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The aquatic and littoral forms of the Patagonian frog *Atelognathus patagonicus* (Batrachylinae): new molecular evidence

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Abstract

Atelognathus patagonicus is one of the eight species included in the Patagonian genus *Atelognathus*, an endemic frog occurring in the system of endorheic basaltic lagoons of the Laguna Blanca National Park (PNLB), Neuquén, Argentina. Based on morphological data, Cei & Roig (1968) described two forms of *A. patagonicus*, which they called "aquatic" and "littoral". These morphotypes were first suggested to belong to different species, but later, Cei (1972) proposed that both forms represent a balanced polymorphism within *A. patagonicus*. More recently, an ecomorphological study showed that aquatic and littoral are reversible forms of the same individual (phenotypic plasticity). In this paper we compare the morphotypes of *A. patagonicus* using nucleotide sequences of the mtDNA (cytochrome b and control region) in order to test the existence of genetic differentiation between the aquatic and littoral forms. In addition, we present data of genetic variability of *A. patagonicus* from the Laguna Blanca system. We did not detect genetic differentiation between littoral and aquatic morphotypes for both genes studied. This observation is consistent with the hypothesis of phenotypic plasticity. In contrast with the expected results for low vagility organisms, the diversity index observed in *A. patagonicus* revealed a low genetic variability.

Key words: *Atelognathus patagonicus*, phenotypic plasticity, mtDNA, morphotypes

Introduction

Atelognathus patagonicus (Gallardo 1962) represents one of the eight species included in the Patagonian genus *Atelognathus* (Basso 1998; Díaz-Páez *et al*. 2011, Basso *et al*. 2011). The species is an endemic frog occurring in the system of endorheic basaltic lagoons of the Laguna Blanca National Park (PNLB), Neuquén, Argentina, where the arid climate determines vegetation of low, thorny, shrubby steppe (Cei & Roig 1968; Cuello *et al*. 2009). *Atelognathus patagonicus* was first described by Gallardo (1962) for Laguna Blanca, the largest lagoon in the system. At present, the species is extinct from its type locality due to the introduction of *Percichthys colhuapiensis* and other fish species (*Oncorhynchus mykiss* and *Salmo trutta*), but persists restricted to several smaller, isolated lagoons lacking fish surrounding Laguna Blanca. Due to its restricted distribution and threats, the species is categorized "in danger of extinction" by the Argentinean Herpetological Society (Lavilla *et al*. 2000) and "Endangered" according to the IUCN Red List of Threatened Species (Úbeda *et al*. 2008).

Based on morphological data, Cei & Roig (1968) described two forms of *A. patagonicus*, which they called "aquatic" and "littoral". The aquatic form is found associated with underwater rocks and is characterized by well-developed interdigital membranes, highly vascularized skin forming undulating folds on the lateral and ventral regions of the trunk and on the thighs (bagginess), and orange-yellow ventral color. The littoral form is found beneath rocks outside the water (up to 80 m from the lagoon, Cuello *et al*. 2008) in an extremely arid environment. The littoral form shows emarginated interdigital membranes, absence of bagginess, and a grayish-white ventral coloration.

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Despite sympatric occurrence of both forms and sharing of available reproductive sites, sufficient differences in morphology have been described to suggest the occurrence of two different specific entities (Cei & Roig 1968). However, Cei (1972), in a subsequent immunological study based on serum proteins, concluded that the aquatic and littoral forms were best explained by genetically determined characters maintained in population balance.

In a recent study, Cuello *et al*. (2008) evaluated the link between both morphotypes and the environment variables in the semi-permanent pond Batea. They found that changes in the phenotype were reversible and consistent with the water level and other limnological conditions in the pond. During wet seasons, while the pond contained water, frogs of the aquatic morphotype were captured. As the water level descended, the frogs began to present littoral, aquatic, and intermediate morphotype characteristics; and when the pond dried up completely, only the littoral morphotype was found. Based on these results, Cuello *et al*. (2008) concluded that the two forms are maintained by phenotypic plasticity, enabling the frogs to take advantage of both terrestrial and aquatic environments.

To date, *Atelognathus patagonicus* taxonomy has been studied from immunological and ecomorphological approaches (Cei & Roig 1968; Cei 1972; Cuello *et al*. 2008), but no study involving DNA variability has been performed. Therefore, the aim of this work is to evaluate the existence of genetic differentiation between the aquatic and littoral forms within and between lagoons, using nucleotide sequences of mitochondrial genes. In Addition, we present data of genetic variability of *A. patagonicus* from the Laguna Blanca system.

Material and methods

A total of 85 *Atelognathus patagonicus* adults and juveniles were analyzed, representing both the aquatic (N = 60) and littoral (N = 25) morphotypes from nine lagoons and surrounding areas within and outside of the Laguna Blanca National Park (Table 1; Figure 1). After collection, tissue samples were preserved in 2-mL tubes filled with 96% ethanol and placed in a freezer at −20 °C until they were analyzed. DNA was extracted from liver or muscle using phenol/chloroform following standard procedures (Sambrook & Russell 2001). The concentration of each DNA sample was estimated by spectrophotometry and diluted to a working concentration for use in polymerase chain reactions (PCR).

TABLE 1. Geographic location of sampling sites and number of individuals sequenced.

Sampling Sites	Coordinates		N	$N_{cyt\ b}$	N_{CR}
Burro	39°06'51.0"S	70°24'42.9"W	9	9	5
Molle	39°00'48.6"S	70°24'58.1"W	11	11	8
Hoyo	39°00'41.9"S	70°25'54.3"W	15	15	11
Batea	39°02'02.9"S	70°24'36.0"W	13	13	6
Tero	39°06'50.6"S	70°25'09.3"W	3	3	2
Overo	39°01'05.3"S	70°25'43.9"W	11	9	9
Jabón	38°58'51.9"S	70°22'14.7"W	14	13	10
Antiñir	38°59'08.6"S	70°23'50.4"W	3	3	2
Flamencos	38°54'28.7"S	70°23'10.1"W	6	5	4
Total			85	81	57

We amplified a ~650 bp section of the cytochrome b gene (c*yt b*) and ~580 bp of the control region (*CR*) mitochondrial DNA. The primers used to amplify cytochrome b were MVZ15-L (GAACT AATGG CCCAC ACWWT ACGNA A) and MVZ16-H (AA ATAGG AARTA TCAYT CTGGT TTRAT) (Goebel *et al*. 1999); and for control region ControlWRev-L (GACAT AYTAT GTATA ATCGA GCATT CA) and ControlP-H (GTCCA TAGAT TCAST TCCGT CAG) (Goebel *et al*. 1999). PCR cocktails (25 µL) were prepared containing, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 0.3 µM each primer, and 0.5 units of *Taq* DNA polymerase (Invitrogen) and approximately 50 ng whole genomic DNA. PCR products were amplified using a standard protocol: initial denaturation at 94ºC for 2 min, 40 cycles of 94ºC for 30 s, 45ºC for 45 s and 72°C for 2 min, and a

final extension at 72°C for 6 min. PCR products were sequenced on an ABI 3130 capillary genetic analyzer (Applied Biosystems, Inc.). Sequencing reactions were executed using the standard protocol for Big Dye Terminators v. 3.1 (Applied Biosystems). All samples were sequenced in both directions and the contigs made using DNA Baser v. 3 (Heracle BioSoft, Pitesti, Romania). Alignments were optimized manually using BioEdit 7.0.9.0 (Hall 1999).

Genetic differentiation among localities was assessed by calculating pairwise Fst values following Hudson *et al*. (1992). Significance of the Fst statistic was determined by bootstrap test with 1000 replications in ARLEQUIN v.3.0 (Excoffier *et al*. 2005). We also used ARLEQUIN to estimate the number of haplotypes (*n*), haplotype diversity (*h*), and nucleotide diversity (π) incorporating Jukes & Cantor's (1969) model of sequence evolution as recommended by Nei & Kumar (2000). Tajima's (1989) *D* and Fu's (1997) F_S tests were calculated to detect departures from neutrality. A statistical parsimony network was constructed using TCS v.1.21 (Clement *et al*. 2000), which identifies unrooted cladograms that have a high probability (> 95%) of being true based on a finite-site model of DNA evolution (Templeton *et al*. 1992).

Results

From each of the nine sample sites, 653 and 581 bp nucleotides were analyzed for *cyt b* and *CR* mitochondrial DNA genes, respectively. The pairwise Fst values were non significant between lagoons. Therefore, in order to estimate the diversity indexes we consider the Laguna Blanca system as a unique population with nine sample sites. The amplified *cyt b* segment was obtained from 81 individuals representing both morphotypes (Table 1). A total of 9 variable sites (1.37 %) produced 7 haplotypes. The haplotype diversity (*h*) was 0.41, and the nucleotide diversity (π) was 0.16 %. The amplified *CR* segment was obtained from 57 individuals representing both morphotypes (Table 1). Only one variable site (0.17 %) produced 2 haplotypes. The haplotype diversity (*h*) was 0.49, and the nucleotide diversity (π) was 0.08 % between samples.

No significant departure from neutrality was found for both studied genes using the *D* statistic from Tajima's test (*cyt b* = -1.08, *p* = 0.13; *CR* = 1.64, *p* = 0.95) and *Fs* statistic in Fu's test (*cyt b* = 0.24, *p* = 0.28; *CR* = 1.97, *p* = 0.79), which is considered to be one of the most powerful tests for detecting population expansion (Ramos-Onsins & Rozas 2002). The neutrality hypothesis, tested using Ewens-Watterson (Ewens 1972; Watterson 1978), was not rejected in any of the cases.

The haplotype network obtained using the software TCS (Figure 2) for *cytb* shows a dominant haplotype present in all lagoons, which belongs to individuals with both aquatic and littoral morphotypes (haplotype H1). Four haplotypes were exclusive to a single lagoon (H4 to Molle, H5 and H6 to Hoyo, and H7 to Tero lagoons). The maximum number of individuals sharing a haplotype was 61, but we identified as many as 4 singletons (Table 2).

TABLE 2. Detail of the number of individuals with aquatic (A) and littoral (L) morphotypes for each haplotype obtained from the cytochrome b (*cyt b*) and control region (*CR*) sequences in each of the lagoons analyzed.

Haplotype	Sampling Sites									Total (Frequency)
	Burro	Molle	Hoyo	Batea	Tero	Overo	Jabón	Antiñir	Flamencos	
cyt b										
H1	3 A / 2 L	5 A / 4 L	3 A / 5 L	5 A / 7 L	1 A / 0 L	8 A / 0 L	11 A / 0 L	2 A / 0 L	5 A / 0 L	61 (75.3 %)
H2	1 A / 3 L	----------	3 A / 1 L	0 A / 1 L	1 A / 0 L	1 A / 0 L	1 A / 0 L	----------	----------	12 (14.8 %)
H3	----------	0 A / 1 L	1 A / 0 L	----------	----------	----------	1 A / 0 L	1 A / 0 L	----------	4 (4.93 %)
H4	----------	1 A / 0 L	----------	----------	----------	----------	----------	----------	----------	1 (1.23 %)
H5	----------	----------	1 A / 0 L	----------	----------	----------	----------	----------	----------	1 (1.23 %)
H6	----------	----------	1 A / 0 L	----------	----------	----------	----------	----------	----------	1 (1.23 %)
H7					1 A / 0 L					1 (1.23 %)
RC										
H8	2 A / 2 L	2 A / 1 L	3 A / 3 L	1 A / 4 L	2 A / 0 L	5 A / 0 L	6 A / 1 L	----------	3 A / 0 L	35 (61.4 %)
H9	1 A / 0 L	3 A / 3 L	5 A / 0 L	0 A / 1 L	----------	4 A / 0 L	3 A / 0 L	2 A / 0 L	1 A / 0 L	23 (40.35 %)

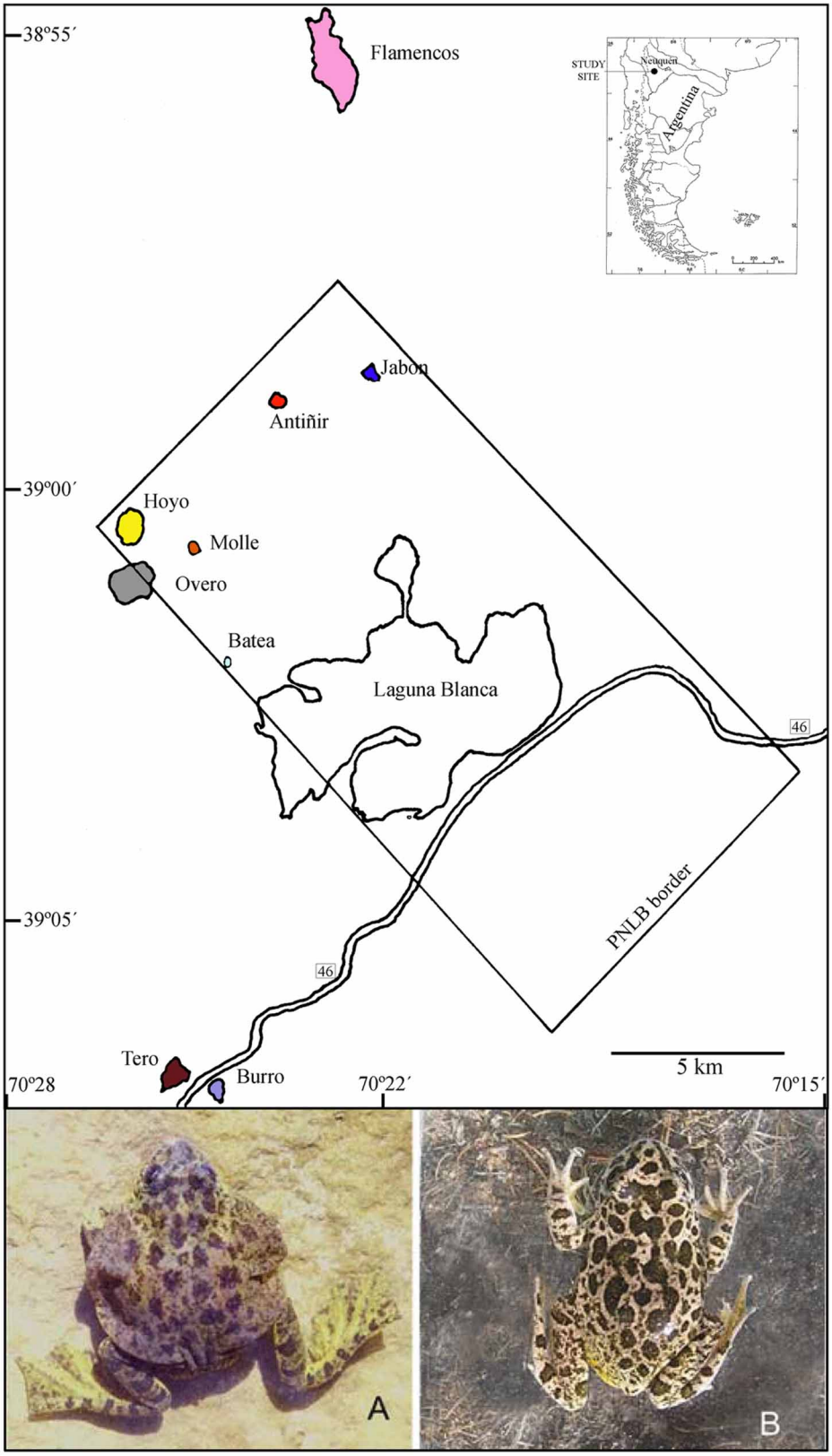

FIGURE 1. Above: Localities sampled in the mid-west of Neuquén Province, Argentina. The rectangle shows the boundary of Laguna Blanca National Park (PNLB). Samples were collected from lagoons located within and outside PNLB (Burro, Molle, Hoyo, Batea, Tero, Overo, Jabón, Antiñir and Flamencos lagoons). Below: *Atelognathus patagonicus.* A): Dorsal view of aquatic morphotype, B): Dorsal view of littoral morphotype. Photographs by C.A. Úbeda & M.E. Cuello, respectively.

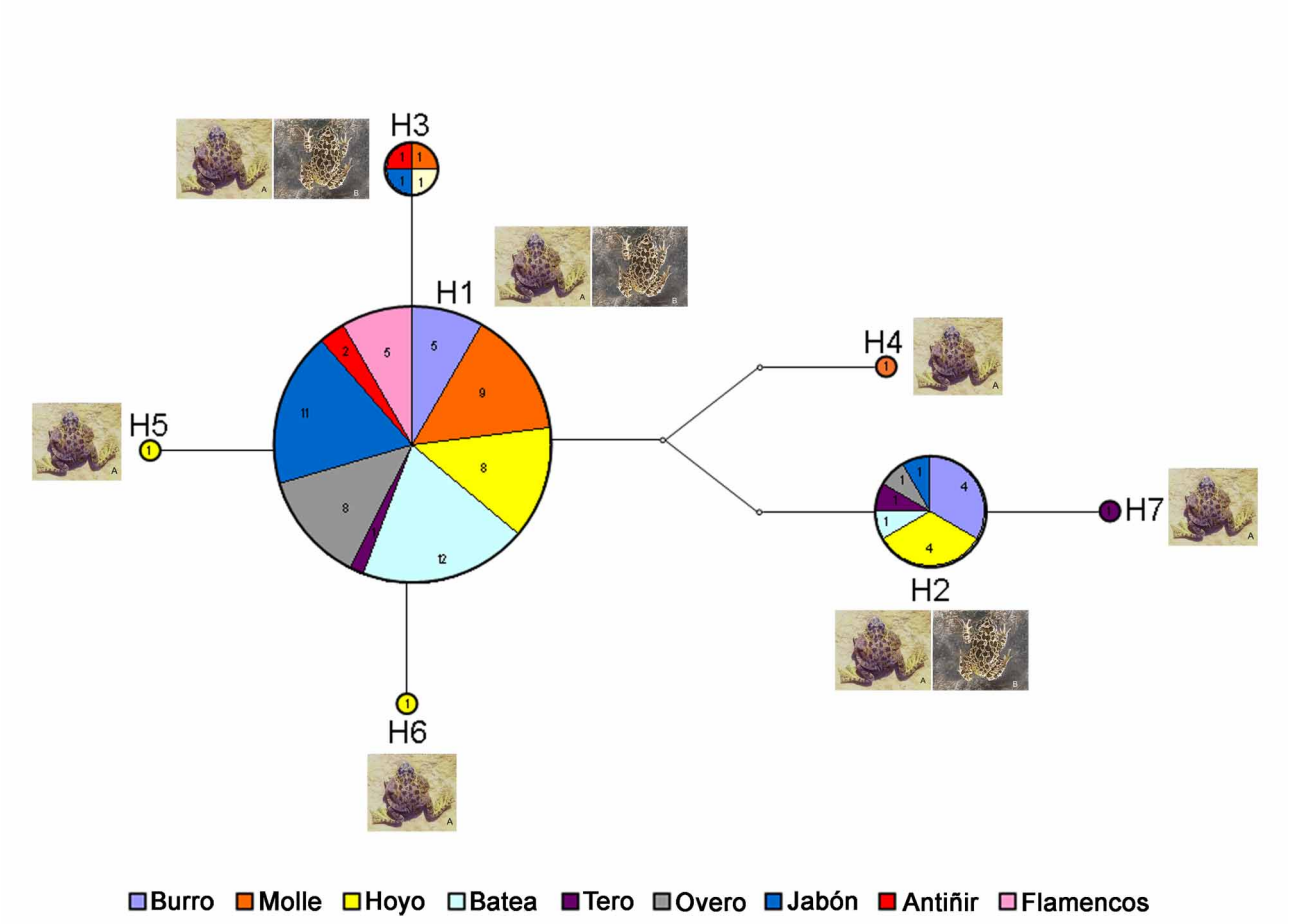

FIGURE 2. TCS Minimum spanning network tree among haplotypes of *cyt b* mitochondrial DNA from *Atelognathus patagonicus*. Each circle represents a unique haplotype; the area of each circle is proportional to the number of individuals. Each color slice within circles represents a lagoon (as shown in Figure 1) and shows the number of individuals for the haplotype in such lagoon. The link between haplotypes represents a single mutational event. Aquatic morphotype, photograph A, Littoral morphotype, photograph B.

Discussion and conclusions

As far as we know, this is the first molecular study performed on *Atelognathus patagonicus* populations. The littoral and aquatic forms of *A. patagonicus* were compared through molecular markers (nucleotide sequences of the genes cytochrome b and control region of the mitochondrial DNA). The results of the haplotype network demonstrates haplotype sharing, where all the haplotypes present in the littoral form are also found in the aquatic form (H1, H2, H3, H8, H9). The presence of rare haplotypes in the aquatic form might be explained by the difference in sample size (aquatic N = 60, littoral N = 25). The result of the haplotype network confirm the hypothesis of phenotypic plasticity proposed by Cuello *et al*. (2008), who suggested that the morphological differences found between the two forms are the result of a reversible phenotypic condition and not due to the existence of two cryptic species, as was suggested by Cei & Roig (1968), nor to the existence of a balanced polymorphism (Cei 1972).

Tajima's *D* and Fu´s *Fs* statistics were not significant, showing the lack of evidence for selective effects, like population expansion, bottleneck, or heterogeneity of mutation rates. Therefore, we may consider that the *A. patagonicus* population from Laguna Blanca system is in demographic equilibrium.

The low number of haplotypes observed in the *CR* gene is quite unexpected, because the mutation rate in *CR* is usually higher than other mtDNA molecules (Baker & Marshall 1997, Saccone *et al.* 2000). Nevertheless, recent studies have shown the existence of the same pattern of variation in different groups of animals (Roukonen & Kvist

2002, Brehm *et al*. 2003, Zink & Weckstein 2003, Samuels *et al.* 2005, Ujvari *et al*. 2005, Tang *et al*. 2006, Matsui *et al*. 2007).

It is well known that amphibians exhibit strong site fidelity (Waldmann & McKinnon 1993), have patchy geographical distributions due to specific and complex habitat requirements and low dispersal capacity (Stebbins & Cohen 1995). However the genetic homegeneity observed in *A. patagonicus* from the lagoons of the Laguna Blanca system may be the result of three possible scenarios: 1) Water level was higher during late Pleistocene times, enabling gene flow among individuals of a single ancestral population, 2) migration occurs mainly due to the movement of individuals with the littoral morphotype because they are better adapted to the xeric environment, or 3) individuals with aquatic morphotype migrate during times of exceptional rainfall and subsequent water flow.

Due to the restricted distribution and the lability of the environments they inhabit, *A. patagonicus* is of high priority for conservation among Argentinean anurans. Not only is it important to have detailed knowledge of the genetic variability of its populations, but it is also necessary to conduct further studies to learn about its eco-physiological requirements, which will provide useful information for designing effective conservation plans.

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References

Baker, A.J. & Marshall, H.D. (1997) Mitochondrial control region sequences as tools for understanding evolution. *In*: Mindell, D.P. (Ed.), *Avian Molecular Evolution and Systematics*. Academic Press, New York, pp. 51–82.

Basso, N.G. (1998) A new telmatobiine leptodactylid frog of the genus *Atelognathus* from Patagonia. *Herpetologica*, 54, 44–52.

Basso, N.G., Úbeda, C.A., Bunge, M.M. & Martinazzo, L.B. (2011) A new genus of neobatrachian frog from southern Patagonian forests, Argentina and Chile. *Zootaxa*, 3002, 31-44.

Brehm, A., Harris, D.J., Alves, C., Jesus, J., Thomarat, F. & Vicente, L. (2003) Structure and evolution of the mitochondrial DNA complete control region in the lizard *Lacerta dugesii* (Lacertidae, Sauria). *Journal of Molecular Evolution*, 56, 46–53.

Cei, J.M. & Roig, V.G. (1968) Telmatobinos de las lagunas basálticas de Neuquén (*Anura, Leptodactylidae*). *Physis*, 27, 265–284.

Cei, J.M. (1972) Herpetología Patagónica. V. Las especies extra-cordilleranas alto patagónicas del género *Telmatobius*. *Physis*, 31, 431–449.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657–1659.

Cuello, M.E., Úbeda, C.A. & Bello, M.T. (2008) Relationship between morphotypes of *Atelognathus patagonicus* (Anura, Neobatrachia) and environmental conditions: evidence and possible explanation. *Phyllomedusa*, 7, 35–44.

Cuello, M.E., Perotti, M.G. & Iglesias, G.J. (2009) Dramatic decline and range contraction of the endangered Patagonian frog *Atelognathus patagonicus* (Anura, Leptodactylidae). *Oryx*, 43, 443–446.

Díaz-Páez, H., Vidal, M.A., Ortiz, J.C., Úbeda, C.A. & Basso, N.G. (2011). Taxonomic identity of the patagonian frog *Atelognathus jeinimenensis* (Anura: Neobatrachia) as revealed by molecular and morphometric evidence. *Zootaxa*, 2880, 20-30.

DNA Baser Sequence Assembler Software, v. 3.0. 2010. Heracle BioSoft, online manual. Available from: http://www.dnabaser.com/help/manual.html.

Ewens, W.J. (1972) The sampling theory of selectively neutral alleles. *Theoretical Population Biology*, 3, 87–112.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47–50.

Fu, Y.X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915–925.

Gallardo, J.M. (1962) Los géneros *Telmatobius* y *Batrachophrynus* (Amphibia, Leptodactylidae) en la Argentina. *Neotropica*,

8, 45–58.
Goebel, A.M., Donnelly, J.M. & Atz, M.E. (1999) PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Molecular Phylogenetics and Evolution*, 11, 163–199.
Hudson, R.R., Slatkin, M. & Maddison, W.P. (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, 132, 583–589.
Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
Jukes, T. & Cantor, C. (1969) Evolution of protein molecules. *In*: Munro, H.N. (Ed) *Mammalian Protein Metabolism.* Academic Press, New York, pp. 21–132.
Lavilla, E.O, Ponssa, M.L. (Coords.), Baldo, D., Basso, N., Bosso, A., Céspedez, J., Chebez, J.C., Faivovich, J., Ferrari, L., Lajmanovich, R., Langone, J.A., Peltzer, P., Úbeda, C., Vaira, M. & Vera Candioti, F. (2000) Categorización de los Anfibios de Argentina. *In*: Lavilla, E.O., Richard, E. & Scrocchi, G.J. (Eds), *Categorización de los Anfibios y Reptiles de la República Argentina*. Asociación Herpetológica Argentina, San Miguel de Tucumán, Argentina, pp. 11–34.
Matsui, M., Tominaga, A. Hayashi, T. Misawa, Y. & Tanabe, S. (2007) Phylogenetic relationships and phylogeography of *Hynobius tokyoensis* (Amphibia: Caudata) using complete sequences of cytochrome b and control region genes of mitocondrial DNA. *Molecular Phylogenetics and Evolution*, 44, 204–216.
Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.
Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, 19, 2092-2100.
Roukonen, M. & Kvist, L. (2002) Structure and evolution of the avian mitochondrial control region. *Molecular Phylogenetics and Evolution*, 23, 422–432.
Saccone, C., Gissi, C., Lanave, C., Larizza, A., Pesole, G. & Reyes, A. (2000) Evolution of the mitochondrial genetic system: an overview. *Gene*, 261, 153–159.
Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, New York, Vol. 1. Third Edition.
Samuels, A.K., Weisrock, D.W., Smith, J.J., France, K.J., Walker, J.A., Putta, S., Voss, S.R. (2005) Transcriptional and phylogenetic analysis of five complete ambystomatid salamander mitochondrial genomes. *Gene*, 349, 43–53.
Stebbins, R.C. & Cohen, N.W. (1995) *A Natural History of Amphibians***.** New Jersey, Princeton University Press, 316 pp.
Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polimorphism. *Genetics*, 123, 585–595.
Tang, Q.G., Liu, H.Z., Mayden, R. & Xiong, B.X. (2006) Comparison of evolutionary rates in the mitochondrial DNA cytochrome *b* gene and control region and their implications for phylogeny of the Cobitoidea (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution*, 39, 347–357.
Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, 132, 619–633.
Úbeda, C., Lavilla, E. & Basso, N. (2008) *Atelognathus patagonicus*. *In*: *IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4*. Available from: http://www.iucnredlist.org (18 February 2011).
Ujvari, B., Madsen, T. & Olsson, M. (2005) Discrepancy in mitochondrial and nuclear polymorphism in meadow vipers (*Vipera ursinii*) questions the unambiguous use of mtDNA in conservation studies. *Amphibia-Reptilia* 26, 287–292.
Waldmann, B. & McKinnon, J.S. (1993) Inbreeding and outbreeding in fishes, amphibians and reptiles**.** *In:* Thornhill, N.W. (Ed) *The natural history of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives.* University of Chicago Press, Chicago, pp 251-282.
Watterson, G. (1978) The homozygosity test of neutrality. *Genetics*, 88, 405–417.
Zink, R.M. & Weckstein, J.D. (2003) Recent evolutionary history of the Fox Sparrows (Genus: *Passerella*). *The Auk*, 120, 522–527.