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Molecular phylogeny of the New World gecko genus *Homonota* (Squamata: Phyllodactylidae)

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The genus *Homonota* was described by Gray (1845) and currently includes 10 species: *Homonota andicola*, *H. borellii*, *H. darwinii*, *H. fasciata*, *H. rupicola*, *H. taragui*, *H. underwoodi*, *H. uruguayensis*, *H. williamsii* & *H. whitii* and one subspecies of *H. darwinii* (*H. darwinii macrocephala*). It is distributed from 15° latitude south in southern Brazil, through much of Bolivia, Paraguay, Uruguay and Argentina to 54° south in Patagonia and across multiple different habitats. Several morphological taxonomic studies on a subset of these species have been published, but no molecular phylogenetic hypotheses are available for the genus. The objective of this study is to present a molecular phylogenetic hypothesis for all the described species in the genus. We sequenced two mitochondrial genes (cyt-*b* & 12S: 1745 bp), seven nuclear protein coding (RBMX, DMLX, NKTR, PLRL, SINCAIP, MXRA5, ACA4: 5804 bp) and two anonymous nuclear loci (30Hb, 19Hb: 1306 bp) and implemented traditional concatenated analyses (MP, ML, BI) as well as species-tree (*BEAST) approaches. All methods recovered almost the same topology. We recovered the genus *Homonota* as monophyletic with strong statistical support. Within *Homonota*, there are three strongly supported clades (*whitii*, *borellii* and *fasciata*), which differ from those previously proposed based on scale shape, osteology, myology and quantitative characters. Detailed morphological analyses based on this highly resolved and well-supported phylogeny will provide a framework for understanding morphological evolution and historical biogeography of this phenotypically conservative genus. We hypothesize that extensive marine transgressions during Middle and Late Miocene most probably isolated the ancestors of the three main clades in eastern Uruguay (*borellii* group), north-western Argentina-southern Bolivia (*fasciata* group), and central-western Argentina (*whitii* group). Phylogeographic and morphological/morphometric analyses coupled with paleo-niche modelling are needed to better understand its biogeographical history.

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Introduction

New World lizards of the genus *Homonota* occupy a unique position among Gekkonid lizards, as it ranges farther into the Southern Hemisphere than any other group (Kluge 1964). They are endemic to southern South America, occurring throughout the Monte, Chaco, Espinal, Patagonian, Andean, and Pampas biomes in Argentina, Bolivia, Brazil, Paraguay and Uruguay (Kluge 1964; Vanzolini 1968a,b; Peters & Donoso Barros 1970; Cei 1986, 1993; Abdala, 1998; Pérez *et al.* 2008), from 15°S to 54°S in

Patagonia. Almost all *Homonota* species are nocturnal and found in a variety of habitats ranging from rocky environments, sedimentary river cliffs, Mediterranean or coastal dunes, shrubby flatlands to urban areas; all known species are oviparous and insectivorous (Abdala 1997; Cei 1986, 1993). The genus contains 10 described species: *H. andicola* Cei 1978a; *H. borellii* (Peracca 1897); *H. darwinii* Boulenger 1885; *Homonota fasciata* (Duméril & Bibron 1836); *H. rupicola* Cacciali, Avila & Bauer 2007; *H. taragui* Cajade, Etchepare, Falcione, Barraso & Alvarez 2013; *H. uruguayensis* (Vaz-Ferreira & Sierra De Soriano 1961); *H. underwoodi* Kluge 1964; *H. whitii* Boulenger 1885; and *H. williamsii* Avila, Perez, Minoli & Morando 2012; and a still enigmatic subspecies of *H. darwinii* (*H. darwinii macrocephala*) described by Cei (1978b) from northern Argentina. Only *H. uruguayensis* and *H. rupicola* are not found in Argentina; the first one is distributed in Brazil and Uruguay and the second one in Paraguay.

Although the genus *Homonota* is the most diverse of the family Phyllodactylidae in terms of species numbers in Argentina, systematic, phylogenetic and ecological studies are scarce (Vaz-Ferreira & Sierra De Soriano 1961; Kluge 1964; Vaz-Ferreira & Sierra 1973; Cei 1978a,b,c; Gudynas & Gambarotta 1980; Abdala 1986, 1992, 1993, 1995, 1996, 1998; Gudynas 1986; Aun & Martori 1994; Abdala & Moro 1996; Piantoni *et al.* 2006), in comparison with other co-distributed lizard genera such as *Liolaemus* or *Phymaturus*.

Kluge (1964) published a review of the South American geckos and based on dorsal scale shape, proposed a character-based taxonomic hypothesis with three groups within *Homonota*: Group I: *H. borellii*, *H. uruguayensis*, *H. horrida*, and *H. fasciata*, all with a heterogeneous dorsal scale pattern that included long, keeled scales forming regular longitudinal rows, separated by small keeled or smooth scales; Group II: *H. darwinii*, *H. underwoodi*, *H. whitii*, and *H. gaudichaudii*, all with homogeneous smooth dorsal scales; and Group III: *H. dorbignii* with heterogeneous small dorsal scales that are either granular and elongated, lenticular, smooth and irregularly arranged. In 1978 Cei described a new species, *Homonota andicola* (1978a), and a new subspecies of *Homonota darwinii*, *H. d. macrocephala* (Cei 1978b), and based on Kluge's (1964) data, published a review (Cei 1978c) recognizing two natural groups based on longitudinal rows of dorsal scales, inferior caudal scales, and rostral scales and rostral groove: (i) *Homonota horrida* (Abdala & Lavilla, 1993 synonymized *H. horrida* with *H. fasciata*), *H. uruguayensis* and *H. borellii* with rows of keeled dorsal scales; and (ii) *H. darwinii darwinii*, *H. darwini macrocephala*, *H. underwoodi*, *H. andicola*, *H. whitii*, all with smooth or slightly keeled rows of dorsal scales.

Abdala (1992) used cranial characteristics of six species to construct a phenogram, which also separated the genus in two groups (*H. horrida*, *H. borellii*, *H. whitii*, and *H. darwinii*, *H. uruguayensis*, *H. underwoodi*), but found that cranial variation is not conclusive unless a large series was studied from each species. The following year, Abdala (1993) presented a phenetic analysis based on 30 quantitative continuous characters, concluding that *H. horrida* and *H. uruguayensis* form a different group from the remaining species. Kluge (1993) published a species list of the world geckos and included 10 species within *Homonota* (*H. andicola*, *H. borellii*, *H. darwinii*, *H. fasciata*, *H. gaudichaudii*, *H. horrida*, *H. penai*, *H. underwoodi*, *H. uruguayensis* and *H. whitii*); he considered the genus *Garthia* as a synonym of *Homonota*. Abdala (1996) completed cladistic analyses of cranial characters and proposed that *Garthia* is a more derived clade than the monophyletic *Homonota*. In the same year, Abdala and Moro (1996) published a cladistic analysis of 20 myological characters of South American geckos, and found *Homonota* to be closely related to *Phyllodactylus* and *Hemidactylus*, and they recoved *Gymnodactylus geckonoides*, *Garthia gaudichaudii* and *G. penai* as the sister clade of *Homonota*. In a later study, Abdala (1998) studied 25 qualitative, meristic and osteological characters and concluded that *Garthia* is a different taxon than *Homonota*. Abdala also concluded that *Homonota darwinii* is the most basal species of the genus and that *H. underwoodi* is the sister taxon of (*H. fasciata* + *H. uruguayensis*), but with no resolution for *H. andicola*, *H. whitii* and *H. borellii*. More recently, Cacciali *et al.* (2007) described a new species for Paraguay, *Homonota rupicola*, and constructed a dichotomous key for all the described species of the genus. Gamble *et al.* (2011a) published a molecular phylogeny for New World geckos based on five nuclear genes and included two species of *Homonota* (*H. darwinii* and *H. fasciata*); this study recovered *Phyllodactylus* as the sister genus and also confirmed Abdala and Moro (1996) in resolving *Garthia* as a separate genus, and placing the genera *Phyllopezus*, and *Gymnodactylus* as phylogenetically closely related to *Homonota*.

All of these previous studies (Kluge 1964; Cei 1978a,b,c; Abdala 1992, 1993, 1996, 1998; Abdala & Moro 1996) included different subsets of species and different classes of morphological data (cranial, myological, meristic and qualitative characters), while no inclusive study has been published for this genus based on molecular data. The objective of this work is to provide a molecular phylogeny for all recognized species of *Homonota* based on a multilocus data set and analysed with multiple tree-construction methods based on different assumptions.

Materials and methods

Taxon sampling

The genus *Homonota* includes 10 recognized species; eight type localities are located in Argentina, one in Uruguay

and one in Paraguay. The majority of the samples were obtained from the LJAMM-CNP tissue collection. Samples of five species are from type localities and for the other five species, we record the locality distance (straight line) from their respective type localities: (i) *H. whittii*, 90 km north-east; (ii) *H. underwoodi*, 140 km south-west; (iii) *H. uruguayensis*, samples from Brazil located 120 km north-east from the type locality in Uruguay; (iv) *H. fasciata*: 300 km south; (v) *H. borellii*, 540 km south-east (Fig. 1, TL: type localities, arrows: sample localities; Table 1).

To test monophyly of the genus, we selected four taxa from other related genera (Gamble *et al.* 2011a) as out groups, including *Garthia gaudichaudii*, *Phyllodactylus kofordi*, *Gymnodactylus geckoides*, *Phyllopezus pollicaris przewalskyi* and *P.p. pollicaris*. We rooted all trees using *Phyllopezus* and *Phyllopezus* + *Gymnodactylus*. This rooting scheme permitted us to test the monophyly of *Homonota* with respect to *Garthia* and *Phyllodactylus*. We used a total of 37 individual lizards (31 ingroup + 6 outgroup terminals). Details of the specimens and localities are summarized in Table 1 and Appendix S1.

Each species was represented by two individuals collected at the same locality for the majority of taxa; exceptions are *Phyllopezus pollicaris przewalkyi*, *P. p. pollicaris* and *Phyllodactylus kofordi* for which we included one individual for each taxon. For a few genes for which some individuals did not give a good PCR product, we selected other individuals from the same species and the same or a nearby locality (see details in Appendix S1).

Gene sampling

We collected sequences from two mitochondrial gene fragments: 1-cytochrome *b*, using the light-strand primers GluDGL (Palumbi 1996) and the heavy-strand primer Cytb 2 and Cytb 3 (Palumbi 1996), and the Cyt.F.1 primers (Whiting *et al.* 2003) were used as internal sequencing primers; 2-12S, using the primers of Wiens *et al.* (1999). Mitochondrial PCR conditions followed Morando *et al.* (2003). We also sequenced nine nuclear genes, seven of which are protein-coding gene loci (NPCL), including: (i) alpha-cardiac actin intron 4 (ACA4, Waltari & Edwards 2002); (ii) dmX-like protein 1 (DMXL1) (Gamble unpublished data; Werneck *et al.* 2012); (iii) encoding matrix remodelling associated intron 5 (MXRA5, Portik *et al.* 2011); (iv) natural killer-tumour recognition sequence (NKTR, Townsend *et al.* 2011); (v) prolactin receptor (PRLR, Townsend *et al.* 2008); (vi) intron 8 and flanking exon regions of RNA binding motif protein (RBMX, Gamble *et al.* 2011b); and (vii) synuclein alpha interacting protein (SNCAIP, Townsend *et al.* 2008). Two nuclear markers are from a non-published set of Anonymous Nuclear Loci (ANL) (primers for loci H30b and H19) developed by one of the authors from a genomic library.

To develop the ANLs, we assembled a genomic library from one individual of *Homonota* following the general protocol of Noonan & Yoder (2009) with small modifications (detailed in Morando *et al.* 2013). We verified a subset of amplified fragments via Blast search on GenBank with

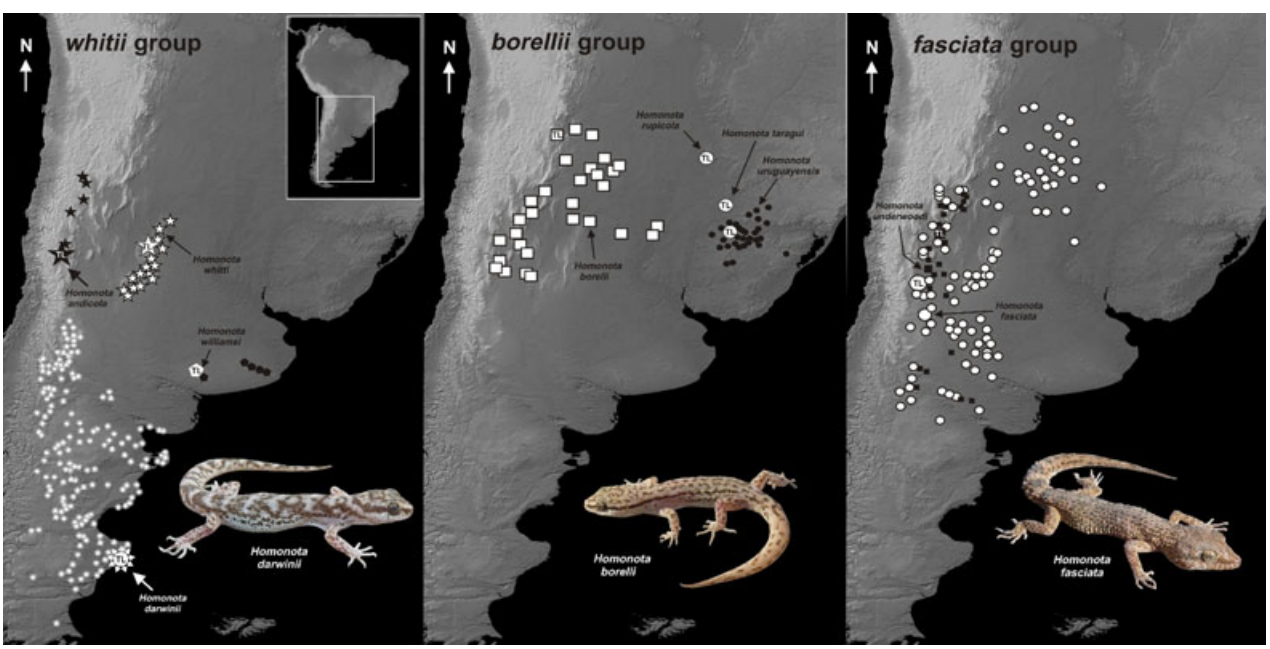

Fig. 1 Distribution maps of the 10 *Homonota* species. TL: type localities, arrows: sample localities.

Table 1 *Homonota* species with original description information, type locality, sampled locality and voucher numbers (LJAMM-CNP: Herpetological collection at CENPAT, UFRG: Universidad Federal do Rio Grande do Sul, LG: Laboratory of Cytogenetics of Vetebrates, IBUSP, Sao Paulo, Brazil; CHUNB: Coleção Herpetológica da Universidade de Brasília; MNHNPA: Colección Herpetológica del Museo Nacional de Historia Natural del Paraguay; TG: Tony Gamble Field collection, IBE: Institute of Evolutionary Biology, Barcelona, Spain)

Species	Author (year)	Type locality	Sample locality	Voucher numbers
H. andicola	Cei (1978a)	Argentina. Mendoza. 40 km N Uspallata, 2300 m.	Mendoza. Las Heras. National Road 149 (Provincial 39) from Uspallata to Barreal, 3 km S limit between Mendoza & San Juan.	LJAMM-CNP 12493–12495
H. borellii	Peracca (1897)	Argentina. Salta. Salta.	Santiago del Estero. Ojo de Agua. Camino from Villa Ojo de Agua to Lomitas Blancas, Sierra de Ambargasta.	LJAMM-CNP 12116–12125
H. darwinii	Boulenger (1885)	Argentina. Santa Cruz. Puerto Deseado (Port Desire), approximately latitude 48°S	Santa Cruz. Deseado. National Road 3, 46 km S Caleta Olivia	LJAMM-CNP 11424–9266
H. darwinii macrocephala	Cei (1978b)	Argentina. Salta. El Quebrachal.	–	–
H. fasciata	Duméril & Bibron (1836)	Argentina. Mendoza. In den Schluchten der Sierra bei Challao	Mendoza. San Rafael. 190, 1 km S Punta del Agua.	LJAMM-CNP 10577–5047
H. rupicola	Cacciali *et al.* (2007)	Paraguay. Cordillera. Compañía Los Naranjos, cerro El Pedregal, Piribebuy	Paraguay. Cordillera. Compañía Los Naranjos, Co. El Pedregal, Piribebuy	MNHNPA H_rupi_1, H_rupi_2
H. underwoodi	Kluge (1964)	Argentina. San Juan. Valle Fértil. Agua de la Pena, Hoyada de Ischigualasto, 82 km north-west of San Agustín de Valle Fértil	San Juan. Albardon. Baños del Salado, 5 km N Baños de la Laja	LJAMM-CNP 10923–10931
H. uruguayensis	Vaz-Ferreira & Sierra De Soriano (1961)	Uruguay. Artigas. Arroyo de la Invernada	Brazil. Rio Grande do Sul, Rosário do Sul.	UFRGS 1568–2139
H. whitii	Boulenger (1885)	Argentina. Córdoba. Cosquín	Córdoba. Tulumba. Provincial Road 18, 9.5 km N Ruta Provincial 16.	LJAMM-CNP 14387–14388
H. williamsii	Avila *et al.* (2012)	Argentina. Buenos Aires. Sierra de la Ventana	Buenos Aires. Tornquist. Parque Provincial Ernesto Tornquist	LJAMM-CNP 4467–6517
Outgroups				
Phyllodactylus kofordi	Dixon & Huey (1070)	Peru, Lambayeque, Co de la Vieja, Motupe	Peru. Chongoyape, near lago Tinajones. Lambayeque	TG00266-TGPK7
Garthia gaudichaudii	Duméril & Bibron (1836)	Chile. Coquimbo Coquimbo	Chile.	IBE- G1 (1), G1 (2).
Phyllopezus pollicaris przewalkyi	Koslowsky (1895)	"Goyaz (Brasilien)" [Phyllopezus govazensis Peters 1877]	Argentina. Chaco. Fuerte Esperanza.	CHUNB 57388
Phyllopezus pollicaris pollicaris	Spix (1825)	"Goyaz (Brasilien)" [Phyllopezus govazensis Peters 1877]	Brazil. Ceará. Tianguá.	LJAMM-CNP12089
Gymnodactylus geckoides	Spix (1825)	Brazil. Bahia.	Brasil. Alagoas. Xingo. Brasil. Barra dos Coqueiros, SE	LG 911 LG 1050

megablast (highly similar sequences), and with more dissimilar sequences (discontiguous megablast) criteria. Those sequences that resulted in 'non significant similarity was found' output were considered as anonymous markers. Primers were developed for 15 loci that met ANL criteria in the searches, and these were tested on a subset of two *Homonota* species. Of the 15 loci tested, we chose two for this project (Homo19b_F: CCTAAGAAAAGAGAAGGCA ATTCA; Homo19b_R: TGCATGCTACTCAGATTCC TG; Homo30b_F: CAATCCAGTCGAAGGAAGGA; Homo30b_R: AAACTTGGTTGGGTGCAGAG) that met the following requirements: (i) they amplified without significant optimization; (ii) they were variable among the two test species; and (iii) they amplified/sequenced for the remaining samples.

Molecular data

Genomic DNA was extracted using the Quiagen® DNeasy® 96 Tissue Kit for animal tissues following the protocol provided by the manufacturer. Protocols for PCR and sequencing procedures follow Morando *et al.* (2003, 2004) for 12S and *cyt*-b. The seven NPCL were amplified with one of the following standard touchdown cycles: 94°C–2:45 min, 40X [94°C–15 s, 51°C or 57°C –20 s

(−0.1°C/cycle), 72°C–1 min], 72°C–1 min, final rest at 4°C (called 1-touch51 or 1-touch57); or 95°C–1:30 min, 10X [95°C–35 s, 63°C–35 s (−0.5°C/cycle), 72°C–1 min], 10X (95°C–35 s, 58°C–35 s, 72°C–1 min), 15X (95°C–35 s, 52°C–35 s, 72°C–1 min; final rest at 10°C; called ANL63). The touchdown cycle described by Noonan and Yoder (2009), with standard reaction conditions (per sample: 2 μL dNTPs (1.25 mM), 2 μL 5× Taq buffer, 1 μL each primer (10 μM), 1 μL MgCl (25 mM), and 0.1 μL Taq DNA polymerase (5 U/μL; Promega Corp., Madison, WI, USA); 14 μL total reaction volume) was used for the ANL genes.

Sequencing reactions used the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Inc, Carlsbad, CA, USA) in a GeneAmp PCR 9700 thermal cycler (Applied Biosystems, Inc.). Sequencing products were cleaned with Sephadex G-50 Fine (GE Healthcare Bio-Sciencies AB, Piscataway, NJ, USA) and sequenced in an ABI 3730xl DNA Analyzer (Applied Biosystems, Inc.) at the BYU DNA Sequencing Centre. All sequences were edited using the program SEQUENCHER v4.8 (™Gene Codes Corporation Inc. 2007, Ann Arbor, MI, USA), and as for most genes the alignment was straightforward, we only used MAFFT (Katoh & Standley 2013) for alignment of the mitochondrial fragment 12S and the nuclear gene ACA4. We confirmed open reading frames in all protein-coding genes by translation into amino acids. Missing data in all cases were coded as '?', and sequences are deposited in GenBank (Accession Nos. in Appendix S1).

Gene tree and species-tree analyses

The best-fit evolutionary model for each gene (cyt-*b* [794 bp]: TRN+I+G; 12S [951 bp]: GTR+G; MXRA5 [961 bp]: TPM1μf+G, NKTR [1074 bp]: TRN+G, SINCAIP [449 bp]: TPM2 μf+G, RBMX [600 bp]: HKY+G, DMXL [959 bp]: HKY+G, ACA4 [1218 bp]: HKY+G, PLRL [543 bp]: TRN+G, Homo_30b [664 bp]: TIM1 + G, Homo_19b [642 bp]: HKY+G,) was selected using the corrected Akaike information criterion in JMODELTEST v0.1.1 (Posada 2008). For all nuclear genes, recombination was tested and excluded using RDP: Recombination Detection Program v3.44 (Martin & Rybicki 2000; Heath *et al.* 2006). Separate Bayesian analyses (BI) were conducted for each gene using MRBAYES v3.1.2 (Ronquist & Huelsenbeck 2003). Each analysis used four heated Markov chains (using default heating values) and run for one million generations. The equilibrium samples (after 25% of burn-in) were used to generate a 50% majority-rule consensus tree, and posterior probabilities (PP) were considered significant when ≥0.95 (Huelsenbeck & Ronquist 2001). Maximum Parsimony (MP) analyses were performed for each gene with PAUP v4.0b4b (Swofford 2001), based on a traditional heuristic search with 1000 replicates. Maximum Likelihood (ML) analyses for each gene were performed with RAXML v7.0.4 (Stamatakis 2006), based on 1,000 rapid bootstrap analyses for the best ML tree.

We performed concatenated analyses with MP, ML and BI for the following datasets: 1-two mitochondrial genes combined, 2-nine nuclear genes combined, 3-all genes combined. Parsimony analyses were conducted using PAUP v4.0b4b (Swofford 2001) to run a traditional heuristic search with 10 000 replicates and 10 000 bootstrap pseudoreplicates (Felsenstein 1985); strong nodal support being inferred for bootstrap values ≥70 (Hillis & Bull 1993; with caveats). Likelihood analyses were conducted using RAXML v7.0.4 (Stamatakis 2006), based on 1000 rapid bootstrap analyses. Bayesian analyses were conducted using MRBAYES v3.1.2 (Ronquist & Huelsenbeck 2003), with four heated Markov chains (using default heating values) and run for five million generations for (i) combined mtDNA, 10 million generations, for (ii) combined nuDNA and 50 million generations, and for (iii) all genes combined, with Markov chains sampled at intervals of 1000 generations. The equilibrium samples (after 25% of burn-in) were used to generate a 50% majority-rule consensus tree, and posterior probabilities (PP) were considered significant when ≥0.95 (Huelsenbeck & Ronquist 2001).

To reconstruct a species tree incorporating the multispecies coalescent approach, we used the hierarchical Bayesian model implemented in *BEAST v1.6.0 (Drummond & Rambaut 2007). Species were identified on the basis of published morphological characters, in combination with their geographic distributions (sampling localities). Two separate analyses were run for 100 million generations (sampling every 1000 generations) for all genes and for only nuclear genes. We considered clades with PP > 0.95 to be strongly supported; however, we are aware that the relationship between PP from *BEAST and the probability of a species-tree clade being correctly reconstructed remains under-explored. To estimate support for branches for a gene tree or for a concatenated tree is different than estimating support for branches for a species tree, as this last one is principally related to the number of genes presenting a particular relationship in a tree node (Liu *et al.* 2008). Also, concatenation assumes that all gene regions have evolved along a single evolutionary history (Degnan & Rosenberg 2009), and when the genes have different evolutionary histories this assumption is violated. Under these conditions, such a violation may result in a well-supported but incorrect species tree (Degnan & Rosenberg 2009; Heled & Drummond 2010).

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

Bayesian MCMC phylogenetic analyses (MrBayes and *BEAST) by examining likelihood and parameter estimates over time in TRACER v1.5.0 (Rambaut & Drummond 2009). All parameters had effective sample sizes (ESS) greater than 200, and most were >300 upwards to over 15 000; thus, most runs had at least several hundred independent samples from the MCMC chains, a good indication that the analyses adequately sampled the posterior distributions.

Although there are few *Homonota* fossils available (Albino 2005; Albino & Brizuela 2014), they are from the late Quaternary, and their recent age makes them a poor choice for dating in this context, and thus, we estimated divergence times with an indirect calibration. We performed a likelihood ratio test (LRT) using JMODELTEST v0.1.1 (Guindon & Gascuel 2003; Posada 2008) to test for deviation from a strict molecular clock for each gene. Based on these results, we used a strict molecular clock model for all genes in a *BEAST v1.6.0 analysis (Drummond & Rambaut 2007) to estimate divergence times based on a species tree. We used a Yule prior and a normal prior on the global substitution rate to calibrate the estimation (mean = 0.0065 substitutions/my; SD = 0.0025 for the ucld.mean parameter) based on the mtDNA substitution rate of 0.65% changes/million years (Macey *et al.* 1998), a metric widely used in dating squamate phylogenies. Two independent analyses were performed for 100 million generations and sampled every 1000 generations. The ESS values for parameter convergence were checked using TRACER v1.5 (Rambaut & Drummond 2009).

Results

Phylogenetic hypotheses for all described *Homonota* species are depicted in the species tree recovered with *BEAST (Fig. 2A) and in the concatenated Bayesian tree (Fig. 2B), which are highly concordant across all methods implemented in this study. Stars on the species tree (Fig. 2A) denote nodes with posterior probabilities (ST-PP) and concatenated Bayesian posterior probabilities (IB-PP) >0.98, and ML- and MP-bootstrap support values >95% on the concatenated mitochondrial gene trees, concatenated nuclear gene trees and all genes concatenated tree (this last one shown on Fig. 2B) (with one exception on the concatenated mitochondrial tree: the sister relationship between *H. fasciata* and *H. underwoodii* had ML & MP boostrap support values <95%). Individual Bayesian gene trees are included in Supplemental Material, Appendix S2; also, in all nuclear single-gene trees results obtained with different methods (MP, ML) were topologically very similar, and none recovered any strongly supported conflicting nodes (results not shown). We obtained a well-resolved picture of phylogenetic relationships for *Homonota* species with two exceptions (see below).

Homonota is a strongly supported clade (Fig. 2), with *Phyllodactylus* as its sister genus, and based on concatenated results (Fig. 2B), *Garthia* is the sister genus to these two genera with high statistical support (PP = 1). Three main clades are recovered within *Homonota* with strong statistical support (Fig. 2) that we refer to as: (i) the *fasciata* group, including *H. fasciata* and *H. underwoodi*; (ii) the *borellii* group, with *H. borellii*, *H. taragui*, *H. rupicola* and *H. uruguayensis*; (iii) the *whitii* group, including *H. whitii*, *H. andicola*, *H. darwinii* and *H. williamsii*. Relationships between these three groups are moderately in conflict: in the species tree (Fig. 2A), the sister relationship between the *borellii* and the *fasciata* groups has a ST-PP = 0.91 support, while the only nuclear species tree (not shown) and concatenated nuclear gene tree and the all genes concatenated tree (Fig. 2B), recovered the *borelli* and *whittii* groups as sister clades with high support (nuclear ST-PP = 0.89, B-PP = 1 and ML-bootstrap = 97%); there was no support for any of these relationships in the mitochondrial-only concatenated tree. Within the *whitii* group, *H. andicola* is recovered as the sister species of *H. whitii* and *H. darwinii* as the sister species of *H. williamsii*, both highly supported (Fig. 2, stars in panel A and PP = 1 in panel B). *Homonota uruguayensis* is strongly supported as the most basal taxon within the *borellii* group (Fig. 2A, star, and PP = 1 in panel B). The sister relationship recovered in the species tree (Fig. 2A) between *H. taragui* and *H. borelli* had a high statistical support (ST-PP = 0.92, concatenated nuclear genes IB-PP = 1, concatenated nuclear genes ML boostrap = 0.97; white arrow), but this topology is incongruent with the concatenated mitochondrial tree recovered with all three methods (MP = 100%, ML = 100%, IB-PP = 1), and the concatenated all genes (MP = 100%, ML = 100%, IB-PP = 1); these analyses all recovered *H. taragui* as the sister taxon of *H. rupicola* (Fig. 2B, IB-PP = 1).

Although our estimated divergence times are not calibrated with a *Homonota* fossil, they give an approximate temporal window for the origin and diversification of this genus. Divergence date estimations with confidence intervals (grey horizontal bars on nodes) are depicted on Fig. 2. The first divergence on the genus is estimated at ~15 mya (million years ago), and the origins of the three main clades are between ~12 and 7 mya. Divergences within clades are estimated between ~7 and 2 mya, with the youngest ones within the *whitii* group.

Discussion

Our results present the first comprehensive molecular hypothesis for phylogenetic relationships for the genus *Homonota*, including all 10 described species. We used 10 independent markers (nine nuclear and two mitochondrial genes), to infer trees based on different approaches

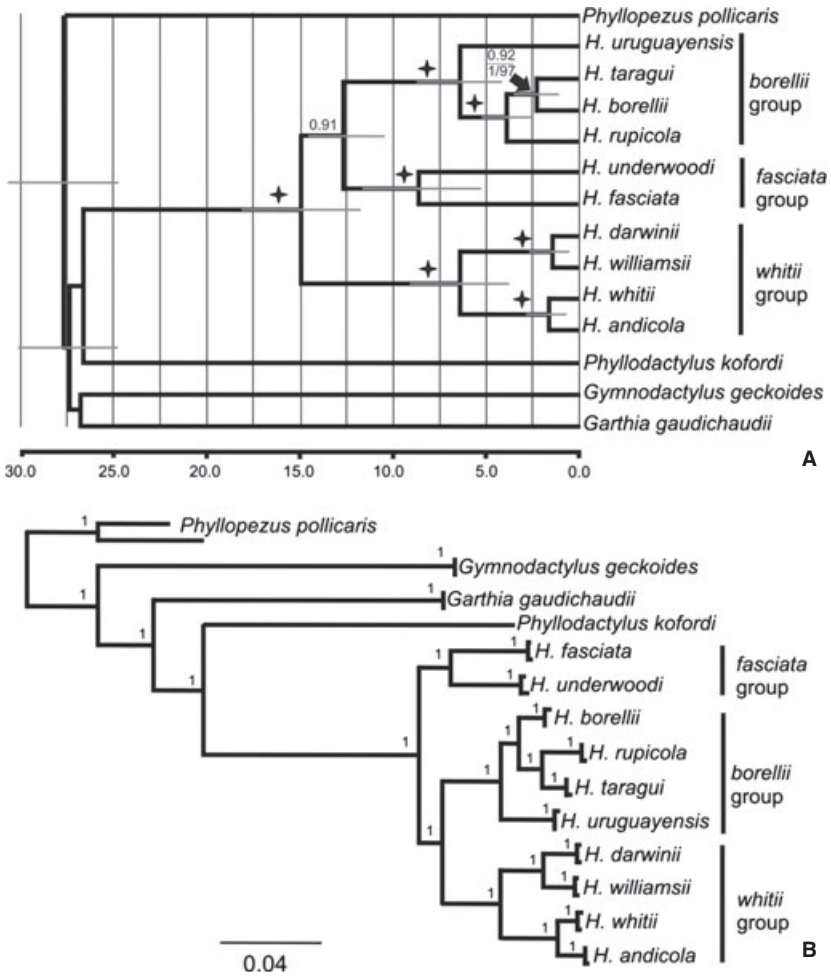

Fig. 2 Species tree inferred with *BEAST; scale corresponds to estimated divergence times in million years with grey reflecting confidence intervals. —A. Stars show nodes with strong support based on different datasets and analytical approaches (see text for details). Arrow: top number: species-tree posterior probability, bottom numbers: Bayesian concatenated nuclear genes posterior probability/ML concatenated nuclear genes bootstrap support (see text for details on alternative topology based on mitochondrial genome and concatenated all genes analyses). Node with 0.91: species-tree posterior probability value (see text for alternative moderately supported topology). —B. Multilocus concatenated Bayesian tree for *Homonota* species; numbers above branches represent posterior probabilities.

(concatenated with MP, ML, BI and *BEAST species trees) and we obtained largely concordant and well-resolved results.

Our best-supported phylogenetic hypothesis is concordant with previous morphological (Abdala & Moro 1996; Abdala 1998) and molecular studies (Gamble *et al.* 2011a) in recovering *Garthia* as a separate genus from *Homonota*, in opposition to Kluge (1964, 1993, 2001), who considered all species in these two genera as *Homonota*. Our species-tree hypothesis proposes *Phyllodactylus* as the sister genus of *Homonota*, which is congruent with the Abdala & Moro (1996) study based on 20 myological characters, and with the Gamble *et al.* (2011a) molecular study. The monophyly of *Homonota* is highly supported and within the genus three main well-supported clades are recognized: *borellii*, *fasciata* and *whitti* (Fig. 2).

Our *fasciata* group includes *H. fasciata* and *H. underwoodi*, the first species with a wide distribution in Chaco and Monte environments from northern Patagonia on a western-central longitudinal axis through Argentina to Bolivia and Paraguay. In contrast, *H. underwoodi* has a much narrower distribution in the Monte desert of central-western Argentina (Fig. 1). The *borellii* group includes four species, two of which were recently described (*H. rupicola* and *H. taragui*) and are only known from their type localities. This group includes the eastern-most distributed species of this genus, *H. uruguayensis*, which is recovered as the oldest species of this group, and *H. taragui*, as the sister species of *H. borellii*, the north-western-most distributed species of this group, while *H. rupicola* is the north-eastern-most distributed species and is the sister taxon of these last two. The *whitii* group includes

H. andicola + *H. whitii*, which have a central-western distribution in Argentina, and the Patagonian species *H. darwinii* as sister to *H. williamsii*, distributed along the north-eastern border of the Patagonian area (Fig. 1).

We found two nodes with conflicting results: our species-tree hypothesis including all genes recovers a sister relationship between the *borellii* and the *fasciata* groups with moderate support (ST-PP = 0.91, Fig. 2A), while the species tree based on only nuclear genes (not shown) and the concatenated analyses found high to moderate support for a sister relationship between the *borellii* and the *whittii* groups (ST-PP = 0.89, IB-PP: 1, Fig. 2B, ML-Boostrap: 93%, MP-Boostrap: 75%; results not shown). We suggest that more markers are needed to resolve these relationships. The second strongly supported incongruence is the sister relationship between *H. taragui* and *H. borellii*; this topology is highly supported with all nuclear genes and the species tree (Fig. 2A), but the mitochondrial genome of *H. taragui* is more closely related to *H. rupicola* (Appendix S2, also uncorrected pairwise cyt-b distance = 10.7%; *H. taragui* vs. *H. borellii* = 11.2%). The all-genes-concatenated tree resolves this same relationship, almost certainly because the mitochondrial signal may have been strong enough to override the phylogenetic signal of the nuclear genome (Fig. 2B). Three main processes can account for this pattern: (i) the random sorting of independent genes, especially if the diversification occurred over a short period of time; (ii) secondary contact, with mitochondrial introgression, which in this case could have occurred in the past; or (iii) a hybrid origin of the species. A detailed phylogeographic analyses with more nuclear markers, coupled with paleoniche modelling, is necessary to test these hypotheses.

The species composition of these three groups is not concordant with previous hypotheses proposed based on squamation (Kluge 1964; Cei 1978c), cranial (Abdala 1992) and other morphological characters (Abdala 1998). There are two non-exclusive reasons that can account for these incongruences: (i) previous studies were based on a subset of species, but most importantly; (ii) geckos in general are known for their highly conserved morphologies, which can obscure phylogenetic signal. In some cases, this will lead taxonomists to define widely distributed taxa based on certain morphological characters that later, based on new molecular evidence and better sampling, are shown to be symplesiomorphic or convergent (e.g. Bauer *et al.* 1997, 2013; Heinicke *et al.* 2014). In other cases, some gecko taxa, which were interpreted as single widely distributed species, have been shown by detailed molecular analyses to harbour extensive cryptic diversity (e.g. South America, Werneck *et al.* 2012; Europe and northern Africa, Rato *et al.* 2012; Africa, Leaché & Fujita 2010; Australia, Fujita *et al.* 2010; and Asia, Brown *et al.* 2012).

Our study suggests that some of the morphological characters used in earlier taxonomic studies of *Homonota* may include a combination of symplesiomorphic and convergent character states, with possibly none or only a few synapomorphies that could define each of the three main nodes (e.g. Kluge 1964; Abdala 1993, 1998). If true, then any signal from these characters would likely have been obscured by a higher number of homoplastic characters. In the last species description of this genus (Cajade *et al.* 2013), the authors remark '*H. taragui* seems to have morphological similarities with *H. borellii* and *H. rupicola*, on one hand, and with *H. uruguayensis* and *H. williamsii* on the other'. Thus, their observations are partly congruent with our molecular hypothesis but may also represent a pattern in which some squamation characters shared between species of the *borellii* group and *H. willimasii* (our *whittii* group) are either symplesiomorphic or convergent. A review of different types of morphological characters for all *Homonota* species, in the light of this well-supported molecular phylogeny, is needed to test for morphological synapomorphies that could define each of the three molecular well-resolved groups and to understand the broader morphological evolution of this genus.

Based on the species-tree estimation, the genus *Homonota* originated during Middle Miocene (~15 mya, Fig. 2; compared with approximately 20 mya estimated from Fig. 1 in Gamble *et al.* 2011a), and on average, the ancestors of the *borellii*, *fasciata* and *whitii* groups originated during the Late Miocene (~6–8 mya), with the oldest one corresponding to the *fasciata* group. During the Middle and Late Miocene (10–5 mya), there were at least two extensive Atlantic marine transgressions into low-lying basins of southern South America, known collectively as the Paranean, Paranaense or Paranan Sea, which extended over most of eastern Argentina, western Uruguay, southern Paraguay and south-eastern Bolivia (Fig. 3; Ramos & Alonso 1995; Pascual *et al.* 1996; Lundberg *et al.* 1998; Hernández *et al.* 2005; Ruskin *et al.* 2011). Multidisciplinary palaeoenvironmental studies have shown a strong marine Miocene impact on this part of South America (Lundberg *et al.* 1998; Hovikoski *et al.* 2010; Cooke *et al.* 2011). We hypothesize that this Paranean Sea could have isolated the ancestor of the *borellii* group on some emergent land in the east, in what is now the distribution area of *H. uruguayensis*, and this ancestor then later colonized northwestern areas (Fig. 3, number 1 in grey circle, light grey arrows). The ancestor of the *fasciata* group could have been isolated in emergent areas in southern Bolivia and northwestern Argentina (Fig. 3, number 2 in white circle) in the Sierras Subandinas and Cordillera Frontal: western areas of Salta Jujuy and Tucumán provinces in Argentina (Ruskin *et al.* 2011), and later dispersed to the south and east. The ances-

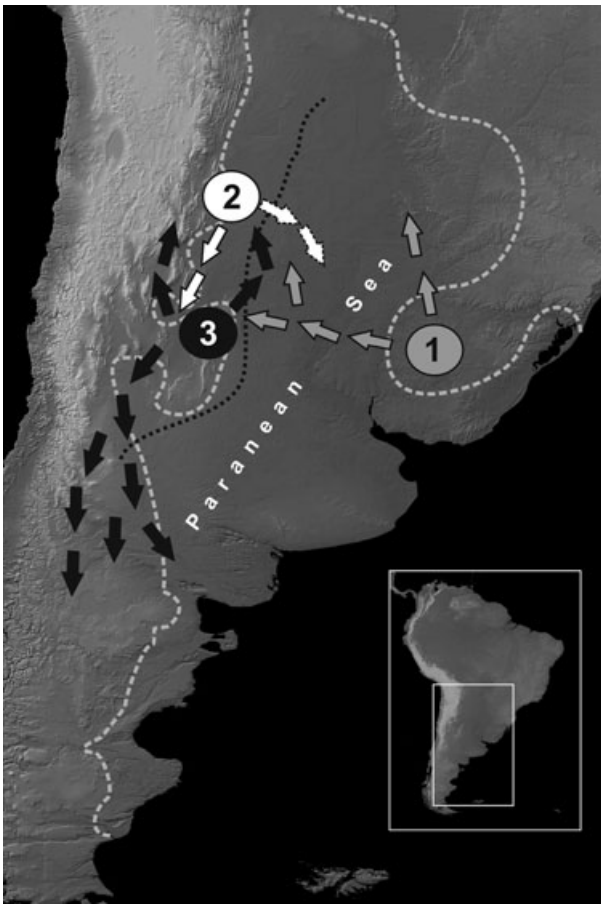

Fig. 3 Map of southern South America illustrating interpreted distribution of Miocene Paraná seaway (grey dash line, after Ramos & Alonso 1995; Hernández *et al.* 2005; black dot line, after Ruskin *et al.* 2011). (i) Emerged land where the ancestor of the *borellii* group could have been isolated; (ii) Emerged land where the ancestor of the *fasciata* group could have been isolated; (iii) Emerged land where the ancestor of the *whittii* group could have been isolated. Arrows reflect hypothesis of posterior distributions from emerged areas.

tor of the *whittii* group could have persisted on another emergent land mass in western-central Argentina (Fig. 3, number 3 in black circle), in the Sierras Pampeanas, in northern San Luis, western Córdoba, south-eastern Catamarca and La Rioja, southern Tucumán and south-western Santiago del Estero provinces (Ruskin *et al.* 2011), and dispersed to the north (*H. andicola* and other *H. whittii* populations) and to the south (*H. darwinii* and *H. williamsii*).

During the mid-Miocene (17–14 mya), the Southern Patagonian Andes experienced significant surface uplift, and major uplifts continued until approximately ~10 Ma, forming a barrier to Pacific winds and the concomitant desertification of the eastern Patagonia (Blisniuk *et al.* 2005, 2006; Folguera *et al.* 2011). Thus, occupation of the Patagonian steppes by the ancestor of *H. darwinii* and *H. williamsii* most probably occurred in the Pliocene, when this biome would have been available. The multiple late Miocene (~6 Ma; Rabassa *et al.* 2011) glaciation cycles that occurred in Patagonia most certainly imposed different environmental pressures on populations across the wide latitudinal range of *H. darwinii* (Fig. 1), and thermal physiological evidence (Weeks & Espinoza 2013) suggests that physiological adaptations may have allowed this species to expand its range into highly variable climates. Half of the described *Homonota* species have restricted distributions, and the majority of the type localities of the 10 described species are distantly located from each other. The extensive distribution of the genus and its Miocene–Pliocene divergence history make it a very interesting candidate clade upon which to develop general biogeographic scenarios for southern South America, but more dense sampling over much of the distribution, coupled with additional data and analyses, are needed to generate more detailed biogeographic hypotheses.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Voucher individuals used for sequencing each gene with GenBank accession numbers.

Appendix S2. Individual Bayesian gene trees.