Revision of *Liolaemus tenuis* subspecies (Duméril & Bibron, 1837) by analysis of population genetic structure

Marcela A. Vidal[1], Juan Carlos Ortiz[1], Marcela Astorga[1], Pedro Victoriano[1], Madeleine Lamborot[2]

[1] Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

[2] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Population differentiation depends on extrinsic factors such as geographical distance, ecological barriers, and different ecotypic regions (Endler, 1977; Pounds and Jackson, 1981; Bossart and Prowell, 1998; Brieva and Formas, 2001) that promote genetic divergence. In some lizards, genetic differentiation occurs after long periods of isolation, when the gene flow is low, the population size is reduced, habitats are restricted, and vagility is low (Bezy and Sites, 1987; Sarre et al., 1990).

Lizard species of the genus *Liolaemus* are widely distributed in the central-southern region of Chile (Donoso-Barros, 1966; Nuñez and Jaksic, 1992). Of these, *Liolaemus tenuis* (Duméril and Bibron, 1837) has one of the largest ranges. This arboreal species inhabits the distinct bioclimatical regions found in the area between Coquimbo (29°58′S, 71°21′W) and Los Lagos (39°51′S, 72°50′W), from sea level up to 1800 m (Donoso-Barros, 1966; Di Castri, 1968).

Two subspecies (*L.t. tenuis* and *L.t. punctatissimus*) have been described within this species, differentiated fundamentally by their dorsal coloration patterns (Müller and Hellmich, 1933). These subspecies were originally described based on samples from two geographically distant localities [*L.t. tenuis* (Santiago; 33°27′S, 70°40′W) and *L.t. punctatissimus* (Lota; 37°04′S, 73°10′W)]. However, the colour pattern in both subspecies shows a clinal variation within one species (Vidal, 2002). Furthermore, the distributional ranges of these species are superimposed (from 37° to 40°) (Núñez and Jaksic, 1992), which is contradictory to the concept of subspecies (Mayr and Ashlock, 1991). This infor-

mation suggests these proposed subspecies do not correspond to real taxonomical entities, but rather are phenotypic variations within the species.

Considering that most of the observable colour and morphology variations in natural populations do not respond to a genetic basis, and because this is a recurrent problem in taxonomic descriptions of highly variable species (i.e. *L. tenuis*) we used allozyme electrophoresis to examine the genetic structure of the *L. tenuis* populations and established the degree of genetic differentiation among the two proposed subspecies.

Two hundred thirty-three lizards were collected manually from 12 localities along the north-south axis of Chile (fig. 1); *L.t. tenuis* inhabited six of the localities and *L.t. punctatissimus* the other six. The localities and the number of specimens examined were as follows: *L.t. tenuis*: Salamanca ($n = 9$), Aconcagua ($n = 12$), Quilpué ($n = 21$), Río Clarillo ($n = 38$), Licantén ($n = 50$), Curicó ($n = 30$); *L.t. punctatissimus*: Cañete ($n = 21$), Nacimiento ($n = 10$), Victoria ($n = 11$), Curacautín ($n = 14$), Lautaro ($n = 9$), and Puesco ($n = 8$) (Appendix I).

The lizards were anaesthetised with Chloroform and kept at $-80°C$ until processed. Eviscerated specimens were homogenised with distilled water. The homogenates from each specimen were analysed by horizontal starch gel (12.5%) electrophoresis, using the Hillis and Moritz (1990) staining procedures. The proteins assayed in the tissue extracts were: phosphoglucomutase (*Pgm-1*, *Pgm-2*; Enzyme commission [EC] 5.4.2.2); esterase (*Est-1*, *Est-2*; EC 3.1.1); tetrazolium oxidase (*To-1*; EC 1.15.1.1); isocitrate dehydrogenase (*Idh-1*; EC 1.1.1.42); malic enzyme (*Me-1*; EC 1.1.1.40); glucosephosphate isomerase (*Gpi-1*; EC 5.3.1.9); aspartate transaminase (*Ata-1*; EC 2.6.1.1); general proteins (*Gp-1*; *Gp-2*); leucine amino peptidase (*Lap-1*; EC 3.4.11.1); malate dehydrogenase (*Mdh-1*; EC 1.1.1.37); L — lactate dehydrogenase (*Ldh-1*; EC 1.1.1.27); sorbitol dehydrogenase (*Sdh-1*; EC 1.1.1.14); and glycerol-3-phosphate dehydrogenase (*G3pdh-1*; EC 1.1.1.8). The alleles at each locus were named alphabetically according to the order of their relative electrophoretic mobility (Bezy and Sites, 1987).

Measures of genetic variability and genetic distances (Rogers, 1972) were computed using BIOSYS 2 (Swofford and Selander, 1981). A non-parametric test (Spearman) was used to test correlations between the observed heterozygosity, percentage of polymorphism, number of alleles per locus, and sample size (Sokal and Rohlf, 1995). Deviations from Hardy-Weinberg equilibrium, the excess and deficit of heterozygotes, and the genetic homogeneity between populations were tested by Fisher's Exact Test, setting 1,000 iterations per 100 batches in the Markov chain method, with GENEPOP software (Raymond and Rousset, 1997).

Interlocalities genetic variation, using Rogers' genetic distance, was examined through multidimensional scaling (MDS; Lessa, 1990), using STATISTICA 5.1 (StatSoft; Tulsa, Okla). The isolation by distance model (Wright, 1943) was analysed from the regression of $F_{st}/(1-F_{st})$ estimates for pairs of populations on the logarithm of the geographic distance for populations (Rousset, 1997). The GENEPOP software (ISOLDE option) (Raymond and Rousset, 1997) was used to test the hypothesis of isolation by distance. The F_{st} statistic was
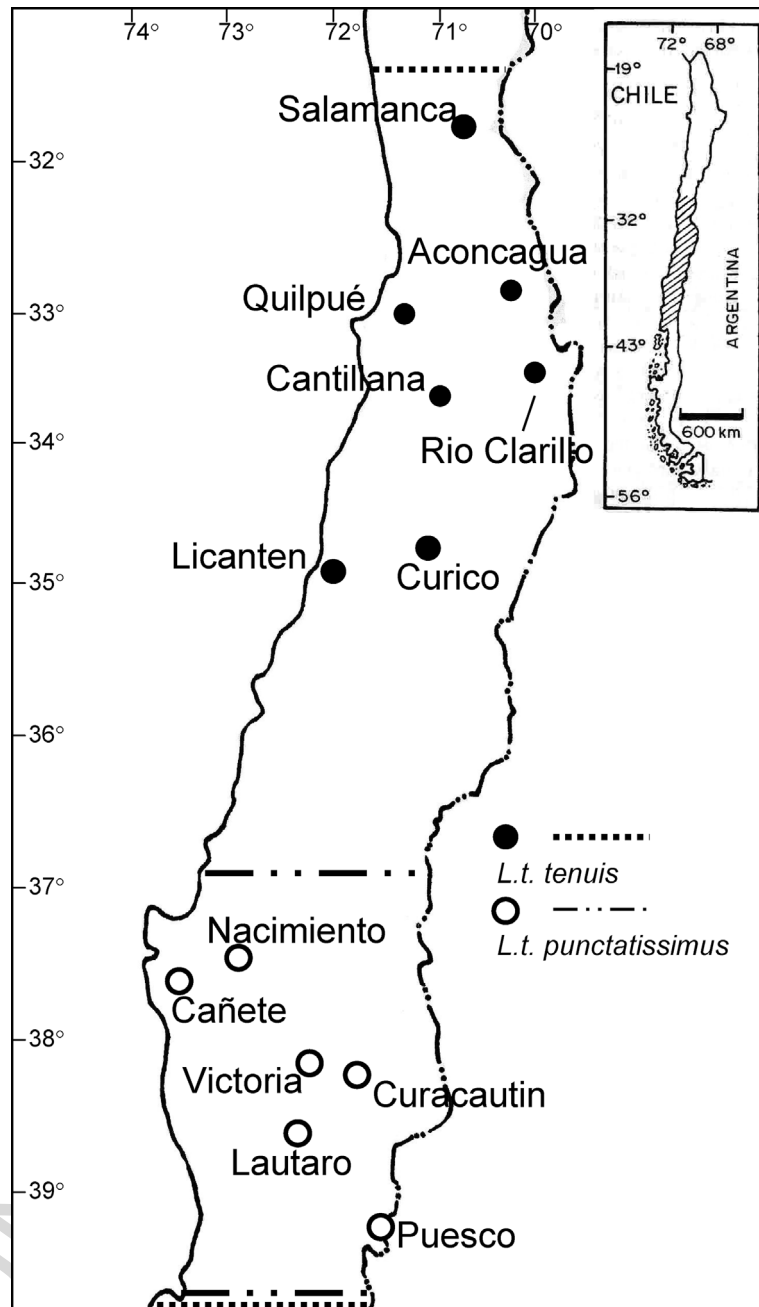
Figure 1. Geographic range (Donoso-Barros, 1966) for *L.t. tenuis* (■■■■■■■) and *L.t. punctatissimus* (▬ ▬ ▬) subspecies, and sampling localities of *Liolaemus tenuis* populations used in this study.

calculated according to Weir and Cockerham (1984) and the gene flow (Nm) values were estimated according to Slatkin (1985).

A total of 16 presumptive loci were resolved: three were polymorphic (*Pgm-2; Est-2; To-1*), 13 were monomorphic (*Est-1, Idh-1, Pgm-1, Me-1, Gpi-1, Ata-1, Gp-1, Gp-2, Lap-1, Mdh-1, Ldh-1, Sdh-1, G3pdh-1* (table 1). The average number of alleles per locus was 1.21. The mean of polymorphic loci was 16.3%, ranging from 12.5% (five localities) to 18.8% (seven localities) (table 1). These values are within the ranges reported by Gorman and Renzi (1979), Bezy and Sites (1987), Sarre et al. (1990), and Martins (1995) for other reptile families.

No significant relationships were found between the sample size and the observed mean heterozygosity ($r_s = 0.309$; $P = 0.327$) and the percentage of polymorphic loci ($r_s = 0.515$; $P = 0.086$), but the correlation between sample size and mean number of alleles per locus was significant ($r_s = 0.802$; $P = 0.001$). However, the ranges of average number of alleles per locus varied little among all the populations studied (1.13-1.38). The observed and expected mean heterozygosities for the localities were 0.066 and 0.059, respectively, and showed no significant differences (Wilcoxon Z value = 1.176; $P = 0.239$). Fisher's Exact Test revealed a deficit of heterozygotes for *Pgm-2* in two localities, for *Est-2* in one locality, and for *To-1* in two localities. An excess of heterozygotes was found in one locality for *Pgm-2* and in one locality for *Est-2*.

No diagnostic loci between subspecies were observed (table 1). The genetic distance (D) between localities ranged from 0.009 to 0.064, which is in the same range as the hypothetical subspecies (*L.t. tenuis*, mean D = 0.026; *L.t. punctatissimus*, mean D = 0.038). In reptiles and amphibians, values of D = 0.10 suggest population similarity within one species (Kalezic and Hedgecock, 1979; Bezy and Sites, 1987; Jacobs, 1987; Sarre et al., 1990). Rogers' distance between subspecies was D = 0.012, suggesting a low level of genetic differentiation; furthermore, this value is lower than the mean genetic distance among localities within each subspecies. Therefore, these results suggest that the subspecies are populations within the *L. tenuis* species.

In the twelve localities analysed, the average value of F_{st} across all loci was 0.083 and the statistical analysis showed that this value was significantly different from zero ($P < 0.001$), indicating the existence of subdivisions among populations. However, this F_{st} value is considered low for reptiles (Sarre et al., 1990). The respective gene flow (N_m) is 2.76 individuals per generation, suggesting an important genetic exchange among populations (e.g. Formas and Brieva, 2000). A non-hierarchical positional analysis (multidimensional scaling) showed no consistent ordering of the localities according to subspecies (fig. 2). However, the northern localities tended to be in close positions. In contrast, southern localities are dispersed in the multidimensional space. In fact, this analysis revealed higher differences among the southern localities (e.g. Victoria, Curacautin, Lautaro, and Puesco) than between the northern and southern localities, since two of the southern localities were closer with the northern ones. This probably explains the lack of correlation found between isolation and genetic and geographic distance ($P_r = 0.860$) when using distance

Table 1. Allele frequencies, polymorphism (%), heterozygosity (observed and expected), mean number of alleles per locus, and F_{st} per locus at three variable loci for twelve *Liolaemus tenuis* localities. Salamanca (SA), Aconcagua (AC), Quilpue (QU), Rio Clarillo (RC), Licanten (LI), Curico (CU), Cañete (CÑ), Nacimiento (NA), Victoria (VI), Curacautin (CT), Lautaro (LA), and Puesco (PU).

Locus		SA	AC	QU	RC	LI	CU	CÑ	NA	VI	CT	LA	PU	F_{st}
	n	9	12	21	38	50	30	21	10	11	14	9	8	
Pgm-2	a	0.944	0.909	0.714	0.750	0.860	0.850	0.555*	0.900	0.955	0.786	0.555*	0.875	0.089
	b	0.056	0.000	0.048	0.224	0.130	0.067	0.175	0.100	0.045	0.071	0.444	0.125	
	c	0.000	0.000	0.238	0.026	0.010	0.000	0.000	0.000	0.000	0.071	0.000	0.000	
	d	0.000	0.091	0.000	0.000	0.000	0.083	0.075	0.000	0.000	0.071	0.000	0.000	
Est-2	a	0.556	0.792	0.667	0.583	0.479	0.383	0.762	0.650	0.682	0.786	0.722	0.875	0.041
	b	0.444	0.083	0.310	0.167	0.240	0.283	0.119	0.300	0.318	0.214	0.278	0.125	
	c	0.000	0.125	0.024	0.250	0.281	0.333	0.119	0.050	0.000	0.000	0.000	0.000	
TO-1	a	1.000	1.000	0.976	0.882	0.690*	0.816*	0.952*	1.000	0.818	0.643	0.555*	0.688	0.118
	b	0.000	0.000	0.024	0.118	0.130	0.050	0.024	0.000	0.045	0.179	0.444	0.250	
	c	0.000	0.000	0.000	0.000	0.180	0.133	0.024	0.000	0.136	0.179	0.000	0.063	
% Polymorphism		12.5	12.5	12.5	18.75	18.75	18.75	18.75	12.5	18.75	18.75	18.75	12.5	
H. observed		0.048	0.026	0.068	0.098	0.085	0.078	0.048	0.073	0.034	0.054	0.131	0.054	
H. expected		0.037	0.032	0.058	0.072	0.084	0.077	0.055	0.041	0.051	0.070	0.086	0.042	
Mean number of alleles per locus		1.13	1.19	1.25	1.31	1.38	1.38	1.38	1.19	1.25	1.38	1.19	1.19	

* Deviation from Hardy-Weinberg equilibrium, $P < 0.01$.
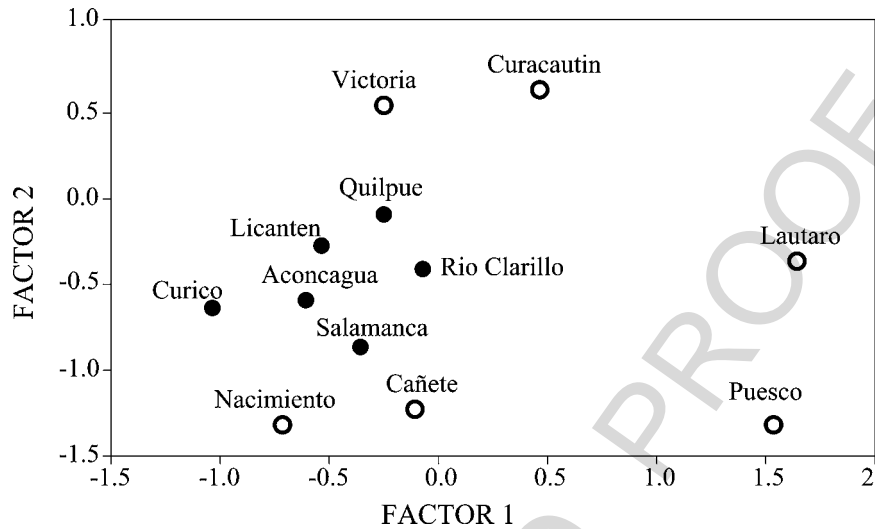
Figure 2. Bi-dimensional ordination of the twelve localities of *Liolaemus tenuis* using the/a multidimensional scaling method. Closed circles = *L.t. tenuis*, open circles = *L.t. punctatissimus*.

analysis. The lack of significant correlations between these variables suggests that the populations could have recently recolonised these areas from glacial refuges (Villagrán et al., 1995). The greater genetic variation between southern localities (as opposed to between southern and northern ones) could have arisen from stochastic events due to the isolation of small populations in the southern range during the last glaciations. The forest habitat of *L. tenuis* in south central Chile has been altered by both glaciations (Heusser, 1981; van Geel et al., 2000) and floral change during different periods (Villagrán and Hinojosa, 1997). In fact, at least four southern *L. tenuis* localities (Victoria, Curacautín, Lautaro, and Puesco) fall within the geographic limits of the most recent glaciation (Formas and Brieva, 2000). The colour patterns of the two proposed subspecies are not concordant with the geographical arrangement of genetic variation between populations obtained from this study. Although *L. tenuis* shows a clinal variation of colour patterns (greenish accents in southern populations and a high proportion of brownish scales in the northern ones), there is a higher variation of colour patterns, including colour designs, within southern populations than between the two proposed subspecies (Vidal, 2002). This suggests that the colour variation is a polymorphism of different populations with a clinal trend, but where the coloration types don't show a geographical arrangement consistent with the previously defined subspecies.

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Appendix 1

List of *Liolaemus tenuis* individuals used in this study and their geographic coordinates. Specimens are deposited in the Museum of Zoology of the University of Concepción (MZUC), the Laboratory of Cytogenetics, Faculty of Science, University of Chile (LN), and the Museum of Natural History of Concepción (CHMHNC).

Salamanca ($31°47'$S, $71°58'$W): MZUC27121-129; *Aconcagua* ($32°55'$S, $71°32'$W): LN2293-2298, LN2302-2304, LN2427, LN2429-2430; *Quilpué* ($33°02'$S, $71°27'$W): MZUC27130-136, MZUC27091, MZUC27109-120; *Río Clarillo* ($33°39'$S, $70°38'$W): LN2657-2658, LN2669-2670, LN2306-2307, MZUC27093-099, LN1651-1653, LN1658, LN1705-1717; *Licantén* ($34°59'$S, $72°00'$W): MZUC26977, MZUC27002-004, MZUC27008, MZUC27011-017, MZUC27030-031, MZUC27036-27037, MZUC26974, MZUC27018023, MZUC27038-039, MZUC26976, MZUC26984-986, MZUC26988, MZUC27026, MZUC26975, MZUC26978-983, MZUC26987, MZUC26989-001, MZUC27005-27007, MZUC27032-035; *Curicó* ($34°58'$S, $71°13'$W): MZUC27009-010, MZUC27027-029, MZUC27137-138, MZUC27103-108, MZUC26974, MZUC27018-21, MZUC27023, MZUC27039, MZUC27007, MZUC27009, MZUC27137-38, LN2127-2128, LN2130, LN2309, LN2410-2411, LN2414, LN2416; *Cañete* ($35°17'$S, $72°14'$W): MZUC26964-972, MZUC27101-102, CHMHNC 652-653, CHMHNC 625, CHMHNC 627-633; *Nacimiento* ($37°30'$S, $72°40'$W): MZUC27040-042, CHMHNC 565-567, CHMHNC 594-595, CHMHNC 612-613; *Victoria* ($38°13'$S, $72°20'$W): MZUC27053-058, MZUC27060-063, MZUC27090; *Curacautín* ($38°26'$S, $71°53'$W): MZUC27064-066, MZUC27068, MZUC27070-072, MZUC27074-080; *Lautaro* ($39°27'$S, $72°47'$W): MZUC27081-089; *Puesco* ($39°34'$S, $71°35'$W): MZUC27044-052.