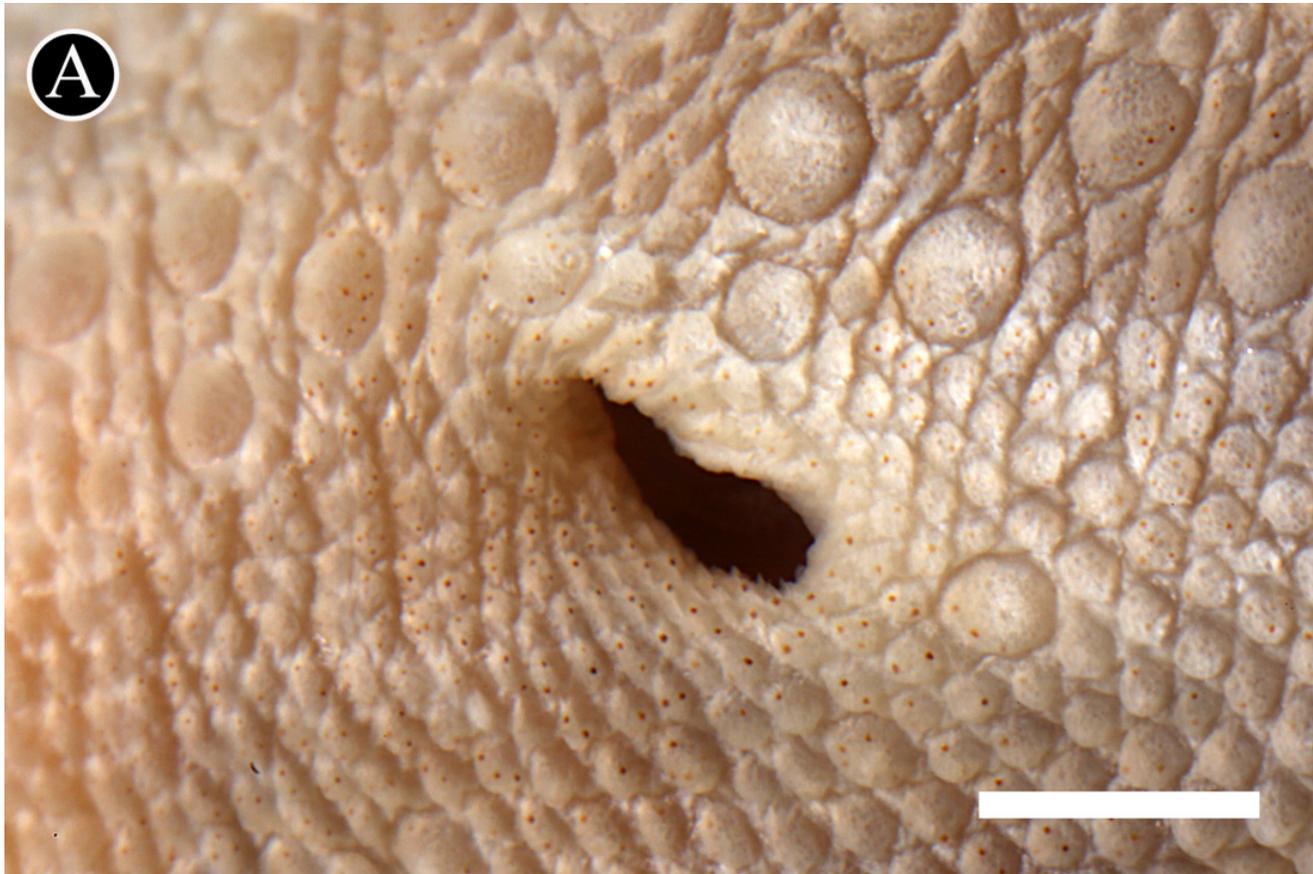



Figure 7

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.

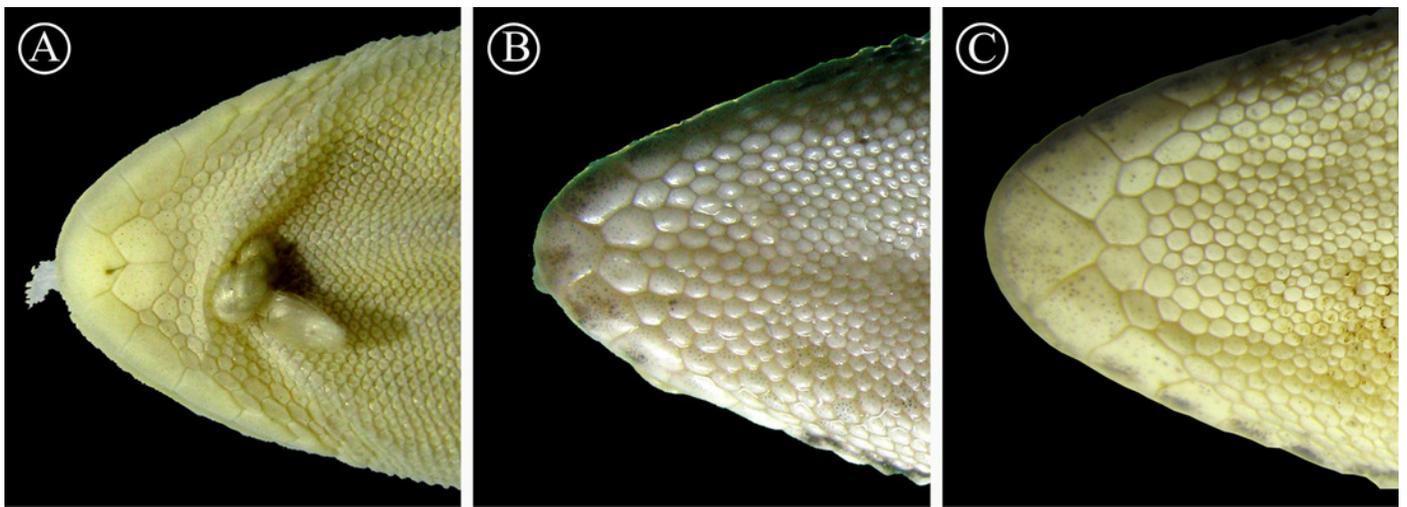



Figure 8

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.

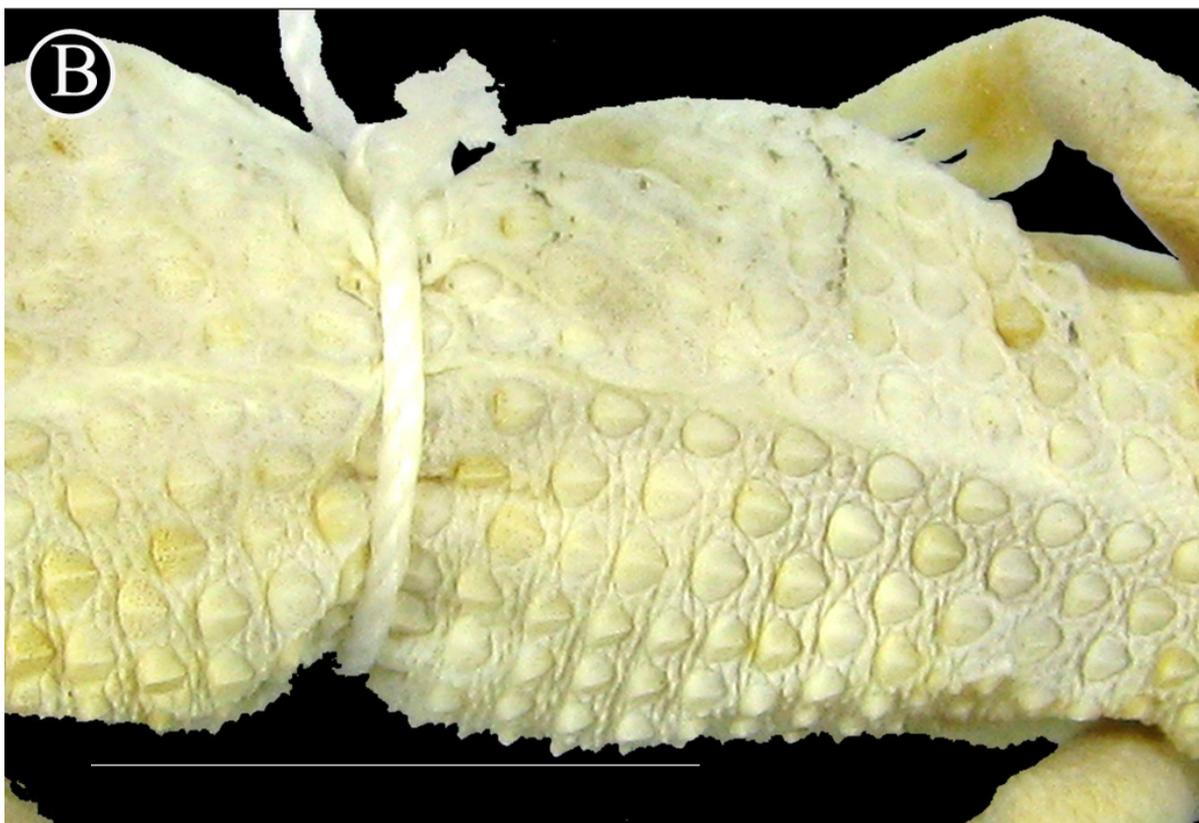
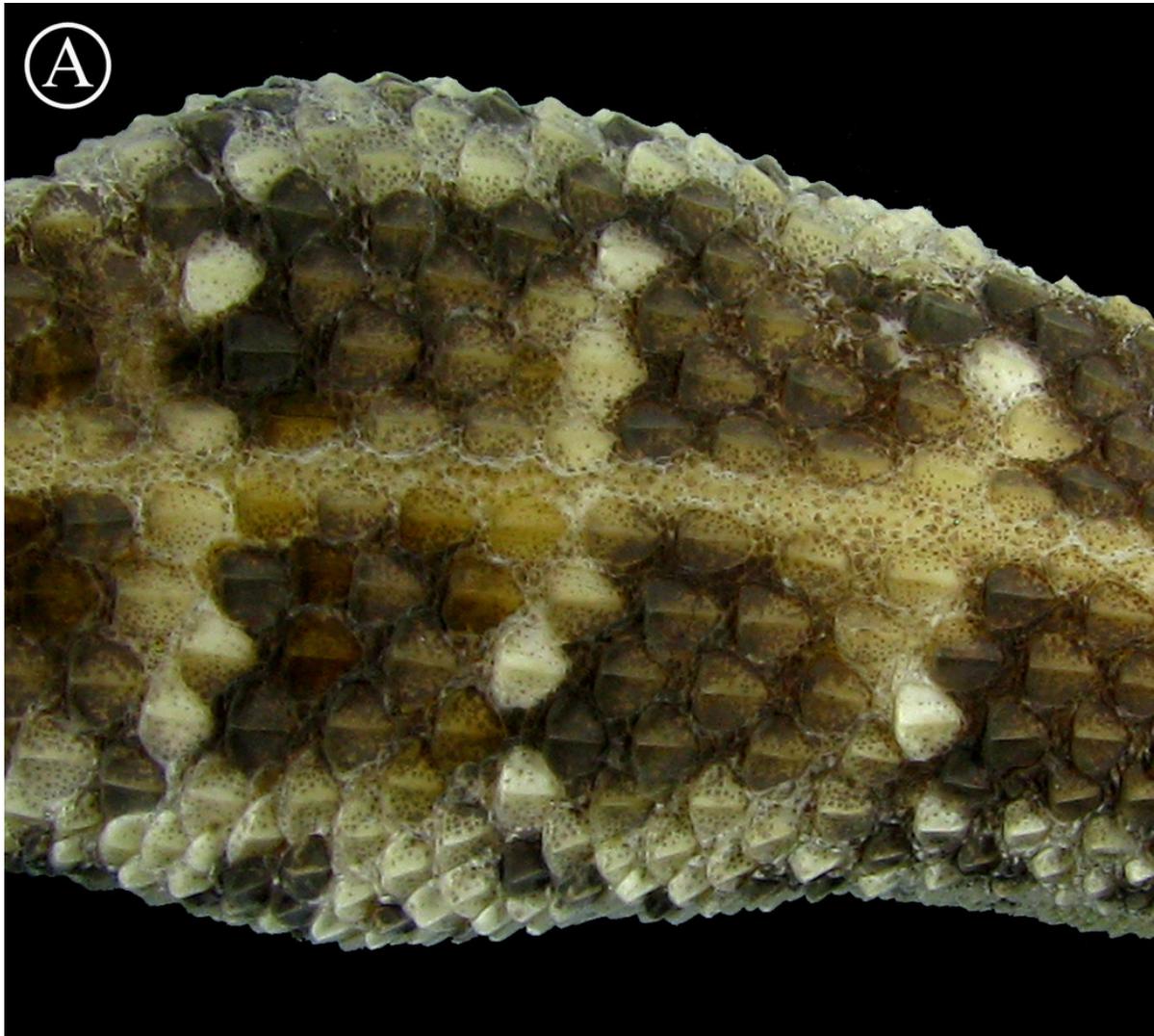



Figure 9

Lectotype of *Homonota horrida* (IZH-R 1).

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).

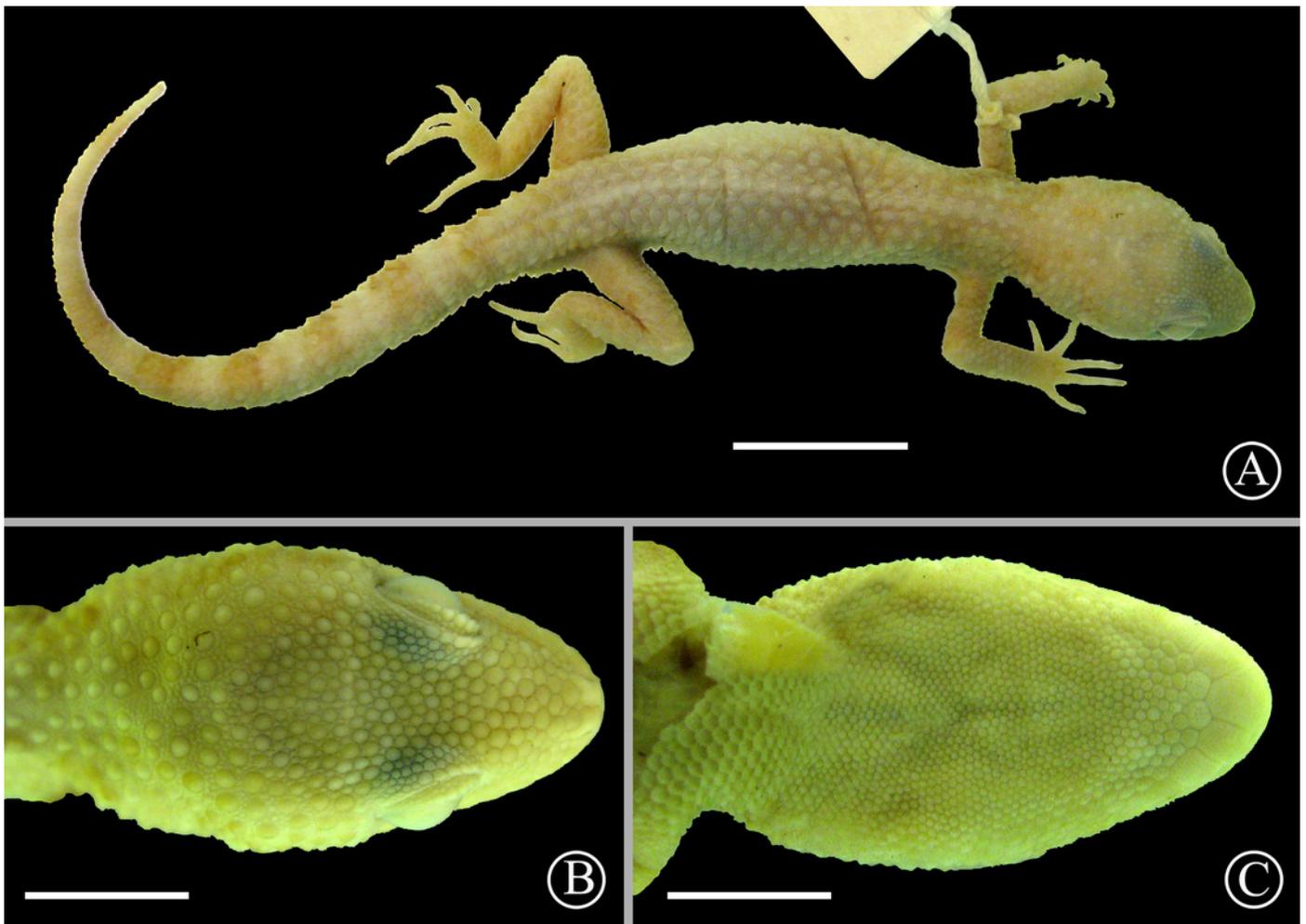



Figure 10

Holotype of *Homonota septentrionalis* (MNHNP 12238).

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

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

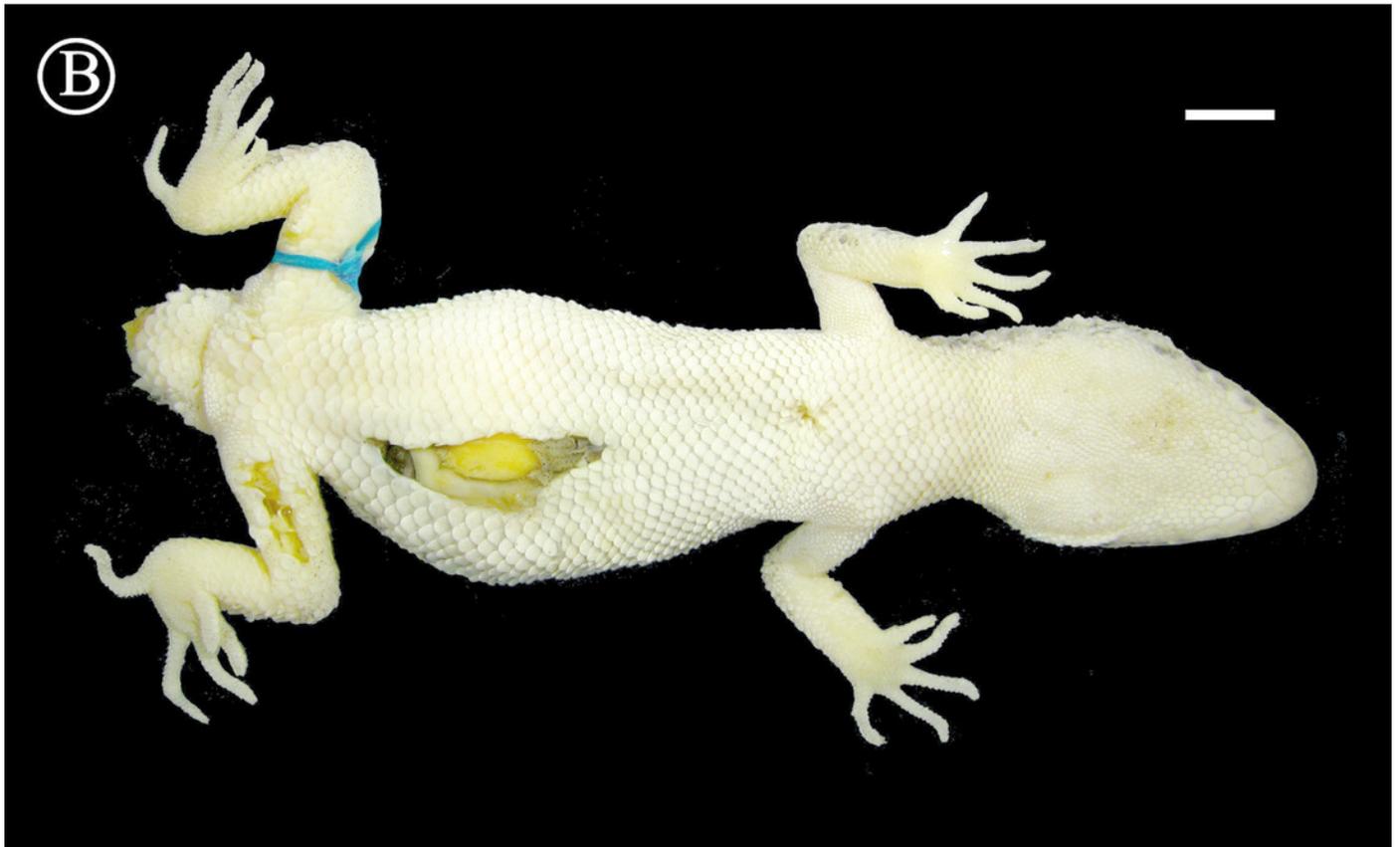
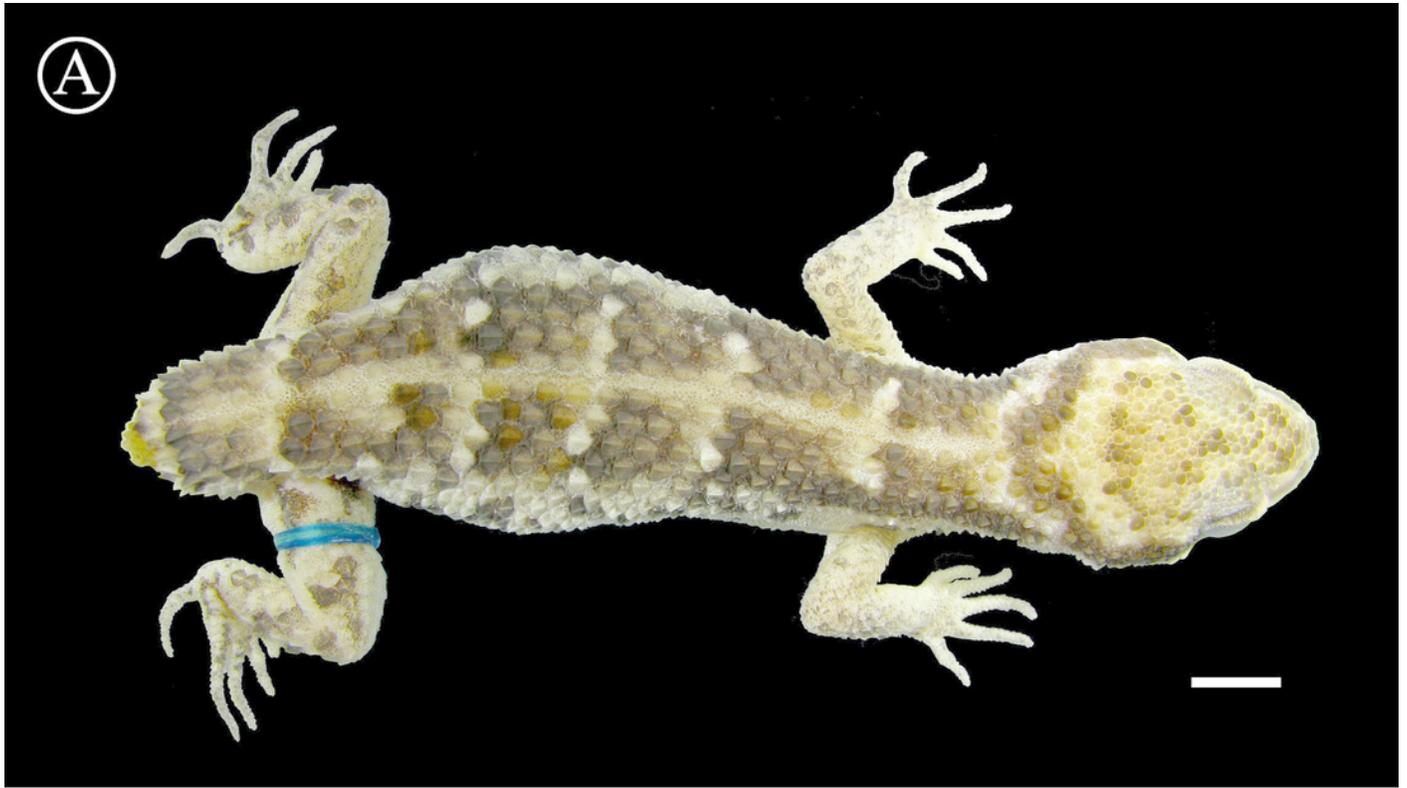



Figure 11

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, MNHNP 11855, 11406, 9131 from E to H respectively.

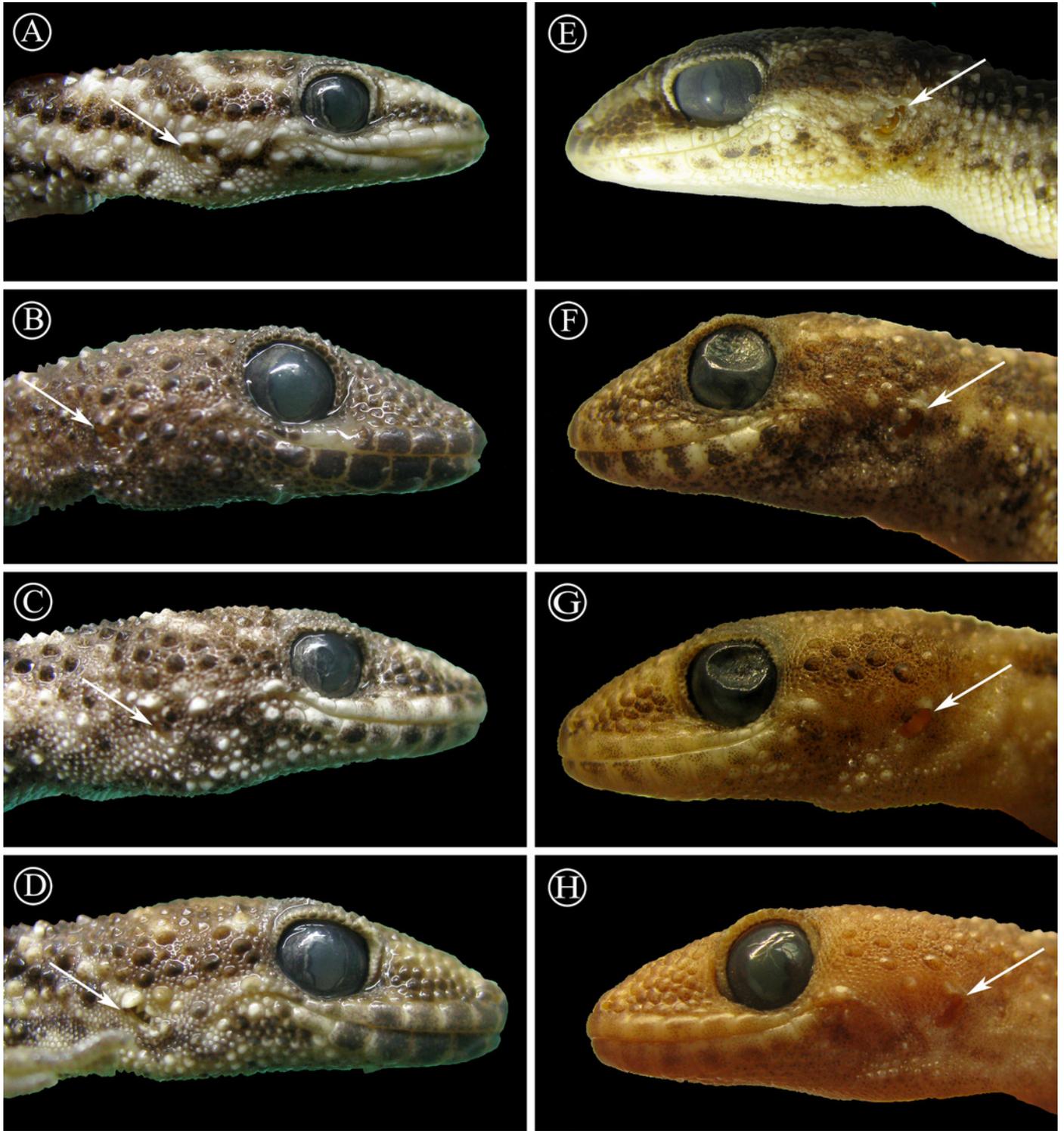
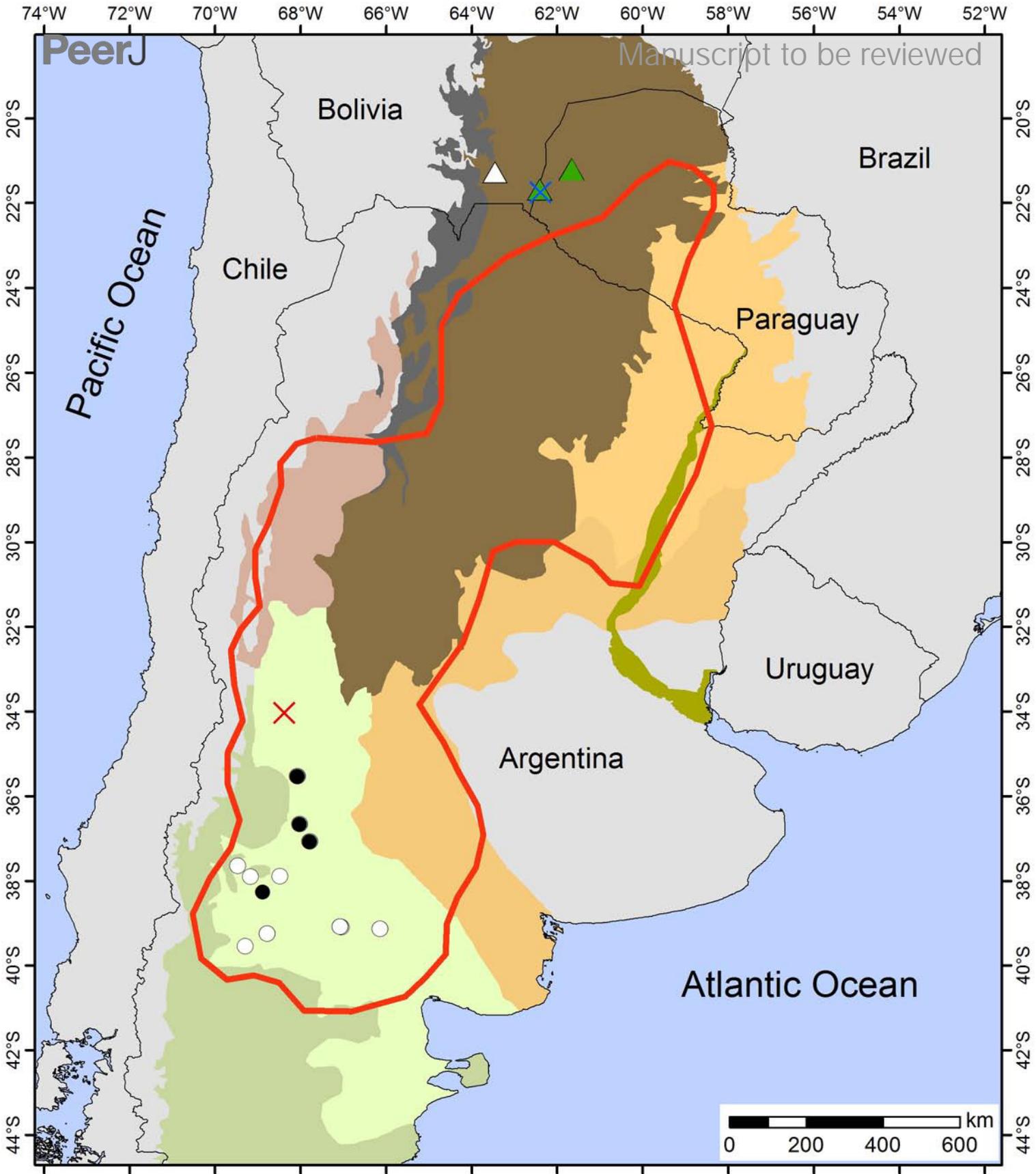



Fig. 12. Distribution of *Homonota septentrionalis*

Locality records of *Homonota septentrionalis* (triangles) highlighting localities of specimens used for genetic analyses (green triangles), and the distribution of *Homonota horrida* 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*.

Ecoregions

■ Dry Chaco	■ Low Monte	━━ Distribution of *H. horrida*
■ Espinal	■ Paraná Flooded Savanna	▢ Country borders
■ High Monte	■ Patagonian Steppe	
■ Humid Chaco	■ Southern Andean Yungas	

Figure 13

Fig. 13. Habitat of Homonota septentrionalis

Environmental characteristics of the type locality of *H. septentrionalis*.

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.

1   **Table 1**

2   Uncorrected pairwise genetic distances (in percentages) based on 16S mtDNA among samples of

3   Species A from Argentina (white cells) and Species B from Paraguay (gray cells) formerly

4   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	-

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.

1          **Table 2**

2  The 11 fixed sites differences on our 16S mtDNA alignment among three samples of Species A

3  from Argentina (Ar) and three of Species B from Paraguay (Pa), formerly referred as *Homonota*

4      *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

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.

1 **Table 3**

2 Normality Shapiro-Wilk (W) values for metric (above) and meristic (below) characters showing

3 the p value. Values shaded in gray did not reach normality. See Materials and Methods section

4 for reference to the acronyms.

	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

Continuous

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

Morphological differences

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

1 **Table 4**

2 Differences in morphological traits between MNHN 6756 (holotype of *Homonota fascia*) and

3 *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