Zootaxa 2880: 20–30 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Taxonomic identity of the patagonian frog *Atelognathus jeinimenensis* (Anura: Neobatrachia) as revealed by molecular and morphometric evidence

HELEN DÍAZ-PÁEZ[1,4], MARCELA A. VIDAL[2], JUAN C. ORTIZ[3,4], CARMEN A. ÚBEDA[5] & NÉSTOR G. BASSO[6]

[1]*Departamento de Ciencias Básicas, Campus Los Ángeles, Universidad de Concepción. Casilla 341, Los Ángeles, Chile*
[2]*Laboratorio de Genómica y Biodiversidad, Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bío-Bío. Casilla 447, Chillán, Chile*
[3]*Departamento de Zoología, Universidad de Concepción. Casilla 160-C. Concepción, Chile*
[4]*Centro de Investigación en Ecosistemas de la Patagonia (CIEP)*
[5]*Departamento de Zoología, Centro Regional Bariloche, Universidad Nacional del Comahue, Quintral 1250, R 8400 FRF - Bariloche, Argentina*
[6]*Centro Nacional Patagónico (CENPAT-CONICET). Bvd. Brown 2915, U9120ACF Puerto Madryn, Chubut, Argentina*
[1]*Corresponding author. E-mail: hediaz@udec.cl*

Abstract

The frog genus *Atelognathus* is currently represented by nine species distributed in Argentinean and Chilean Patagonia. It is mainly distributed in Argentina, and there are only three species in Chile (*A. ceii*, *A. grandisonae* and *A. jeinimenensis*). Regarding the morphological relationships among *Atelognathus* species, Meriggio *et al.* (2004) suggest that *A. jeinimenensis* is more related to *A. salai* than other species. *A. salai* was described from Laguna Los Gendarmes (Argentina), 90 km air line from the type locality of *A. jeinimenensis*. This paper presents a morphological analysis and a study of population genetics using mtDNA nucleotide data from Argentinean and Chilean localities to assess the genetic distance between *A. salai* and *A. jenimenensis*. We obtained 477 bp-long *d-loop* sequences from 51 *Atelognathus* specimens collected in four localities. According to our results, the morphological differences between *A. salai* and *A. jeinimenensis* populations are limited to size, and there are no distinctive characteristics that would separate two species, in addition to which a simple geographic pattern of genetic diversity suggests a single species of *Atelognathus.* Also, the populations from Chile (Cerro Castillo, RN Lago Jeinimeni and Chile Chico) and Argentina (Laguna de Los Gendarmes) have low levels of genetic divergence that may be consistent with glaciations during the Late Pleistocene. We propose *Atelognathus jeinimenensis* as a junior synonym of *A. salai* and that the Chilean populations should be assigned to *A. salai*.

Key words: d-loop, haplotypes network, mtDNA, population genetics, taxonomic, morfology, Anura, *Atelognathus salai*, *Atelognathus jeinimenensis*

Introduction

The frog genus *Atelognathus* Lynch, 1978 is currently represented by nine species with distribution in the Argentinean and Chilean Patagonia, between 38º 40' and 49º S (Basso 1998). According to Lynch (1978), the genus *Atelognathus* is diagnosed primarily by the presence of a large, exposed frontoparietal fontanelle, short palatine bones, large nasals, and the absence of quadratojugals, columella, tympanic annuli, and cavity tympani. According to paleoenvironmental data, the genus would have had a wide ancestral Patagonian distribution, which could have been fragmented as a result of paleoclimatic changes in the area (Lynch 1978; Cei 1984; Cei & Roig 1968). Patagonia was affected by repeated glaciations during Plio-Pleistocene times, which shaped it into its present physical and biological configuration (Villagrán *et al.* 1986; Denton *et al.* 1999; Moreno & León 2003). During the Last Glacial Maximum (LGM) (~26,000–17,500 cal yr BP [calendar years before present]), piedmont glacier lobes covered vast areas on both sides of the Andes range. Palynological studies suggest millennial-scale changes in temperature and hydrological balance during the LGM, with mean summer temperatures during the coldest stadials around 6–8ºC lower than present (Heusser *et al.* 1999; Moreno *et al.* 1999; Lamy *et al.* 2007).

 Accepted by M. Vences: 25 Feb. 2011; published: 17 May 2011

Atelognathus is mainly distributed in Argentina, and only three species are present in Chile (*A. ceii*, *A. grandisonae* and *A. jeinimenensis*). These species are narrowly distributed and only known from their type localities (Lynch 1975; Basso 1998; Meriggio *et al*. 2004). Cei (1984) described *Atelognathus salai* from Laguna de los Gendarmes, Santa Cruz Province (46º 06'S, 71º41'W, ca. 1050 m elevation), Argentina; 90 km air line from the type locality of *A. jeinimenensis*. Based on morphological data, Meriggio *et al.* (2004) suggested that *A. jeinimenensis* is more closely related to *A. salai* than to any other species in the genus. However, when the inter-population variability between *A. salai* and *A. jeinimenensis* is taken into account, the differences in morphology reported by Meriggio *et al.* (2004) are of dubious taxonomic value, probably due to the fact that the comparisons are based entirely on bibliographical references.

During the spring-summer season of 2007–2008, several specimens of *Atelognathus* assignable to both *A. salai* and *A. jeinimenensis* were collected in Chilean localities, close to the Argentinean border, representing new localities covering a distributional gap between the type localities of both species. The morphological similarity, geographical proximity and the absence of geographic barriers between *A. salai* and *A. jeinimenensis*, encouraged us to analyze their taxonomic status based on molecular data and adding new morphometric analyses. Herein, we present a population genetic study of the species complex *Atelognathus salai – jeinimenensis* using mtDNA nucleotide data. Additionally, we discuss the role of paleoenvironmental changes associated with Quaternary glaciations (the last ~2 million years) in the geographic and genetic structure of *A. salai - jeinimenensis* populations.

Material and methods

Morphometrics analyses. The material analysed comes from four Patagonian localities: Laguna de los Gendarmes and Reserva Nacional Lago Jeinimeni (topotype specimens of *A. salai* and *A. jeinimenensis* respectively), and two new locality records: Chile Chico and Cerro Castillo, XI Region, Chile. It consists of 26 specimens from the Chilean localities (Table 1; Figure 1) stored at the Museo de Zoología de la Universidad de Concepción (MZUC 36572-36575, 36577-36580; 36584-36586; 36589; 36404-36408; 36410-36411; 36414, 36418; 36770-36772; 36763; 36767), and 23 *A. salai* specimens from the type locality, stored in the herpetology collection at Centro Nacional Patagónico (CENPAT-LASBA 96.3-96.8; 96.10-96.18; 08.01-08.06).

TABLE 1. Localities and number of *Atelognathus* individuals analyzed for morphology and DNA sequenced in this study.

Locality	Coordinates	N morphology	N genetics
Argentinean locality			
Laguna de los Gendarmes, (Type locality)	46º06'S, 71º41'W	23	8
Chilean localities			
Reserva Nacional Lago Jeinimeni (Type locality)	46º50'S, 71º59'W	4	7
Cerro Castillo	46º04'S, 72º16'W	20	24
Chile Chico	46º32'S, 72º16'W	2	12

The following morphometric features were measured in adult specimens: (1) snout-vent length; (2) head width; (3) head height; (4) nostril-eye distance; (5) nostril-mouth distance; (6) internostril distance; (7) interorbital width; (8) eye diameter; (9) arm length, (10) forearm length, (11) hand length; (12) femur length; (13) tibia length; (14) foot length. Each trait was log10 transformed to conduct parametric statistic analyses. Measurements were taken to the nearest 0.01 mm using a calliper under a stereoscopic microscope, following Cei (1980).

Geographic variation of morphological characters was assessed using ANOVA, and Principal Components Analysis (PCA). All morphological analyses were performed using SPSS13.0 (Rivas 2009). The principal components explaining at least 70% of total variance were graphed.

DNA sampling and laboratory protocols. Total tissue samples of 51 toad digits were analyzed from four Patagonian localities (Table 1). Total genomic DNA was isolated from samples using a salt-extraction protocol (Aljanabi & Martínez 1997). A partial Control Region (*d-loop*, 477 bp) gene was amplified and sequenced with primers ControlWRev-L: (GACAT AYTAT GTATA ATCGA GCATT C) and ControlP-H (GTCCA TAGAT TCAST TCCGT CAG) (Goebel *et al*. 1999). Five microliters of extraction product were electrophoresed on 1% agarose gel to estimate the quality and amount of genomic DNA, and sample dilutions were performed (100ng/µL)

for polymerase chain reaction (PCR) amplification. The *d-loop* gene region was amplified via PCR in 25µL of reaction volume containing 0.25µL Taq (Invitrogen), 5µL of PCR Buffer 10X, 4µL $MgCl_2$ 50mM, 1µL dNTPs 100mM, 1.5µL of each primer (10pmol), and 1µL of template DNA. The thermal cycling amplification conditions were as follows: initial denaturation at 96 °C for 5 min, followed by 39 cycles of strand denaturation at 94 °C for 30 s, annealing at 50°C for 0.45 s, primer extension at 72 °C for 2 min, and a final 10 min extension at 72 °C. Size of PCR products was checked by comparing with a 100 bp DNA ladder (Invitrogen) on 2% agarose gel. Amplified DNA was purified with the QIAquick PCR Kit according to the supplier's protocol (Qiagen, USA). Finally, all samples were sequenced in the forward and reverse direction with an automated DNA-sequencer ABI3130 (Applied Biosystems). Sequence alignments were accomplished using Proseq (Filatov 2002), and checked by eye.

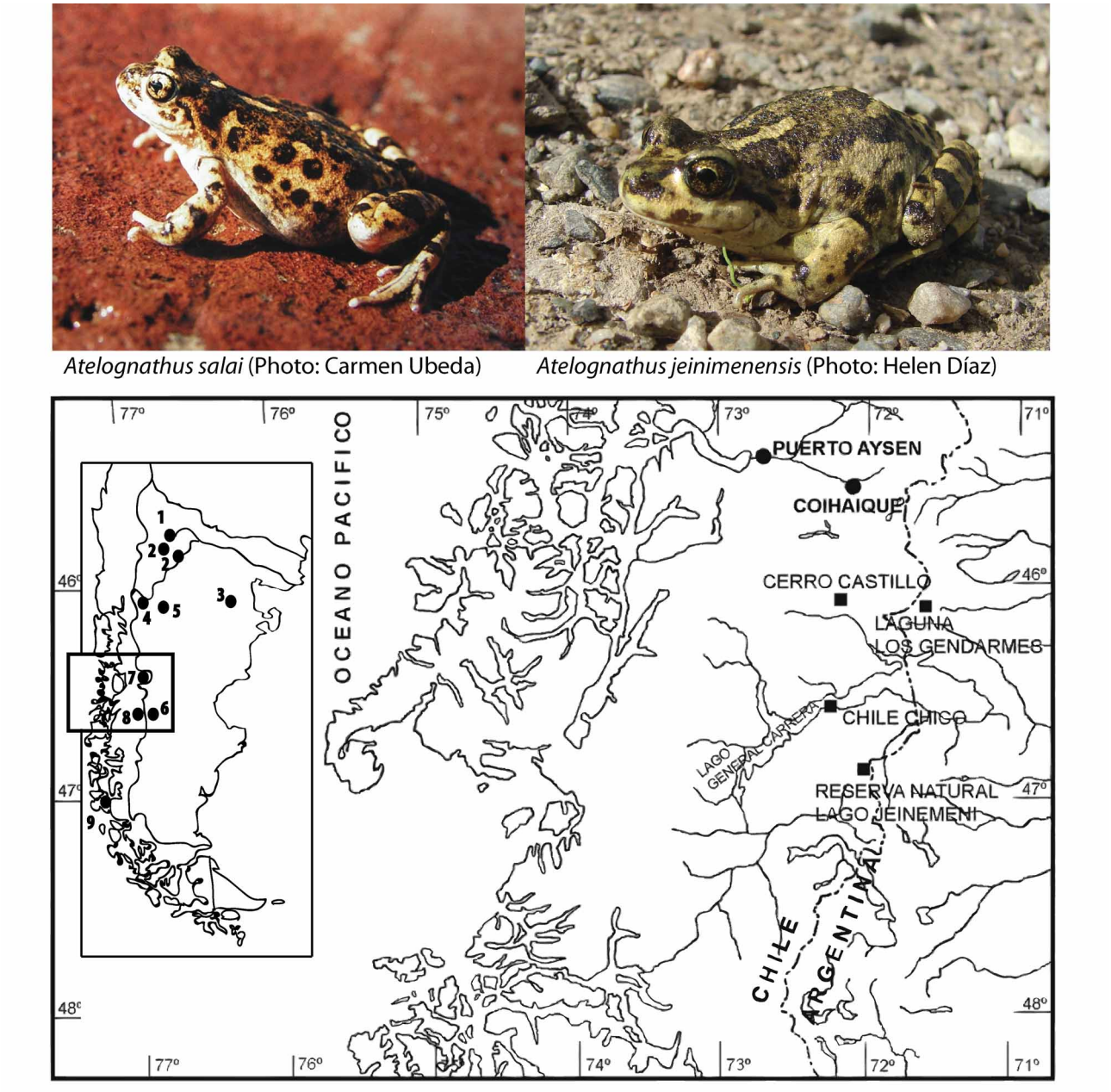

FIGURE 1. On the map, both *Atelognathus* species considered in this study. The photopgraphs correspond to adults of *A. salai* female from Laguna de los Gendarmes (not collected), and *A. jeinimenensis* male from Cerro Castillo (MZUC 36579). Below are types localities of all nine *Atelognathus* species in South America (1: *A. patagonicus*, 2: *A. praebasalticus*, 3: *A. reverberii*, 4: *A. nitoi*, 5: *A. solitarius*, 6: *A. salai*, 7: *A. ceii*, 8: *A. jeinimenensis*, 9: *A. grandisonae*) and the box shows the localities analyzed in this study.

For population genetics analysis we computed median-joining network using the software Network 4.5.0.0 (Bandelt *et al*. 1999) to interpret the relationships and geographical partitioning among haplotypes. To assess mitochondrial genetic diversity within *Atelognathus* populations, we calculated the number of haplotypes (K), number of polymorphic sites (S), nucleotide diversity (H) and number of pairwise differences (∏) using the software DNAsp (Rozas *et al*. 2003). Additionally, we tested whether the pattern of observed polymorphism within populations is consistent with a neutral equilibrium Wright–Fisher model using Fu's Fs (Fu 1997). This statistic takes on a negative value with an excess of rare haplotypes. Such a finding may occur under scenarios of background selection, selective sweeps, or population expansions. However, Fs have proved to be the most sensitive statistics with regard to demographic expansion (Fu 1997; Ramos-Onsins & Rozas 2002). Finally, Tajima's D statistics were calculated to distinguish between a DNA sequences evolving randomly or "neutrally" versus ones evolving under a non-random process as selection, demographic expansion-contraction, or introgression (Tajima 1989). Fu's Fs and Tajima test were computed using the software DNAsp (Rozas *et al.* 2003).

Analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) was performed using the SAMOVA software (Dupanloup *et al*. 2002) to study the proportion of total genetic variation attributable to different hierarchical levels based on the geographic distribution of haplotypes. Several groupings of populations were tested to maximize the among-group component of molecular variance, i.e. to determine the maximum degree of phylogeographical structure present in the data (Liebers & Helbig 2002). Finally, 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 (Schneider *et al*. 2000).

Results

Morphometrics analyses

No sex-linked differences were detected in body size (MANOVA: Wilks' Lambda = 1.703, p = 0.10), therefore subsequent analyses considered all adult individuals together, regardless of sex. Nevertheless, significant differences exist in body size among populations as a function of locality (ANOVA: F = 2.926; g.l. = 1.48; p = 0.094). In general, body size showed an increase in the Argentinean population, with smaller sized individuals corresponding to the Chilean population (MANOVA, Wilks' Lambda = 0.477, p<0.01; Table 2).

TABLE 2. Morphometric measurements of *Atelognathus* species from Argentina and Chile populations. Data are shown as mean ± standard deviation.

	Argentinean Locality (n= 23)	Chilean localities (n=26)	Total (n=49)
Snout-Vent length	35.0±4.8	31.9±4.8	33.3±5.0
Head width	11.9±0.4	11.1±1.6	11.5±1.2
Head height	12.3±0.7	11.2±1.4	11.7±1.2
Nostril-eye distance	2.7±0.3	2.4±0.5	2.6±0.4
Nostril-mouth distance	3.2±0.3	2.8±0.4	3.0±0.4
Internostril distance	2.5±0.2	2.3±0.3	2.4±0.3
Interorbital width	3.0±0.6	2.6±0.4	2.7±0.5
Eye diameter	3.7±0.3	3.6±0.4	3.7±0.3
Arm length	5.2±0.6	4.7±1.2	4.9±1.0
Forearm length	7.0±0.6	6.5±1.1	6.7±0.9
Hand length	9.1±0.6	8.6±1.2	8.8±1.0
Femur length	13.4±1.2	13.2±1.9	13.3±1.6
Tibia length	14.2±0.9	13.0±2.1	13.5±1.7
Foot length	22.1±1.4	20.3±3.4	21.1±2.8

Because of the high colinearity among the predictor variables, we performed a PCA, including all variables in the model. The results of this analysis revealed that the fourteen variables in the model were reduced to two PCA axes, which accounted for 75.35% of the variance (Table 3). The first component axis (PCA1) was strongly positively correlated with most of the variables; and the second axis (PCA2) was significantly correlated with Interorbital width, Femur length and Eye diameter. In the graph there is no grouping in different species (Figure 2).

TABLE 3. PCA axes derived from analysis of morphological features in *Atelognathus* species.

Factor loadings	PCA axis 1	PCA axis 2
Snout-Vent length	0.80	0.04
Head height	0.88	-0.05
Head width	0.92	0.02
Nostril-eye distance	0.87	0.03
Nostril-mouth distance	0.87	-0.19
Internostril distance	0.89	0.02
Interorbital width	0.51	-0.79
Eye diameter	0.69	0.32
Arm length	0.72	0.13
Forearm length	0.89	-0.01
Hand length	0.86	-0.19
Femur length	0.76	0.41
Tibia length	0.97	0.01
Foot length	0.82	0.06
Explained variance (%)	68.21	7.14
Explained variance cumulative (%)	68.21	75.35

The descriptor trait assigned by Meriggio *et al.* (2004) to the species *A. jeinimenensis* as "absence of dermal fringe, is not found in the specimens from Chilean localities. Macroscopic analysis indicates that both Chilean and Argentine populations have a dermal fringe, which is present in all the individuals analysed (Figure 3). Similarly, there is no difference in finger length, which varies within and among populations (Figure 3).

Population genetics. We obtained 477 bp-long *d-loop* sequences from 51 *Atelognathus* specimens collected from four localities (Table 1). The network of *d-loop* sequences (Figure 4) shows the genealogical relationships among the 17 haplotypes connected through a maximum of 18 mutational steps. A single dominant haplotype was observed, in which related sequences were separated by one to four mutational steps. This principal haplotype was shared among all populations.

The number of haplotypes per locality ranged from one (Laguna de los Gendarmes) to eleven (Cerro Castillo). Fourteen nucleotid sites (2.9%) were found to be polymorphic. The mean number of pairwise sequence differences ranged from 0.57 to 2.86, except in the samples from Laguna de los Gendarmes, which are represented by a single haplotype (Table 4). Additionally, Fu's Fs supports the demographic expansion for Cerro Castillo and RN Lago Jeinimeni localities, rejecting stasis at the 0.01 significance level (Table 4). The Fs values were mostly negative as well, but for Chile Chico, this value was not significant (Table 5). Tajima's test showed negative, non-significant ($p < 0.05$) values in all localities. A nested analysis of variance was maximized with a model of three groups (Laguna de los Gendarmes, Cerro Castillo, and RN Lago Jeinimeni-Chile Chico), applied to the *Atelognathus* localities, accounting for 25.7% of the molecular variance among groups and 79% within population. The pairwise Fst values ranged from -0.052 to 0.365. Five of six comparisons were significant ($P < 0.01$), where Chile-Chico-RN Lago Jeinimeni comparison showed no differences.

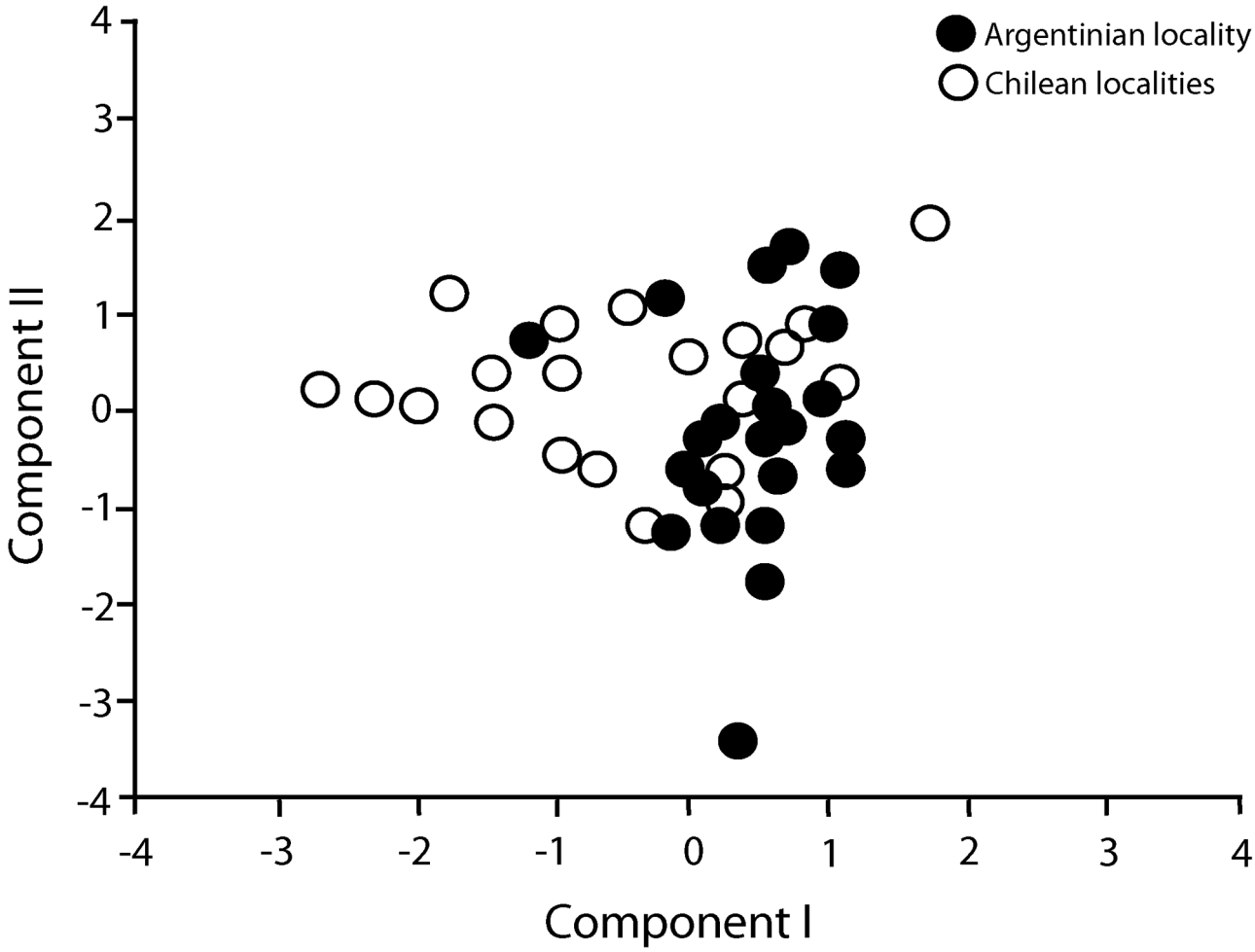

FIGURE 2. Representation of the morphological variation in *Atelognathus* generated from the first two axes in the Principal Component Analysis (PCA).The Principal component (CPi=ith principal component; centroid at 95%) explained the 75.18% of the variance.

TABLE 4 Diversity parameters for four *Atelognathus* localities estimated from mtDNA *d-loop* sequences. Number of individuals (n); number of haplotypes (K), haplotypic diversity (H), number of polymorphic sites (S), mean number of pairwise sequences differences (∏), Fu FS test (Fu), Tajima test (D). Significance levels: **P < 0.01, * P < 0.05.

Locality	n	K	H	S	∏	Fu	D
Laguna de los Gendarmes	8	1	0.000	0	0.00	0	0.00
Reserva Nacional Lago Jeinimeni	7	3	0.524	2	0.57	-0.92*	-1.24
Cerro Castillo	24	11	0.931	12	2.86	-3.30**	-0.38
Chile Chico	12	5	0.576	8	1.74	-0.55	-1.37
Total	51	17	0.787	14	2.19	-8.92**	-1.19

TABLE 5. Fst values (below diagonal) and Fst p-values (above diagonal).

	Chile Chico	Cerro Castillo	Laguna los Gendarmes	Reserva Nacional Lago Jeinimeni
Chile Chico	-	0.0001	0.018	0.999
Cerro Castillo	0.183	-	0.004	0.0002
Laguna de los Gendarmes	0.085	0.365	-	0.0001
Reserva Nacional Lago Jeinimeni	-0.052	0.184	0.106	-

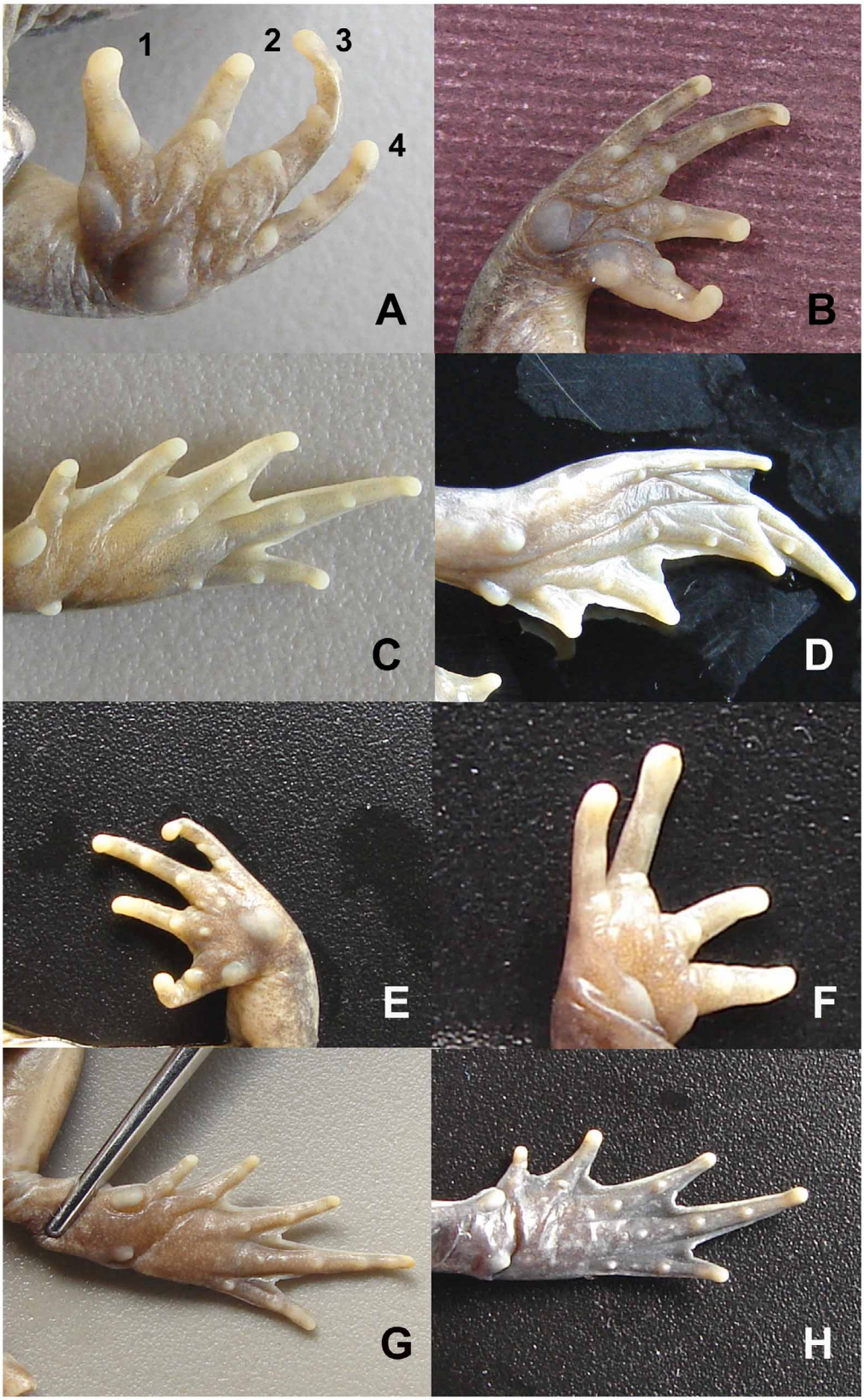

FIGURE 3. Photograph of front and hind legs of *Atelognathus salai* (A-D) from Laguna de los Gendarmes and *Atelognathus jeinimenensis* (E–H) from Cerro Castillo and Chile Chico. A) Length of fingers is 3>4>2=1 (female, CENPAT-LASBA 96-28), B) Length of fingers is 3>4>1>2 (male, CENPAT-LASBA 96-29), C) and D) detail of foot with presence of dermal fringes (male CENPAT-LASBA 08-04, and female CENPAT-LASBA 08-08 respectively), E) Length of fingers is 3>4>2=1 (female from Cerro Castillo, MZUC 36405), F) Length of fingers is 3>4>1=2, (female from Cerro Castillo, MZUC 36771), G) and H) Detail of foot with presence of dermal fringes (female, MZUC 36772 and male MZUC 36580, both from Chile Chico).

Discussion

The morphometric divergence and genetic homogeneity of Argentinean and Chilean populations can be addressed by considering abiotic factors. We favour the probable effect of water temperature on morphology and larval development, since it is a relevant factor affecting body size in ectotherms (Atkinson 1996). The phenotypic plasticity reflects the organism's ability to change in the habitat. In response to pond drying, the larvae of some anuran species exhibited accelerated developmental rates at the expense of growth (Crump 1989; Newmnan 1989). Nevertheless, this effect is not analyzed here.

The identity for *A. jeinimenensis* is poorly sustained by its morphology when a larger series of specimens are compared with topotypes of *A. salai*. Meriggio *et al.* (2004) distinguish *A. jeinimenensis* from the closest *Atelognathus* species, *A. salai*, on the basis of the relative length of the fingers and absence of dermal fringe (but see in Figure 3 in Meriggio *et al.* 2004). The comparisons made by Meriggio *et al.* (2004) are based entirely on bibliographical data. Our analysis by direct comparison with the 23 *A. salai* specimens shows no difference in either relative length of digits or development of the dermal fringe, which was conspicuous in all specimens. The analysis of principal components allowed us to analyse the morphological variation in an integrated way. The first axis, which was strongly and positively correlated with general body length (e.g., Snout-Vent length, Head height, Nostril-eye distance, Nostril-mouth distance) varies in the same direction and magnitude as the second axis, so do not show differences.

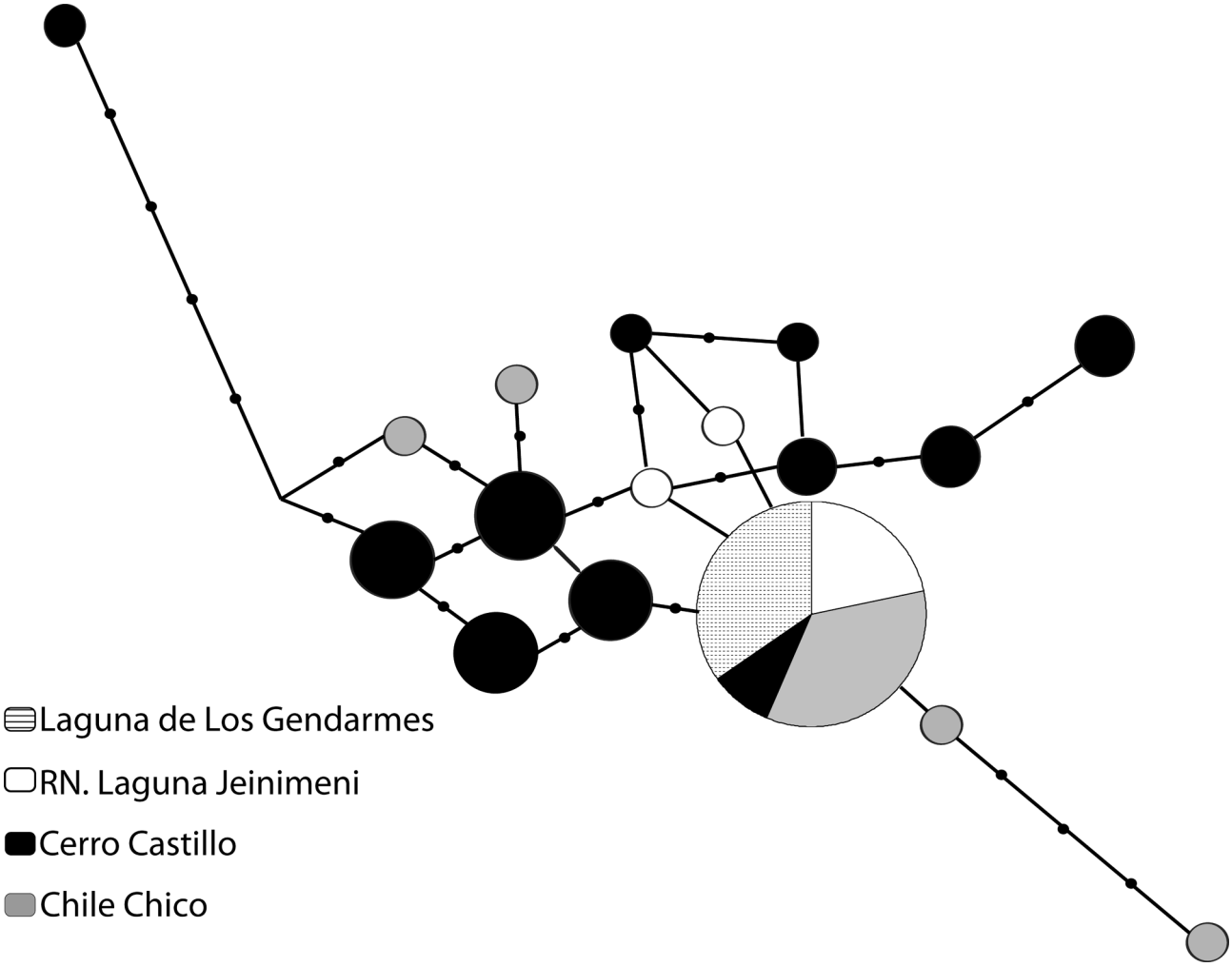

FIGURE 4. Median-joining networks of mitochondrial Control Region (*d-loop*) haplotypes of four *Atelognathus* localities. Size of circles is proportional to frequency. Point indicates mutational steps. Laguna de los Gendarmes and PN. Laguna Jeinimeni are type localities.

According to our results, a simple geographic pattern of genetic diversity of the mtDNA data suggests a single species of *Atelognathus*. No significant haplotypic and nucleotide differences are present among the four studied populations of the *salai-jeinimenensis* complex. On the one hand, our results indicate that the *Atelognathus* populations studied in Chile (Cerro Castillo, RN Lago Jeinimeni and Chile Chico) and Argentina (Laguna de los Gendarmes) have low levels of genetic divergence (Figure 4), which could be consistent with glaciations during the Late Pleistocene. This is supported by negative value of Fu' Fs in all Chilean localities, although Chile Chico is not significant. We calculated an expansion time of ~135,000 years before the present in Chilean localities, based on a mutation rate of 1.69% for anuran *d-loop* (Crawford 2003). According to Lynch (1978) and Cei (1984), the presence of *Atelognathus* in western Patagonia is consistent with the past distribution of the austral forests in the region (Markgraft *et al.* 1996; Menéndez 1969). On the other hand, our results indicate that *Atelognathus* populations that currently occupy this region originated from geographically similar gene pools, thus suggesting that post-glacial colonization occurred from a single source. Vidal (2008), in a review of Chilean faunal distribution, confirms the suggestion of other authors that many species of amphibians and reptiles from south-central Chile might correspond to a mixture of species (Donoso-Barros 1966; Cei 1962; Veloso & Navarro 1988) product of cis-trans Andean dispersal, because the Andes decrease in altitude southward. A similar example is the report in Chile of Atelognathus ceii described for Patagonia on the border of Chile and Argentina (Basso 1998; Díaz-Páez et al. 2008).

The paleoclimatic events that occurred during the Pleistocene appear to support the way in which this genus is distributed in Chilean and Argentinean Patagonia. Considering only the LGM, it has been suggested that it may have been a determining factor in promoting changes in the distribution of plant and animal species (Premoli *et al*. 2000; Victoriano *et al*. 2008; Brieva & Formas 2001). It is thus highly plausible that the LGM could have promoted the loss of genetic diversity among *Atelognathus* populations in the Aysén Region in Chile and Río Negro in Argentina. In fact, data related to the *Atelognathus* distribution area indicate rapid changes affecting the geographical structure of Taitao Peninsula (46º25'S), Puerto Edén (49°08'S) and adjacent areas (Ashworth *et al*. 1991; Lumley & Switsur 1993; McCulloh *et al*. 2000). This would indicate that the region is currently undergoing major climate changes, thus creating conditions that would allow *Atelognathus* specimens to expand from their Patagonian distribution in Argentina. Finally, on the basis of the results from this study, we conclude that *Atelognathus jeinimenensis* Meriggio *et al*., 2004 is a junior synonym of *Atelognathus salai* Cei, 1984 and all *Atelognathus* specimens coming from the Chilean localities RN Lago Jeinimeni, Chile Chico and Cerro Castillo should be assigned to *Atelognathus salai* (Cei 1984).

Acknowledgments

We thank José Núñez, Myriam Iturra and Jonathan Guzmán for their field assistance. *Atelognathus* specimens were captured under license from *Servicio Agrícola y Ganadero* (Nº 6494 December 27, 2006). This research was supported by projects 205.113.066-1sp to JCO and 210.412.045-1sp to HDP, and partially by CONICYT 79090026 to MVM. and PIP 11220090100525 CONICET to N.B.

References

Aljanabi, S.M. & Martínez, I. (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693.

Ashworth, A.C., Markgraft, V. & Villagrán, C. (1991) Late Quaternary climatic history of the Chilean Channels based on fossil pollen and beetle analyses, with an analysis of the modern vegetation and pollen rain. *Journal of Quaternary Science*, 6, 279–291.

Atkinson, D. (1996) Ectotherm life-history responses to developmental temperature. *In:* Johnston IA & AF. Bennet (Eds.), *Animals and temperature phenotypic and evolutionary adaptation*. Cambridge University Press, Cambridge, United Kingdom, pp. 183–204.

Bandelt, H.J., Forster, P. & Rohl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37–48.

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

Brieva, L.M. & Formas, J.R. (2001) Allozyme variation and geographic differentiation in the Chilean leptodactylid frog *Batrachyla taeniata* (Girard, 1854). *Amphibia-Reptilia,* 22, 413–420.
Cei, J.M. (1962) *Batracios de Chile*. Ediciones Universidad de Chile, Santiago. cviii + 128 pp.
Cei, J.M. (1980) *Amphibians of Argentina*. Monitore zoologico italiano N.S. Monogr. 2, xii + 609 pp.
Cei, J.M. (1984) A new leptodactylid frog, genus *Atelognathus*, from southern Patagonia, Argentina. *Herpetologica,* 40, 47–51.
Cei, J.M. & Roig, V.G. (1968) Telmatobiinos de las lagunas basálticas de Neuquén (Anura, Leptodactylidae). *Physis (Buenos Aires),* 27, 265–284.
Crawford, A.J. (2003) Relative rates of nucleotide substitution in frogs. *Journal of Molecular Evolution,* 57, 636–641.
Crump, M.L. (1989) Effect of habitat drying on development time and size at metamorphosis in *Hyla pseudopuma*. Copeia, 1989, 794–797.
Díaz-Páez, H., Núñez, J., Núñez, H. & Ortiz, J.C. (2008) Estado de conservación de los herpetozoos en Chile. *In:* Vidal, M. & Labra, M.A. (Eds), *Herpetología de Chile*. Science Verlag Ediciones, Santiago, Chile, pp 233–268.
Denton, G.H., Heusser, C.J., Lowell, T.V., Moreno, P.I., Andersen, B.G., Heusser, L.E., Schuchter, C. & Marchant, D.R. (1999) Interhemispheric linkage of paleoclimate during the last glaciation. *Geografiska Annaler,* 81A, 107–153.
Donoso-Barros, R. (1966) *Reptiles de Chile*. Ediciones Universidad de Chile, Santiago de Chile. cxxxvi + 458 pp.
Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology,* 11, 2571–2581.
Excoffier, L., Smouse, P. & Quattro, J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics,* 131, 479–491.
Filatov, D.A. (2002) ProSeq: A software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes,* 2, 621–624.
Fu, Y.X. (1997) Statistical neutrality of mutations against population growth, hitchhiking and background selection. *Genetics,* 147, 915–925.
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 others frogs, and a overview of PCR primers which have amplified DNA in Amphibians successfully. *Molecular Phylogenetics and Evolution,* 11, 163–199.
Heusser, C.J., Heusser, L.E. & Lowell, T.V. (1999) Paleoecology of the southern Chilean Lake District-Isla Grande de Chiloé during middle-Late Llanquihue glaciation and deglaciation. *Geografiska Annaler,* 81A, 231–284.
Hudson, R.R., Slatkin, M. & Maddison, W.P. (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, 132, 583–589.
Lamy, F., Kaiser, J., Arz, H.W., Hebbeln, D., Ninnemann, U., Timm, O., Timmermann, A. & Toggweiler, J.R. (2007) Modulation of the bipolar seesaw in the Southeast Pacific during Termination 1. *Earth and Planetary Science Letters*, 259, 400–413.
Liebers, D. & Helbig, J. (2002) Phylogeography and colonization history of Lesser Black-backed gulls (*Larus fuscus*) as revealed by mtDNA sequences. *Journal of Evolutionary Biology,* 15, 1021–1033.
Lynch, J.D. (1975) A new chilean frog of the extra-Andean assemblage of Telmatobius (Amphibia: Leptodactylidae). *Southern California Academy of Sciences Bulletin,* 74, 160–161.
Lynch, J.D. (1978) A re-assessment of the Telmatobiine leptodactylid frogs of Patagonia. *Occasional Papers of the Museum of Natural History. The University of Kansas. Lawrence, Kansas*, 72, 1–57.
Lumley, S.H. & Switsur, R. (1993) Late Quaternary chronology of the Taitao Peninsula, southern Chile. *Journal of Quaternary Science*, 8, 161–165.
Markgraf, V., Romero, E. & Villagrán, C. (1996) History and paleoecology of South American *Nothofagus* Forests. *In:* Veblen, T.T., Hill, R.S. & Read, J. (Eds), *The ecology and biogeography of Nothofagus forests*. Yale University Press, New Haven., pp 354–386.
Mc Culloch, R.D., Bentley, M.J., Purves, R.S., Hulton, N.R.J., Sugden, D.E. & Clapperton, C.M. (2000) Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. *Journal of quaternary Science*, 15, 409–417.
Menéndez, C.A. (1969) Die fossilen floren Sudamerikas. *In:* Fittkau, E.J., Illies, H., Klinge, H., Schwabe, G.H. & Sioli, H. (Eds). *Biogeography and Ecology in South America*, 2. Dr. W. Junk, The Hague, The Netherlands, pp 519–561.
Meriggio, V., Veloso, A., Young, S. & Nuñez, H. (2004) *Atelognathus jeinimenensis* n.sp. de Leptodactylidae para el Sur de Chile. *Boletín del Museo Nacional de Historia Natural (Chile),* 53, 99–123.
Moreno, P.I. & Leon, A.L. (2003) Abrupt vegetation changes during the last glacial to Holocene transition in mid-latitude South America. *Journal of Quaternary Science*, 18, 787–800.
Moreno, P.I., Jacobson, G.L., Andersen, B., Lowell, T.V. & Denton, G.H. (1999) Abrupt vegetation and climate changes during the last glacial maximum and the last Termination in the Chilean Lake District: A case study from Canal de la Puntilla (41ºS). *Geografiska Annaler,* 81 A, 285–311.
Newman, R.A. (1989) development plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology,* 70, 1775–1787.
Premoli, A.C., Kitzberger, T. & Veblen, T.T. (2000) Isozyme variation and recent biogeographical history of the long-lived conifer *Fitzroya cupressoides*. *Journal of Biogeography,* 27, 251–260.
Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biol-*

ogy and Evolution, 19, 2092–2100.
Rivas, A. (2009) *Taller estadística descriptiva multivariada. Análisis de componentes principales.* Dirección de Docencia. Universidad de Concepción, 33pp.
Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19, 2496–2497.
Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin: A software for population genetic data.* Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123, 585–595.
Veloso, A. & Navarro, J. (1988) Lista sistemática y distribución geográfica de anfibios y reptiles de Chile. *Bollettino del Museo Regionale di Scienze Naturali, Torino*, 6, 481–539.
Victoriano, P., Ortiz, J.C., Benavides, E., Adams, B.J. & Sites, J.W. (2008) Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. *Molecular Ecology*, 17, 2397–2416.
Vidal, M.A. (2008) Biogeografía de Anfibios y Reptiles. *In* Vidal, M.A. & Labra, M.A. (Eds), *Herpetología de Chile.* Science Verlag Ediciones, pp 195–231.
Villagrán, C., Armesto, J.J. & Leiva, R. (1986) Recolonización postglacial de Chiloé insular: evidencias basadas en la distribución geográfica y los modos de dispersión de la flora. *Revista Chilena de Historia Natural*, 59, 19–39.