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An integrative approach to elucidate the taxonomic status of five species of *Phymaturus* Gravenhorst, 1837 (Squamata: Liolaemidae) from northwestern Patagonia, Argentina

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The lizard genus *Phymaturus* comprises two reciprocally monophyletic clades: *Phymaturus palluma* and *Phymaturus patagonicus*. Species belonging to the *P. patagonicus* clade occur in extra-Andean Patagonia, and some of them remain with unresolved phylogenetic relationships after studies based on morphological and nuclear and mitochondrial DNA (mtDNA) evidence. Here, we elucidate the taxonomic status of five putative species originally described according to morphotypes based upon morphometric and meristic characteristics and dorsal colour pattern: *Phymaturus agilis*, *Phymaturus excelsus*, *Phymaturus manuelae*, *Phymaturus spectabilis* and *Phymaturus spurcus*. We amplified a 657 bp fragment of *COI* mtDNA from 23 individuals and a 2708 bp fragment of cytochrome *b*, *ND1*, *ND2* and eight transfer RNAs from another 114 individuals. We found strong support for two reciprocally monophyletic genetic lineages: a small clade for *P. manuelae* and a large polytomic clade for the rest of the presumed species. A haplotype network analysis unveiled a genetic structure for lineages in the large clade, suggesting that morphological differences are a consequence of population divergence. To complement the molecular analyses, we estimated morphotype proportions in populations and estimated morph heritability with the regression of offspring on females bearing species-typical phenotypes. Our results indicate that *P. agilis*, *P. excelsus*, *P. spectabilis* and *P. spurcus* compose a single highly structured species.

ADDITIONAL KEYWORDS: Bayesian phylogenetic inference – haplotype networks – heritability – morphotypes – *Phymaturus agilis* – *Phymaturus excelsus* – *Phymaturus manuelae* – *Phymaturus patagonicus* – *Phymaturus spectabilis* – *Phymaturus spurcus*.

INTRODUCTION

Species are generally considered as separately evolving metapopulation lineages (de Queiroz, 2007). However, in instances in which populations are close to or have experienced recent divergence, it is difficult to state their limits owing to subtle differences in morphology, coloration or melanism pattern, incomplete reproductive isolation or incomplete lineage sorting (Maddison & Knowles, 2006; de Queiroz, 2007). Different methods and species definitions may yield different outcomes when applied to one set of taxa (Camargo *et al.*, 2010). The application of a morphologically based method as the sole criterion may induce misinterpretations of

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population variability in two ways: by not recognizing morphologically similar cryptic species (Bickford *et al.*, 2007), or by ascribing genuine intraspecific variation to more than one species. Genetic distance-based methods, although seen as practical tools focusing only on the degree of genetic divergence among taxa, also have limited predictive use for identifying or delimiting species (Will & Rubinoff, 2004). Therefore, in the presence of highly variable morphological characters in closely related populations, an integrative approach to taxonomy must be undertaken (Raxworthy *et al.*, 2007) and, if practical, the inclusion of genetic crosses between incipient taxa to reveal hybrid fitness (Corl *et al.*, 2012).

The lizard genus *Phymaturus* presents several challenges to species delimitation. The genus comprises Andean and extra-Andean Patagonian viviparous, saxicolous and mostly herbivorous lizards of Argentina and Chile, occupying rocky outcrops principally of volcanic origin (Cei, 1986, 1993). The use of rocky habitats seems to have a profound effect on the morphological variation of these lizards, characterized by robust and flattened bodies that allow them to hide in small crevices (Cei, 1986; Scolaro, 2005, 2006). Two groups were clearly differentiated by morphological characteristics (Cei, 1986; Etheridge, 1995; Lobo & Quinteros, 2005a), and further studies confirmed their reciprocal monophyly based on molecular characters (Espinoza *et al.*, 2004; Morando, 2004, 2013): the *P. palluma* clade in the north (between 25 and 39°S) and the *P. patagonicus* clade in the south (between 36 and 46°S), the latter being restricted to the extra-Andean Patagonian steppe. There are currently 49 *Phymaturus* species recognized: 23 in the *P. palluma* clade (Corbalán *et al.*, 2016) and 26 in the *P. patagonicus* clade (Marín *et al.*, 2016; Scolaro *et al.*, 2016), with four and five subclades proposed for each, respectively (Morando *et al.*, 2013).

The *P. spurcus* subclade within the *P. patagonicus* clade includes five putative morphospecies (Fig. 1) endemic to northwestern Patagonia that have the same ecological requirements. *Phymaturus manuelae* Scolaro & Ibargüengoytía, 2008 was described from one isolated population, having a notorious polychromatism not seen in other species of the group (Scolaro & Ibargüengoytía, 2008). The remaining four putative species are found in a dozen rocky outcrops over a relatively small area (~1000 km^2), where the distance between any two populations does not exceed 36 km (Fig. 2). *Phymaturus spurcus* Barbour, 1921 was the first to be described from Estancia Huanuluan (Barbour, 1921) and revalidated later by Lobo & Quinteros (2005b). Subsequently, south of this locality, *Phymaturus spectabilis* Lobo & Quinteros, 2005, *Phymaturus excelsus* Lobo & Quinteros, 2005 and *Phymaturus agilis* Scolaro, Ibargüengoytía & Pincheira-Donoso, 2008 were described, the latter living in syntopy with *P. spectabilis* (Scolaro *et al.*, 2008).

With the exception of *P. manuelae*, the taxonomic status of the other species has been questioned based on a record of a pregnant female of *P. spectabilis* that gave birth to one offspring with the colour pattern of *P. spectabilis* and another one with that of *P. agilis*. Lobo *et al.* (2012a) concluded that *P. agilis* is a junior synonym of *P. spectabilis*. Corbalán *et al.* (2016) also mentioned unpublished data of a *P. spurcus* female that gave birth to morphs of *P. excelsus* and females of *P. excelsus* that gave birth to individuals with morphs of *P. excelsus* and *P. spectabilis*. Avila *et al.* (2011) noted similar colour patterns of *P. spectabilis*, *P. excelsus* and another closely related species, *Phymaturus tenebrosus* Lobo & Quinteros, 2005, questioning their separate taxonomic status and pointing to a significantly polymorphic population. Different morphs of *P. excelsus* were also recognized by Lobo *et al.* (2012b) as bold and brown, and despite the latter morph being similar to *P. spurcus*, they described it as intraspecific dimorphism of *P. excelsus*.

Previous phylogenetic studies on the *Phymaturus* genus using molecular data resulted in partial resolutions of the evolutionary relationships within some groups. By using several nuclear markers and mitochondrial cytochrome *b* (*Cytb*) and 12S genes, Morando *et al.* (2013) were able to resolve deep phylogenetic relationships among *Phymaturus* species, such as the reciprocal monophyly for the *P. palluma* and *P. patagonicus* groups. However, evolutionary relationships at the species level produced incongruent topologies for the *P. spurcus* subclade when adding nuclear genes to the analysis. This group remained unresolved in a more recent barcode study based on cytochrome *c* oxidase I (*COI*; Corbalán *et al.*, 2016), which did not include *P. manuelae*. These results based on nuclear and moderately evolving mitochondrial DNA (mtDNA) regions are expected with recently isolated lineages because of retention of ancestral polymorphism owing to incomplete lineage sorting, for which well-resolved molecular phylogenies are difficult to achieve (Moore, 1995).

Phylogenetic analyses can be powerful tools when using the right molecular markers for the right taxonomic levels. Animal mtDNA is usually effective to resolve species-level and genus-level phylogenetic relationships among lineages given its relatively rapid rates of substitution (Rubinoff & Holland, 2005). Generally, nuclear DNA sequences evolve at slower rates owing to a larger effective population size (Hare, 2001), making them useful to resolve deeper divergences. In contrast, mtDNA is haploid, maternally inherited, and its effective population size is one-quarter that of nuclear genes; therefore, a mitochondrial

haplotype tree can better resolve short internodes than a nuclear-gene tree (Avise *et al.*, 1987; Moore, 1995), allowing inferences about their recent evolutionary history. Introgression of maternal mtDNA has also been observed in diverse reptile groups, resulting in nuclear and mtDNA mixtures in some clades (McGuire *et al.*, 2007). In addition, the presence of genetic structure would indicate, at least, potential pre-mating isolation (Ferguson, 2002) and/or introgressive hybridization after secondary contact. Studies that analyse the spatial distribution of gene lineages in populations, as much as traditional phylogeographical studies do, can help to unveil on-going or recent speciation processes (Avise, 2000). In this sense, haplotype networks can be useful to assess genetic variation across geographical distributions (Templeton, 2004), especially in cases in which the data support phylogenies with rather short branches.

To contribute to the understanding of the evolutionary history of this group, we followed an integrative approach by performing phylogenetic and phylogeographical analyses with traditionally used mitochondrial regions, such as *COI*, and an extended portion that includes *Cytb* and the more variable NADH dehydrogenase subunits 1 (*ND1*) and 2 (*ND2*) and eight transfer RNA (tRNA) genes. Furthermore, we measured genetic distances and constructed a haplotype network to assess the genetic structure of these populations. Finally, to complement results found at the molecular level, we analysed morphotype proportions in four populations and estimated morph heritability in one of them through the regression of offspring on females bearing species-typical phenotypes.

MATERIAL AND METHODS

Sample collection for molecular data analyses

We collected two sample sets, $N_1 = 23$ and $N_2 = 114$ (Table 1), from seven and six rocky outcrops, respectively (each set including the terra typica for each species) out of 13 sites known to be inhabited by species belonging to the *P. spurcus* clade (Fig. 2; Appendix 1). Collections took place in December 2010, February 2011 and December 2012. All individuals were caught by slipknot or hand, kept individually in cloth bags while being transported to the laboratory, and photographed. Species assignation was based on external morphology. Most lizards ($N = 130$) were released at their exact site of capture within 48 h after tissue sampling from the tip of the tail, and only seven individuals were euthanized and tissue samples taken from liver.

DNA isolation, amplification and sequencing

Genomic DNA was manually extracted using the AccuPrep Genomic DNA Extraction kit (Bioneer, South Korea).

The 23 samples from sample set 1 were amplified at the International Barcode of Life Argentinean reference Barcode Laboratory at the Museo Argentino de Ciencias Naturales (MACN) in Buenos Aires, Argentina (Appendix 2). A 657 bp fragment of *COI* mtDNA gene was amplified using cocktail primers C_VF1LFt1 and C_VR1LRt (Ivanova *et al.*, 2007). Cycling conditions consisted of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 52 °C for 40 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Polymerase chain reaction (PCR) products were visualized on 1.2% agarose gels and sequenced on an ABI 3730XL capillary sequencer in the Canadian Centre for DNA Barcoding (CCDB) in Guelph, ON, Canada. Sequences were manually edited with CodonCode Aligner v.3.5.2 (CodonCode Corp., USA) and manually aligned with BioEdit v. 7.0.5.2 (Hall, 1999). Sequences are available in the Barcode of Life Data Systems website (http://www.boldsystems.org/).

Amplification of the 114 samples from sample set 2 was performed at the Gene Amplification Laboratory at INIBIOMA, Bariloche, Argentina. A 798 bp fragment of *Cytb* mtDNA gene was amplified with primers GLUDGL 5′-TGACTTGAARAACCAYCGTTG-3′ and CB3-3RC 5′-GAATGATAYTTCCTATTTGCC-3′ (Palumbi, 1996). Cycling conditions consisted of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 55 °C for 35 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Two other PCRs were performed to amplify an extra region of 1910 bp from the mitochondria spanning 258 bp at the end of *ND1*, entire sequences of tRNA-Ile, tRNA-Gln, tRNA-Met, *ND2* gene, tRNA-Trp, tRNA-Ala, tRNA-Asn, the putative Origin of Replication of Light chain (O_L), tRNA-Cys, tRNA-Tyr and 29 bp at the beginning of the *COI* gene. The first half of this *ND1–ND2*–tRNAs region was amplified using primers L3878 5′-GCCCCATTTGACCTCACAGAAGG-3′ and H4980 5′-ATTTTTCGTAGTTGGGTTTGRTT-3′ (Macey *et al.*, 1997, 1998). For the second half, custom primers ND2midFa 5′-AGGCTCAACCACAGTAACTG-3′

Figure 1. *Phymaturus* specimens of the putative morphospecies comprising the *Phymaturus spurcus* subclade showing dorsal coloration patterns: A, male *Phymaturus agilis*; B, female *P. agilis*; C, male *Phymaturus excelsus*; D, female *P. excelsus*; E, male *P. spurcus*; F, female *P. spurcus*; G, male *Phymaturus spectabilis*; H, female *P. spectabilis*; I, male *Phymaturus manuelae*; and J, female *P. manuelae*. Photograph credit: J. A. Scolaro.

Table 1. Number of individuals sampled at each site according to their taxonomic assignments used for DNA amplification of the *COI* region and the combined *ND1–ND2*–tRNAs and *Cytb* mtDNA region (*ND1*) for all localities

Sites Region	NYH		EYH		SYH		EOA		WOA		EHu		EBV		N	
	COI	*ND1*	*COI*	*ND1*	*COI*	*ND1*	*COI*	*ND1*	*COI*	*ND1*	*COI*	*ND1*	*COI*	*ND1*	*COI*	*ND1*
P. agilis	3	8	–	–	4	10	–	–	–	–	–	–	–	–	7	18
P. spectabilis	1	15	2	23	3	9	–	–	–	–	–	–	–	–	6	47
P. excelsus	–	–	–	–	–	–	3	–	2	12	–	–	–	–	5	12
P. spurcus	–	–	–	–	–	–	–	–	1	11	2	14	–	–	3	25
P. manuelae	–	–	–	–	–	–	–	–	–	–	–	–	2	12	2	12
N *COI*	4	–	2	–	7	–	3	–	3	–	2	–	2	–	23	–
N *ND1*	–	23	–	23	–	19	–	–	–	23	–	14	–	12	–	114

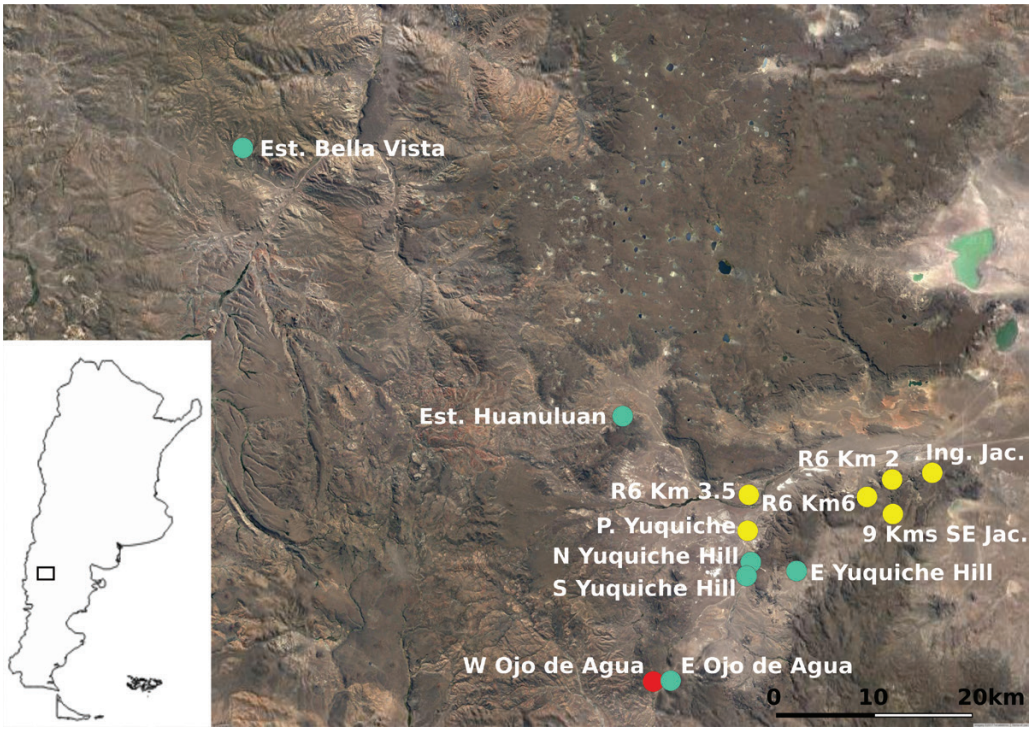

Figure 2. Rocky outcrop sites known for the presence of taxa belonging to the *Phymaturus spurcus* subclade within the *Phymaturus patagonicus* clade of genus *Phymaturus*. Turquoise circles indicate sites from which individuals were amplified for *COI*, *ND1–ND2*–tRNAs and *Cytb* mtDNA regions. Red circle indicates a site from which individuals were amplified only for *COI*. Yellow circles indicate sites not sampled for the present study. The inset map shows the location of the study area in Argentina.

and COIiniRa 5′-CTATACCAGCTCAGGCACC-3′ were designed based on alignments of *Phymaturus* sequences. Cycling conditions for both reactions were 2 min at 94 °C, 30 cycles of 35 s at 94 °C, 35 s at 55 °C and 2 min at 72 °C, and a final extension of 10 min at 72 °C. The PCR products were sent to Macrogen Inc. (Seoul, Republic of Korea) for sequencing after visualization on 1.2% agarose gels. Sequences were manually edited and aligned using BioEdit 7.0.5.2 (Hall, 1999) and deposited in GenBank under accession numbers MH491578-MH491805.

Phylogenetic reconstructions

We used MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) to reconstruct by Bayesian inference the phylogenetic relationships of the 23 *COI* sequences, to which we added 13 sequences previously published by Corbalán *et al.* (2016) from individuals collected at some of the same sites sampled in this study (GenBank accession numbers KU565035–KU565037, KU565059–KU565061 and KU565118–KU565124). We identified the substitution model of sequence evolution with the Akaike criterion using jModelTest 2.1.10 (Posada,

2008), whose best-fit model for *COI* was nst = 6 and rates = gamma. We included three *COI* sequences of *P. patagonicus* Koslowsky, 1898 as outgroups (GenBank accession numbers KU565081–KU565083).

We combined the mtDNA regions *ND1–ND2*–tRNAs and *Cytb* for the 114 samples to assess their phylogenetic relationships by Bayesian inference using non-redundant haplotypes identified with DnaSP v. 5.10 (Librado & Rozas, 2009). We defined three partitions on the combined 2708 bp, obtaining the following substitution models: *Cytb*, nst = 6 and rates = equal, *ND1–ND2*, nst = 6 and rates = gamma and a model with nst = 2 and rates = equal for the tRNAs partition. We used sequences from *P. patagonicus*, *Phymaturus zapalensis* Cei & Castro, 1973, *Phymaturus indistinctus* Cei & Castro, 1973 and *Phymaturus somuncurensis* Cei & Castro, 1973 as outgroups (GenBank accession numbers AF049865, AY661893–AY661894, GQ502773, JX948865, JX949155, JX969038 and JX969042). For each reconstruction, we performed two independent runs, totalling four chains, for 50 000 000 generations, sampling every 1000 trees. The initial 25% of the resulting trees was discarded as burn-in, and a 50% majority-rule consensus tree was constructed.

Haplotype network and genetic divergence

We constructed a haplotype network for the combined *ND1–ND2*–tRNAs and *Cytb* region using the median joining algorithm developed by Bandelt *et al.* (1999), implemented in PopART v. 1.7 (Leigh & Bryant, 2015), and edited it with the vector graphics editor Inkscape (https://www.inkscape.org). We calculated between-populations genetic divergence based on the Kimura's two-parameter model (Kimura, 1980) with MEGA 7.0 (Kumar *et al.*, 2016).

Births in captivity and structure of populations

We captured pregnant females from South Yuquiche Hill in January 2011 ($N_{P.\ spectabilis}$ = 12; $N_{P.\ agilis}$ = 2) and February 2013 ($N_{P.\ spectabilis}$ = 13; $N_{P.\ agilis}$ = 4) and from East Ojo de Agua in February 2011 ($N_{P.\ spurcus}$ = 2). We kept them in the laboratory until parturition with food and water *ad libitum* in an open-top glass terrarium (117 cm × 40 cm × 50 cm) with a sand floor, a rock from the site of capture as a refuge and an infrared lamp (150 W) on one side to provide a temperature gradient. Air temperature in the room ranged from 18.8 to 23.1 °C. For each newborn, we registered the date of parturition, body mass and snout–vent length. We also analysed its morphology, coloration and dorsal pattern and assigned it to one of the morphospecies (Fig. 1). Litter size was determined by the number of offspring born in captivity. We used the offspring–parent regression method (Falconer, 1989) to estimate heritability of phenotype with 31 pregnant females collected from South Yuquiche Hill that produced a mean of 1.97 offspring. We calculated the regression of offspring on dam from South Yuquiche Hill for dorsal morphotypes *P. spectabilis* and *P. agilis*, giving them a score of zero and one, respectively. By ignoring the sires, we obtain half the heritability (Falconer, 1989); therefore, the slope of the regression line was doubled to estimate the heritability.

To assess morphotype proportions within the different populations, we performed intensive fieldwork to capture and release 631 lizards in four localities (North, East and South Yuquiche Hill sites and East Ojo de Agua) during March 2006, November 2006, October 2007, December 2010, February–March 2011, November–December 2011, February 2012, December 2012 and December 2013. We analysed, using the binomial test, the differences in the proportion of individuals with *P. agilis* and *P. spectabilis* morphs in North Yuquiche Hill, East Yuquiche Hill and South Yuquiche Hill, and the differences in proportions of *P. excelsus* and *P. spurcus* morphs in East Ojo de Agua. We performed statistical analyses using SPSS 15.0 and Sigma Plot 11.0.

Key to abbreviations

EBV, Estancia Bella Vista; EHu, Estancia Huanuluan; EYH, East Yuquiche Hill; EOA, East Ojo de Agua; NYH, North Yuquiche Hill; P. agi, *P. agilis*; P. exc, *P. excelsus*; P. man, *P. manuelae*; P. spe, *P. spectabilis*; P. spu, *P. spurcus*; SYH, South Yuquiche Hill; WOA, West Ojo de Agua.

RESULTS

Phylogenetic reconstructions

Bayesian inference based on the *COI* sequences (Fig. 3) resulted in two highly supported, reciprocally monophyletic clades, one for sequences of *P. manuelae* collected in Estancia Bella Vista (posterior probability [PP] = 1) and one big polytomic clade containing the rest of the sequences (PP = 0.98). The combined *ND1–ND2*–tRNAs and *Cytb* region yielded 48 non-redundant haplotypes, and its Bayesian tree (Fig. 4) resembled that derived from *COI*, with each clade fully supported (PP = 1). In both inferences, relationships among the terminals in the large polytomic clade were shallow, although they did reveal a strong geographical pattern. In the *COI* gene tree, the sequences from Estancia Huanuluan were grouped, as were the sequences from East Yuquiche Hill. Those from East and West Ojo de Agua grouped together, which could be expected from proximate sites. In the combined *ND1–ND2*–tRNAs and *Cytb* gene tree, all but one of the haplotypes from Estancia Huanuluan and most haplotypes from East Yuquiche Hill were also clustered.

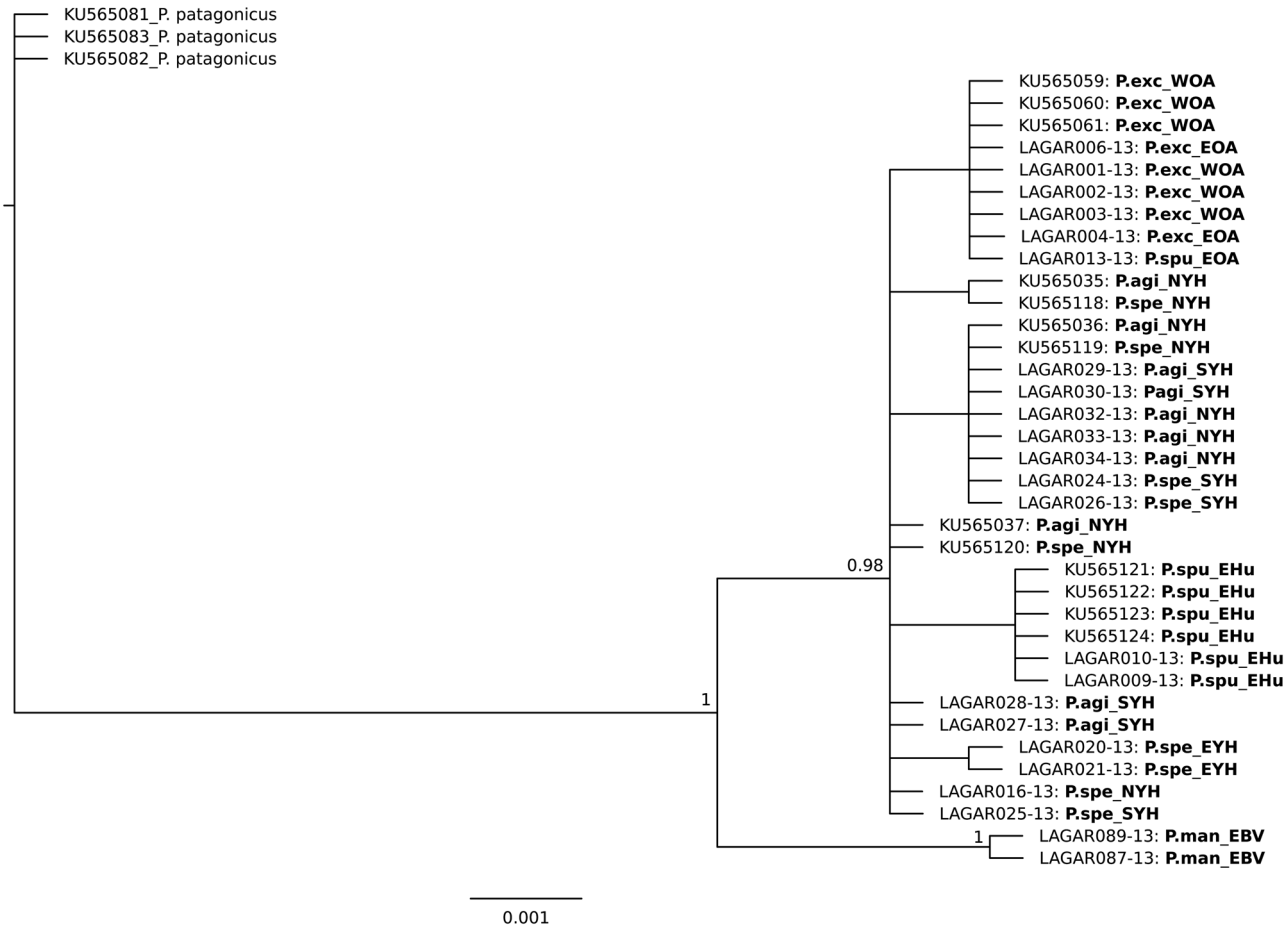

Figure 3. Bayesian phylogenetic tree based on 23 *COI* sequences obtained in the present study (see Appendix 2) and 13 *COI* sequences (GenBank accession numbers KU565035– KU565037, KU565059–KU565061 and KU565118–KU565124) previously published by Corbalán *et al.* (2016). *Phymaturus patagonicus* was used as the outgroup. Bayesian posterior probabilities are shown on nodes.

Haplotype network and genetic divergence

The haplotype network for the combined *ND1*–*ND2*–tRNAs and *Cytb* region resulted in a star-shaped pattern, with an ancestral haplotype in the centre (Fig. 5). All haplotypes seem to have derived from this ancestral haplotype that was not found among the samples analysed. A recent divergence can be hypothesized, considering that there are several haplotypes with only one mutation step from the ancestral haplotype. In addition, a population genetic structure was evidenced, as haplotypes seem to segregate according to rocky outcrops. With the exception of North and South Yuquiche Hill sites, which shared three haplotypes, Estancia Huanuluan, East Yuquiche Hill and West Ojo de Agua presented their own haplotype groups. Haplotypes sampled from *P. manuelae* at Estancia Bella Vista showed a much higher degree of divergence, as expected (Table 2).

Births in captivity, morphotype proportions and heritability estimation

Six females of the *P. agilis* morph gave birth from mid-February to mid-March to one or two offspring. Two females gave birth to offspring of the *P. agilis* morph, two to the *P. spectabilis* morph, and two gave birth to offspring of both morphs. Of the 25 females of the *P. spectabilis* morph, 21 gave birth from mid- to late February, and four gave birth in the first week of March, with litter sizes of one to three offspring. Twelve females gave birth only to offspring of the *P. spectabilis* morph, three gave birth only to offspring of the *P. agilis* morph, and 10 gave birth to offspring of both morphs. The proportion of newborn with the *P. spectabilis* vs. *P. agilis* morphs from females of the *P. spectabilis* morph was significantly different (proportion of *P. spectabilis* vs. *P. agilis* = 77:23, binomial test, $N_{P.\ spectabilis} = 36$, $N_{P.\ agilis} = 11$, $P < 0.001$), whereas the proportion of newborn with the *P. spectabilis* vs.

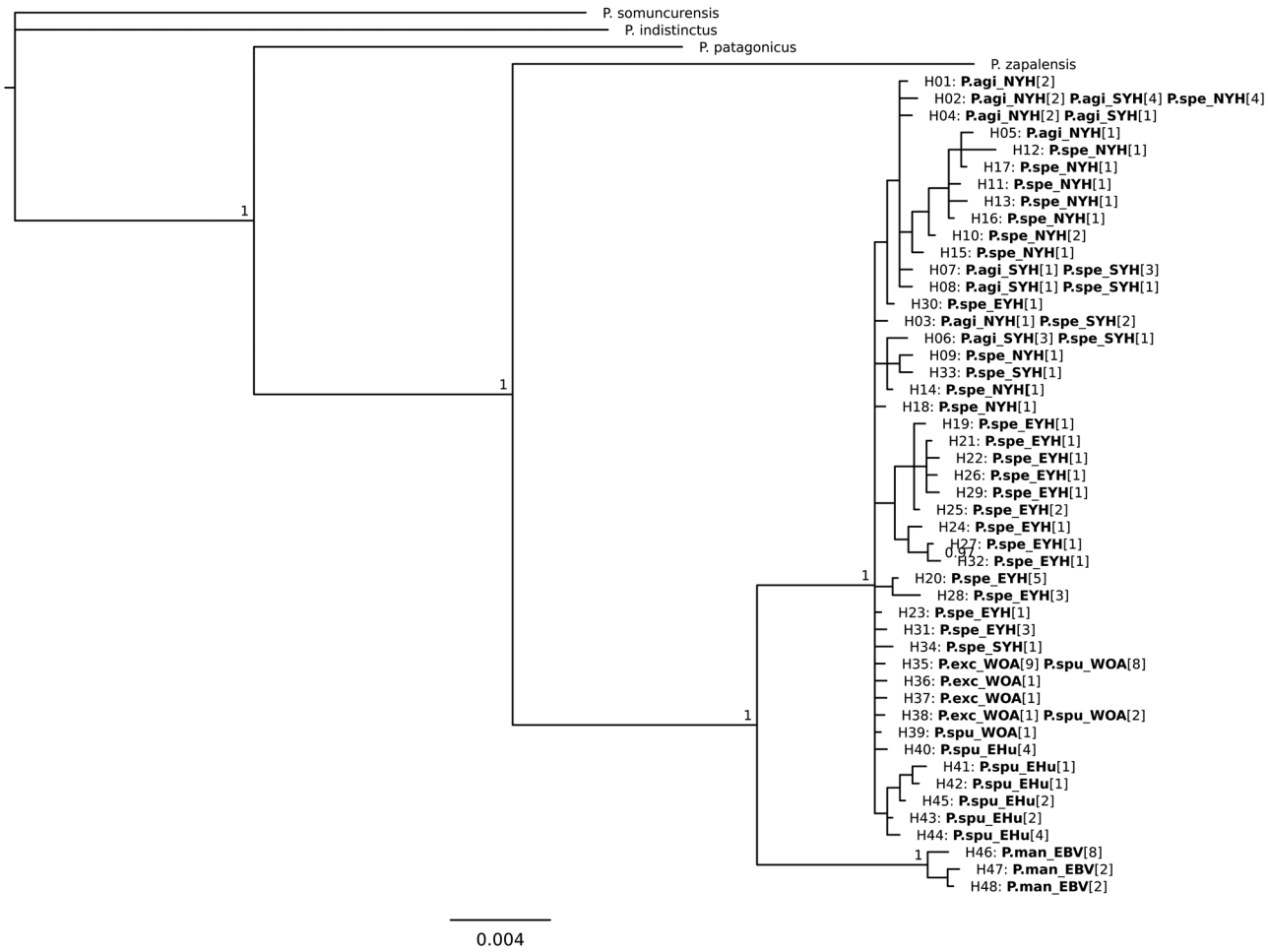

Figure 4. Bayesian phylogenetic tree based on 48 haplotypes of the combined *ND1–ND2*–tRNAs and *Cytb* mtDNA region found among the 114 individuals included in the present study. The fragment of *Cytb* consisted of 798 bp, and the 1910 bp fragment of *ND1–ND2*–tRNAs encompassed the last 258 nucleotides of *ND1* to the first 29 nucleotides of *COI*, including the entire *ND2* gene, eight tRNAs and the putative Origin of Replication of Light chain (O_L). Numbers in square brackets indicate the number of individuals bearing the respective haplotype at a particular site. Outgroup sequences corresponded to *Phymaturus indistinctus*, *Phymaturus patagonicus*, *Phymaturus somuncurensis* and *Phymaturus zapalensis*. Bayesian posterior probabilities are shown on nodes.

P. agilis morphs from females of the *P. agilis* morph was similar (proportion of *P. spectabilis* vs. *P. agilis* = 60:40, binomial test, $N_{P.\ spectabilis} = 6$, $N_{P.\ agilis} = 4$, $P = 0.754$).

Regarding the two females of the *P. spurcus* morph, both gave birth to offspring of the *P. excelsus* morph. One female gave birth to two offspring in late February and the other female gave birth to one offspring at mid-March.

Heritability estimation of the *P. spectabilis* vs. *P. agilis* dorsal morphotypes resulted in a value of 0.77 ± 0.34 ($P < 0.0317$; Fig. 6). The dominant morphotypes at South Yuquiche Hill (the most exhaustively surveyed population) and at East Ojo de Agua were *P. spectabilis* and *P. excelsus*, respectively (80% at each site; Table 3).

DISCUSSION

Our Bayesian analyses based on *COI* and the combined *ND1–ND2*–tRNAs and *Cytb* mtDNA genes coincide with and have higher posterior probabilities than those obtained by Morando *et al.* (2013) based on *Cytb* for the *P. spurcus* subclade of the *P. patagonicus* clade of *Phymaturus*. The use of nuclear markers by these authors resulted in incongruent tree topologies that placed *P. manuelae* within the subclade along with the rest of the *P. spurcus* species. In light of the small genetic distances observed in the mitochondrial regions assessed by the present study, it is not surprising that the use of slower-evolving nuclear markers was unable to resolve the phylogenetic relationships of this group owing to

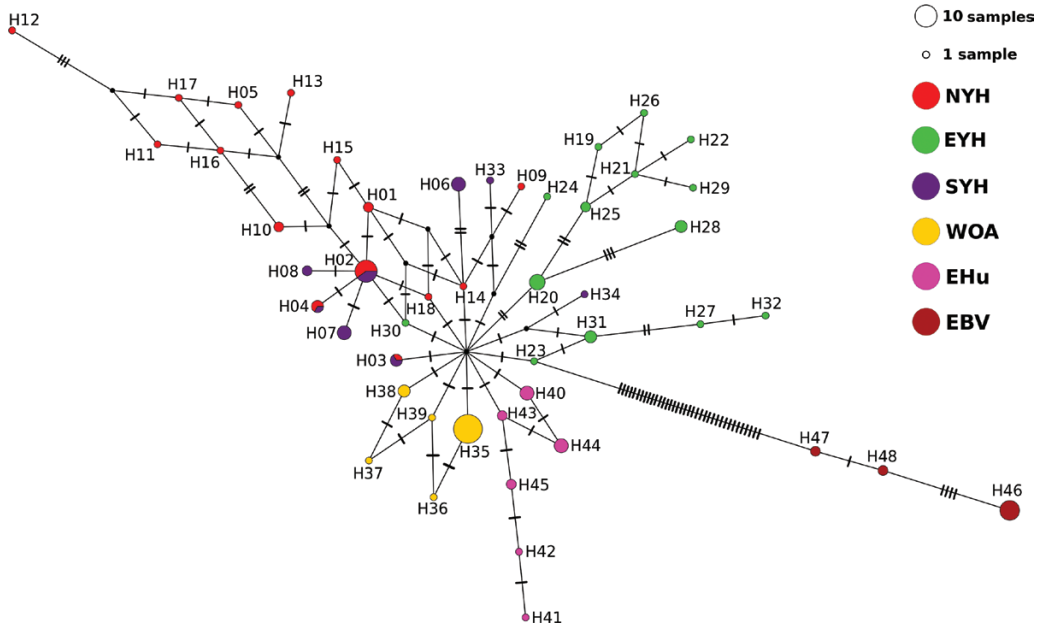

Figure 5. A median joining haplotype network revealed a genetic structure on the combined *ND1–ND2*–tRNAs and *Cytb* region of 2708 bp. Haplotypes derived from a common ancestor, which was not found among the 114 individuals analysed.

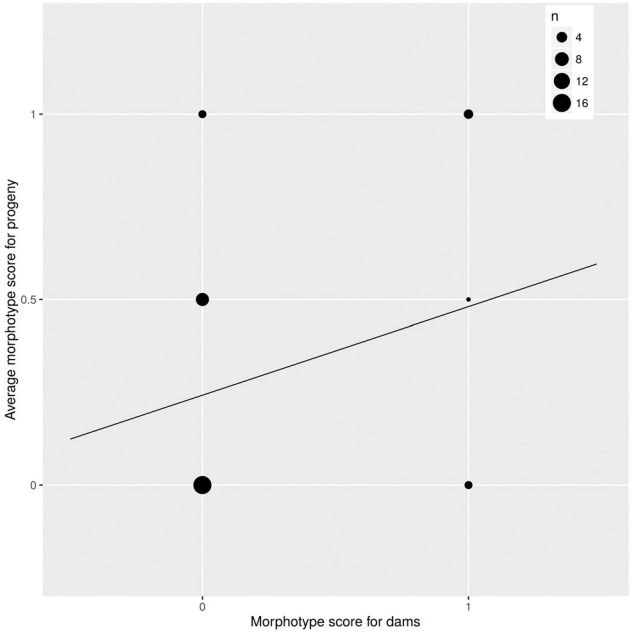

Figure 6. Regression of offspring on dams for dorsal morphotype. Dam dorsal morphotype scores (*Phymaturus spectabilis* = 0, *Phymaturus agilis* = 1) are shown on the horizontal axis and mean scores of progeny along the vertical axis. Point sizes are according to the total number of observations for each offspring–dam morph combination. As only one parent (dam) was included, the slope of the regression line is doubled to estimate heritability, hence $b = 0.38 \pm 0.17$ indicates $h^2 = 0.77 \pm 0.34$ ($P < 0.0317$).

Table 2. Mean genetic distances between sites based on Kimura's two-parameter model calculated for *COI* mtDNA gene (above diagonal) and for the combined *ND1–ND2*–tRNAs and *Cytb* mtDNA region (below diagonal)

	1.	2.	3.	4.	5.
1. NYH & SYH	–	0.000	0.002	0.004	0.013
2. EYH	0.002	–	0.002	0.004	0.013
3. WOA	0.002	0.002	–	0.006	0.015
4. EHu	0.002	0.002	0.001	–	0.015
5. EBV	0.016	0.016	0.016	0.016	–

North and South Yuquiche Hill were joint for calculations as these two localities shared haplotypes.

incomplete lineage sorting (Maddison & Knowles, 2006). A large proportion of *P. spectabilis* females gave birth in captivity to *P. agilis* offspring, and vice versa, and these observations are in agreement with the results of Lobo *et al.* (2012a). Thus, our breeding observations support neither *P. spectabilis* and *P. agilis* nor *P. spurcus* and *P. excelsus* as distinct species. In fact, considering the strong support for the large monophyletic clade containing individuals assigned to all four species, the small genetic distances among them, the similarity of their life histories and their ecological niches, and the previous breeding observations of Avila *et al.* (2011) and Corbalán *et al.* (2016), we propose that *P. agilis*, *P. spectabilis* and *P. excelsus* be synonymized under *P. spurcus*, considering the taxonomic principle of priority.

Table 3. Summary of the proportion of the *P. spectabilis* and *P. agilis* morphs in North, East and South Yuquiche Hill and the proportion of the *P. excelsus* and *P. spurcus* morphs in East Ojo de Agua

Proportions	*P. spectabilis*:*P. agilis*			*P. excelsus*:*P. spurcus*
Location	NYH	EYH	SYH	EOA
Juveniles	65:35 (N = 13:7) P = 0.263	100:0 (N = 6:0)	82:18 (N = 82:18) P < 0.001	80:20 (N = 12:3) P = 0.035
Adult females	45:55 (N = 5:6) P = 1.000	100:0 (N = 25:0)	71:29 (N = 113:47) P < 0.001	83:17 (N = 40:8) P < 0.001
Adult males	56:44 (N = 5:4) P = 1.000	94:6 (N = 15:1) P = 0.001	78:22 (N = 116:32) P < 0.001	73:27 (N = 27:10) P < 0.001

Accordingly, the colour pattern of *P. spurcus* results from the combination of the patterns previously described for each morphotype. Some populations have individuals characterized by a dorsal pattern with a single and uniform colour that could be dark or light brown (Barbour, 1921; Lobo & Quinteros, 2005b) or even reddish brown (Scolaro *et al.*, 2008). This dorsal pattern often includes a variable number of marked ocelli, which can be brownish and lighter than the background dorsal coloration. Other populations include individuals with conspicuous white ocelli against the melanic background dorsal colour or an irregular and discontinuous pattern characterized by speckled black spots (Lobo & Quinteros, 2005a). *Phymaturus manuelae* instead has a dorsal pattern characterized by an irregular and discontinuous pattern of speckled light-brown spots, and a group of sub-elliptic spots arranged in two paravertebral discontinuous lines. On the dorsum, from the nape to the base of the tail, is a group of small, irregular and dense grey or light brown spots arranged in two paravertebral discontinuous lines (Scolaro & Ibargüengoytía, 2008).

Despite their relatively narrow distribution, gene flow seems to be restricted among *P. spurcus* populations as revealed by genetic structure, except between the North and South Yuquiche Hill, whose haplotypes were found in common. Thus, *P. spurcus* appears to be a single highly structured species whose populations seem to be experiencing a process of divergence in morphometric and meristic characteristics and dorsal colour patterns from a common, ancestral population. The star-shaped haplotype network agrees with this hypothesis, and the small genetic pairwise distances among haplotypes are in accordance with a recent diversification. The climatic and geological events that occurred during the Pliocene and Pleistocene have probably contributed to population diversifications and speciation processes that formed the current biodiversity (Avise & Walker, 1998). Northwestern Patagonia, profoundly altered by the uplift of the Andes on the west and massive basaltic flows towards the plains in the east until the mid-Miocene (Ramos & Ghiglione, 2008), was subjected to the alternation of glacial and interglacial periods since the late Miocene (Rabassa *et al.*, 2011). In addition to the fluvial erosion during deglaciations, the Patagonian plains now inhabited by *Phymaturus* were also affected by intense volcanic activity during the Pliocene and Pleistocene (Coira, 1979). Therefore, the continuing transformation of the northwestern Patagonian landscape during the Quaternary may have had a profound effect on the genetic structure of *Phymaturus*. Divergent populations could be the result of isolation owing to the inherent fragmentation of their habitat (Hewitt, 2000, 2004), in addition to low dispersal rates and short dispersal distances as described in closely related lizards, even as juveniles (Habit & Ortiz, 1994; Araya-Díaz, 2007). Rocky outcrops are separated by gravel valleys, on which lizards may not find suitable habitat (i.e. no hiding places from predators), as they are always found on rock substrate or perched on the branches of low woody vegetation rooted within rock crevices (Cei, 1986; Ibargüengoytía *et al.*, 2008; Scolaro & Ibargüengoytía, 2008; Scolaro *et al.*, 2008) and seemingly never away from the rocky crops (Ibargüengoytía NR, Scolaro JA, pers. obs.). The higher degree of divergence of *P. manuelae* at Estancia Bella Vista from the large clade that consists of individuals from the other populations might be the result of this species inhabiting distant basaltic tablelands (Scolaro & Ibargüengoytía, 2008), with several valleys in between acting as barriers to gene flow. The same effect, albeit at a smaller scale, is observed within the large clade itself. The individuals at Estancia Huanuluan diverge minimally from individuals at the rest of the sites, which are located across a single valley.

The differences in female reproductive biology, shown by a tendency of *P. spectabilis* to reproduce earlier and have larger litters, may indicate a complex social context in which the *P. spectabilis* morph appears to dominate over the *P. agilis* morphotype, although larger sample sizes of *P. agilis* morph females are needed to confirm such a hypothesis. The fact that the *P. spectabilis* morph is found in higher proportions

when sympatric with the *P. agilis* morph may also suggest a selective advantage. Although we did not have enough data on pregnant females of the *P. spurcus* and *P. excelsus* morphotypes to analyse their proportions at birth, our observations in East Ojo de Agua showed that the *P. excelsus* morph is four times more frequent than the *P. spurcus* morphotype. In the same way, this seems to indicate some selective advantage favouring the *P. excelsus* morph over the *P. spurcus* morphotype.

Colour and pattern polymorphisms are widespread in nature. Colour polymorphism was first defined by Huxley (1955) as the presence of distinct, genetically determined colour forms in a population in which the proportion of the least abundant cannot be explained by recurrent mutation. Genetically based polymorphism, as shown here with high heritability for dorsal morphotype, is widespread in lizards and varieties can coexist at equilibrium at different proportions within one single population in males (Sinervo & Lively, 1996; Sinervo & Zamudio, 2001; Sacchi *et al.*, 2007) and in females (Sinervo *et al.*, 2000; Vercken *et al.*, 2008). The combination of colour and pattern polymorphisms can evolve under both natural and sexual selection (Roulin, 2004), and their implications in the evolution of reproductive isolation and sympatric speciation have been thoroughly discussed (e.g. Fisher, 1930; Van Valen & Grant, 1970; Gray & McKinnon, 2007). Moreover, in many lizards the dorsal pattern polymorphisms are coupled with ventral colour polymorphisms (Lancaster *et al.*, 2008; Lancaster & Sinervo, 2011), with both being important to female mate choice (Lancaster *et al.,* 2009, 2014) and related to the process of incipient speciation (Bastiaans *et al.*, 2014). For instance, there may be latent hybrid lack of fitness with embryonic incompatibilities among different morphotypes, as in the side-blotched lizard *Uta stansburiana* (Lancaster *et al.*, 2014), which appears to give rise to incipient speciation as morphotypes are lost (Corl *et al.*, 2012). The *P. spurcus* populations studied here might be useful in identifying similar processes of incipient speciation, given that their dorsal pattern polymorphisms are also associated with ventral colour polymorphisms (Fernández JB, Boretto JM, Ibargüengoytía NR, and Sinervo B, unpublished observations). Further research certainly could shed light on the morphotype-specific ecophysiology and behaviours of *P. spurcus*, such as male territorial (Sinervo & Zamudio, 2001), reproductive (Sinervo *et al.* 2000), antipredatory (Lancaster *et al.*, 2009) and thermoregulatory behaviours (Paranjpe *et al.*, 2013), as reported in other species of Patagonian liolaemids.

Here, we computed broad-sense heritability, because our full-sibling estimates through the dam include additive genetic variation (narrow-sense heritability), dominance variation and, potentially, epigenetic maternal effect variation (Falconer, 1989), as shown in controlled genetic crosses in reptiles for a variety of morphological or physiological traits. For instance, purely additive genetic variation on throat colour morphs has been detected through laboratory crosses with field release of progeny (Sinervo & Zamudio 2001) and by field pedigrees and gene mapping (Sinervo *et al.*, 2006). Additive genetic and epigenetic effect variation on dorsal patterns was assessed by genetic crosses and egg steroid manipulations (Lancaster *et al.*, 2008), and purely epigenetic effects on thermoregulatory behaviour (thermal preference) were assessed through genetic crosses (Paranjpe *et al.*, 2013). Further research in *P. spurcus* will include controlled genetic crosses with half-siblings through the sire and full-siblings through the dam to discriminate among these components of familial resemblance, and the alternative hypothesis for within-population polymorphism, that is intogressive hybridization with purifying selection against hybrids formed between diverging populations (Corl *et al.*, 2012).

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REFERENCES

Araya-Díaz S. 2007. *Aspectos autecológicos relevantes para la conservación de Phymaturus flagellifer (Reptilia, Tropiduridae) en la reserva nacional Altos de Lircay, Región del Maule*. Engineer Thesis. Escuela de Agronomía. Universidad de Chile.

Avila LJ, Pérez CHF, Pérez DR, Morando M. 2011. Two new mountain lizard species of the *Phymaturus* genus (Squamata: Iguania) from northwestern Patagonia, Argentina. *Zootaxa* **2924:** 1–21.

Avise JC. 2000. *Phylogeography – the history and formation of species*. Cambridge, MA: Harvard University Press.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18:** 489–522.

Avise JC, Walker DE. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society B: Biological Sciences* **265:** 457–463.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Barbour T. 1921. On a small collection of reptiles from Argentina. *Proceedings of the Biological Society of Washington* **34:** 139–141.

Bastiaans E, Bastiaans MJ, Morinaga G, Gaytán JGC, Marshall JC, Bane B, de la Cruz FM, Sinervo B. 2014. Female preference for sympatric vs. allopatric male throat color morphs in the Mesquite lizard (*Sceloporus grammicus*) species complex. *PLoS ONE* **9:** e93197.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* **22:** 148–155.

Camargo A, Sinervo B, Sites JW. 2010. Lizards as model organisms for linking phylogeographic and speciation studies. *Molecular Ecology* **19:** 3250–3270.

Cei JM. 1986. *Reptiles del centro, centro-oeste y sur de la Argentina: herpetofauna de las zonas áridas y semiáridas*. Monografia IV. Museo Regionale di Scienze Naturali. Torino, Italy.

Cei JM. 1993. *Reptiles del noroeste, nordeste y este de la Argentina: herpetofauna de las selvas subtropicales, Puna y Pampas.* Monografia XIV. Museo Regionale di Scienze Naturali. Torino, Italy.

Coira BL. 1979. Descripción geológica de la Hoja 40d Ingeniero Jacobacci, Provincia de Rio Negro. Carta Geológico-económica de la República Argentina. *Servicio Geológico Nacional Boletín* **168:** 1–101.

Corbalán V, Debandi G, Scolaro JA, Ojeda AA. 2016. DNA barcoding of *Phymaturus* lizards reveals conflicts in species delimitation within the *patagonicus* clade. *Journal of Herpetology* **50:** 654–666.

Corl A, Lancaster LT, Sinervo B. 2012. Rapid formation of reproductive isolation between two populations of side-blotched lizards, *Uta stansburiana*. *Copeia* **2012:** 593–602.

Espinoza RE, Wiens JJ, Tracy CR. 2004. Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 16819–16824.

Etheridge RE. 1995. Redescription of *Ctenoblepharys adspersa* Tschudi, 1845, and the taxonomy of Liolaeminae (Reptilia: Squamata: Tropiduridae). *American Museum Novitates* **3142:** 1–34.

Falconer DS. 1989. *Introduction to quantitative genetics*. Harlow, Essex, UK: Longman Scientific and Technical.

Ferguson JWH. 2002. On the use of genetic divergence for identifying species. *Biological Journal of the Linnean Society* **75:** 509–516.

Fernández JB, Bastiaans E., Medina M, Méndez de la Cruz FR, Sinervo BR, Ibargüengoytía NR. 2018. Behavioral and physiological polymorphism in males of the austral lizard *Liolaemus sarmientoi*. *Journal of Comparative Physiology A* **204:** 219–230.

Fisher R. 1930. *The genetical theory of natural selection*. Oxford: Oxford University Press.

Gray SM, McKinnon JS. 2007. Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution* **22:** 71–79.

Habit EM, Ortiz JC. 1994. Ámbito de hogar de *Phymaturus flagellifer* (Reptilia: Tropiduridae). *Boletín de la Sociedad de Biología de Concepción (Chile)* **65:** 149–152.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hare MP. 2001. Prospects for nuclear gene phylogeography. *Trends in Ecology & Evolution* **16:** 700–706.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Hewitt G. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359:** 183–195.

Huxley JS. 1955. Morphism in birds. In: Portman A, Sutter E, eds. *Acta XI congressus internationalis ornithologicus*. Basel, Stuttgart: Birkhäuser Verlag, 309–328.

Ibargüengoytía NR, Acosta JC, Boretto JM, Villavicencio HJ, Marinero JA, Krenz JD. 2008. Field thermal biology in *Phymaturus* lizards: comparisons from the Andes to the Patagonian steppe in Argentina. *Journal of Arid Environments* **72:** 1620–1630.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN. 2007. Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* **7:** 544–548.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33:** 1870–1874.

Lancaster LT, Hazard LC, Clobert J, Sinervo B. 2008. Corticosterone manipulation reveals differences in hierarchical organization of multidimensional reproductive trade-offs in r-strategist and K-strategist females. *Journal of Evolutionary Biology* **21:** 556–565.

Lancaster LT, Hipsley CA, Sinervo B. 2009. Female choice for optimal combinations of multiple male display traits increases offspring survival. *Behavioral Ecology* **20:** 993–999.

Lancaster LT, McAdam AG, Hipsley CA, Sinervo B. 2014. Frequency-dependent and correlational selection pressures have conflicting consequences for assortative mating in a color-polymorphic lizard, *Uta stansburiana*. *The American Naturalist* **184:** 188–197.

Lancaster LT, Sinervo B. 2011. Epistatic social and endocrine networks and the evolution of life history tradeoffs and plasticity. *Mechanisms of Life History Evolution*: 329–348.

Leigh JW, Bryant D. 2015. POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6:** 1110–1116.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Lobo F, Abdala CS, Valdecantos S. 2012a. Morphological diversity and phylogenetic relationships within a South-American clade of iguanian lizards (Liolaemidae: *Phymaturus*). *Zootaxa* **3315:** 1–41.

Lobo F, Cruz FB, Abdala CS. 2012b. Multiple lines of evidence show that *Phymaturus agilis* **Scolaro, Ibargüengoytía & Pincheira-Donoso, 2008 is a junior synonym of** *Phymaturus spectabilis* **Lobo & Quinteros, 2005.** *Cuadernos de Herpetología* **26:** 21–27.

Lobo F, Quinteros S. 2005a. A morphology-based phylogeny of *Phymaturus* (Iguania: Liolaemidae) with the description of four new species from Argentina. *Papéis Avulsos de Zoologia (São Paulo)* **45:** 143–177.

Lobo F, Quinteros S. 2005b. Taxonomic studies of the genus *Phymaturus* **(Iguania: Liolaemidae): redescription of** *Phymaturus patagonicus* **Koslowsky 1898, and revalidation and redescription of** *Phymaturus spurcus* **Barbour 1921.** *Journal of Herpetology* **39:** 533–540.

Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ. 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* **14:** 91–104.

Macey JR, Schulte JA, Larson A, Fang Z, Wang Y, Tuniyev BS, Papenfuss TJ. 1998. Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: a case of vicariance and dispersal. *Molecular Phylogenetics and Evolution* **9:** 80–87.

Maddison WP, Knowles LL. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* **55:** 21–30.

Marín AG, Pérez C, Minoli I, Morando M, Avila LJ. 2016. A new lizard species of the *Phymaturus patagonicus* group (Squamata: Liolaemini) from northern Patagonia, Neuquén, Argentina. *Zootaxa* **4121:** 412–430.

McGuire JA, Linkem CW, Koo MS, Hutchison DW, Lappin AK, Orange DI, Lemos-Espinal J, Riddle BR, Jaeger JR. 2007. Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution* **61:** 2879–2897.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49:** 718–726.

Morando M. 2004. *Sistemática y filogenia de grupos de especies de los géneros Phymaturus y Liolaemus (Squamata: Tropiduridae: Liolaeminae) del oeste y sur de Argentina*. Unpublished PhD Thesis, Universidad Nacional de Tucumán.

Morando M, Avila LJ, Perez CHF, Hawkins MA, Sites JW Jr. 2013. A molecular phylogeny of the lizard genus *Phymaturus* (Squamata, Liolaemini): implications for species diversity and historical biogeography of southern South America. *Molecular Phylogenetics and Evolution* **66:** 694–714.

Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. *Molecular systematics*: 205–247.

Paranjpe DA, Bastiaans E, Patten A, Cooper RD, Sinervo B. 2013. Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. *Ecology and Evolution* **3:** 1977–1991.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

de Queiroz K. 2005. Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences of the United States of America* **102:** 6600–6607.

de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56:** 879–886.

Rabassa J, Coronato AM, Martínez O. 2011. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. *Biological Journal of the Linnean Society* **103:** 316–335.

Ramos VA, Ghiglione MC. 2008. Tectonic evolution of the Patagonian Andes. *Developments in Quaternary Sciences* **11:** 57–71.

Raxworthy CJ, Ingram CM, Rabibisoa N, Pearson RG, Weins J. 2007. Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Systematic Biology* **56:** 907–923.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Roulin A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews* **79:** 815–848.

Rubinoff D, Holland BS. 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Systematic Biology* **54:** 952–961.

Sacchi R, Scali S, Pupin F, Gentilli A, Galeotti P, Fasola M. 2007. Microgeographic variation of colour morph frequency and biometry of common wall lizards. *Journal of Zoology* **273:** 389–396.

Scolaro JA. 2005. *Reptiles Patagónicos: Sur. Una guía de campo.* Trelew, Chubut: Universidad Nacional de la Patagonia San Juan Bosco.

Scolaro JA. 2006. *Reptiles Patagónicos: Norte. Una guía de campo.* Comodoro Rivadavia, Chubut: Universidad Nacional de la Patagonia San Juan Bosco.

Scolaro JA, Corbalán V, Tappari F, Streitenberger L. 2016. Lizards at the end of the world: a new melanic species of *Phymaturus* of the *patagonicus* clade from rocky outcrops in the northwestern steppe of Chubut province, Patagonia, Argentina. *Boletín del Museo Nacional de Historia Natural, Chile* **65:** 137–152.

Scolaro JA, Ibargüengoytía NR. 2008. A new fragment for the understanding of the puzzling evolutive process of the *Phymaturus* genus: a new species of the *patagonicus* group from Patagonia, Argentina (Reptilia: Iguania: Liolaemidae). *Zootaxa* **1939:** 38–50.

Scolaro JA, Ibargüengoytía NR, Pincheira-Donoso D. 2008. When starvation challenges the tradition of niche conservatism: on a new species of the saxicolous genus *Phymaturus* from Patagonia Argentina with pseudoarboreal foraging behaviour (Iguania, Liolaemidae). *Zootaxa* **1786:** 48–60.

Sinervo B, Calsbeek R, Comendant T, Both C, Adamopoulou C, Clobert J. 2006. Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *The American Naturalist* **168:** 88–99.

Sinervo B, Lively CM. 1996. The rock–paper–scissors game and the evolution of alternative male strategies. *Nature* **380:** 240–243.

Sinervo B, Miles DB, Frankino WA, Klukowski M, DeNardo DF. 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior* **38:** 222–233.

Sinervo B, Zamudio KR. 2001. The evolution of alternative reproductive strategies: fitness differential, heritability, and genetic correlation between the sexes. *Journal of Heredity* **92:** 198–205.

Templeton AR. 2004. Using haplotype trees for phylogeographic and species inference in fish populations. *Environmental Biology of Fishes* **69:** 7–20.

Van Valen L, Grant PR. 1970. Variation and niche width reexamined. *The American Naturalist* **104:** 589–590.

Vercken E, Sinervo B, Clobert J. 2008. Colour variation in female common lizards: why we should speak of morphs, a reply to Cote *et al*. *Journal of Evolutionary Biology* **21:** 1160–1164.

Will KW, Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* **20:** 47–55.

Appendix 1. Sites (rocky outcrops) known with presence of the putative species belonging to the *P. spurcus* subclade within the *P. patagonicus* clade of genus *Phymaturus* (see Fig. 2 for geographical locations)

Locality	Latitude	Longitude	Putative species	Terra typica for species
EBV†	41°2.445′S	70°24.865′W	*P. manuelae*	*P. manuelae*
EHu†	41°17.470′S	69°55.181′W	*P. spurcus*	*P. spurcus*
NYH†	41°25.666′S	69°45.144′W	*P. spectabilis*, *P. agilis*	*P. agilis*
SYH†‡	41°26.053′S	69°44.941′W	*P. spectabilis*, *P. agilis*	*P. spectabilis*
EYH†	41°26.355′S	69°41.878′W	*P. spectabilis*	–
EOA†	41°32.653′S	69°51.364′W	*P. excelsus*, *P. spurcus*	*P. excelsus*
WOA*	41°32.759′S	69°52.167′W	*P. excelsus*	–
R6 Km 2	41°21.217′S	69°34.598′W	*P. excelsus*	–
R6 Km 3.5	41°22.003′S	69°44.799′W	*P. agilis*	–
R6 Km 6	41°22.117′S	69°36.400′W	*P. spurcus*	–
Ing. Jacobacci	41°20.734′S	69°31.383′W	*P. spectabilis*	–
9 km SE Jacobacci	41°23.005′S	69°34.551′W	*P. spectabilis*, *P. excelsus*	–
Paraje Yuquiche	41°24.183′S	69°45.023′W	*P. agilis*	–

*Site from which individuals were sampled only for *COI* amplification.
†Sites from which individuals were sampled for both *COI* and *ND1–ND2*–tRNAs and *Cytb* mtDNA amplification.
‡Rocky outcrop located 2 km north of putative terra typica for *P. spectabilis*.

Appendix 2. Samples (N = 23) from which *COI* fragments were amplified at the International Barcode of Life Argentinean reference Barcode Laboratory at the Museo Argentino de Ciencias Naturales (MACN) in Buenos Aires, Argentina

iBoL sample ID	Collection ID	GenBank accession no.	Species assignation	Locality
LAGAR001-13	MACN-Bar-Herp-ct 00086	MH587773	*P. excelsus*	WOA
LAGAR002-13	MACN-Bar-Herp-ct 00087	MH587781	*P. excelsus*	WOA
LAGAR003-13	MACN-Bar-Herp-ct 00088	MH587771	*P. excelsus*	WOA
LAGAR004-13	MACN-Bar-Herp-ct 00089	MH587784	*P. excelsus*	EOA
LAGAR006-13	MACN-Bar-Herp-ct 00091	MH587791	*P. excelsus*	EOA
LAGAR009-13	MACN-Bar-Herp-ct 00094	MH587789	*P. spurcus*	EHu
LAGAR010-13	MACN-Bar-Herp-ct 00095	MH587782	*P. spurcus*	EHu
LAGAR013-13	MACN-Bar-Herp-ct 00098	MH587776	*P. spurcus*	EOA
LAGAR016-13	MACN-Bar-Herp-ct 00101	MH587792	*P. spectabilis*	NYH
LAGAR020-13	MACN-Bar-Herp-ct 00105	MH587788	*P. spectabilis*	EYH
LAGAR021-13	MACN-Bar-Herp-ct 00106	MH587790	*P. spectabilis*	EYH
LAGAR024-13	MACN-Bar-Herp-ct 00109	MH587777	*P. spectabilis*	SYH
LAGAR025-13	MACN-Bar-Herp-ct 00110	MH587786	*P. spectabilis*	SYH
LAGAR026-13	MACN-Bar-Herp-ct 00111	MH587780	*P. spectabilis*	SYH
LAGAR027-13	MACN-Bar-Herp-ct 00112	MH587779	*P. spectabilis*	SYH
LAGAR028-13	MACN-Bar-Herp-ct 00113	MH587772	*P. spectabilis*	SYH
LAGAR029-13	MACN-Bar-Herp-ct 00114	MH587774	*P. spectabilis*	SYH
LAGAR030-13	MACN-Bar-Herp-ct 00115	MH587787	*P. spectabilis*	SYH
LAGAR032-13	MACN-Bar-Herp-ct 00117	MH587775	*P. spectabilis*	NYH
LAGAR033-13	MACN-Bar-Herp-ct 00118	MH587778	*P. spectabilis*	NYH
LAGAR034-13	MACN-Bar-Herp-ct 00119	MH587785	*P. spectabilis*	NYH
LAGAR087-13	MACN-Bar-Herp-ct 00172	MH587783	*P. manuelae*	EBV
LAGAR089-13	MACN-Bar-Herp-ct 00174	MH587793	*P. manuelae*	EBV

DNA extracts are maintained at the MACN Herpetological collection.