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Systematics and evolutionary relationships of the mountain lizard *Liolaemus monticola* (Liolaemini): how morphological and molecular evidence contributes to reveal hidden species diversity

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The delimitation of species is a major issue in systematic biology and has been a re-emerging discipline in the last decade. A number of studies have shown that the use of multiple data sets is critical for the identification of cryptic species, particularly in groups with complex evolutionary histories. *Liolaemus monticola* is a montane lizard species distributed in central Chile (32°–42°S), with four described subspecies in a latitudinal gradient from north to south: *L. m. monticola*, *L. m. chillanensis*, *L. monticola* ssp. and *L. m. villaricensis*. In order to test the systematic status and phylogenetic relationships of the taxa included in the *L. monticola* group, we analysed morphological (morphometric and meristic) and molecular (allozyme and mitochondrial DNA) data sets. The results of the morphological analyses showed that meristic variables correctly assigned individuals with higher accuracy than did morphometric characters. The results of the analyses of allozyme data revealed eight diagnostic loci that are evidence for significant differences among the four *L. monticola* subspecies. Phylogenetic analyses with mitochondrial DNA data, including additional species, showed that the *L. monticola* group is polyphyletic. We postulate that the four current subspecies represent independent evolutionary lineages and must be raised to the specific level as *L. monticola*, *L. chillanensis* and *L. villaricensis*. The taxonomic status of the unnamed *L. monticola* ssp. remains unresolved, although we provide a preliminary proposal.

ADDITIONAL KEYWORDS: allozymes – Andean range – biometric data – Chile – cytochrome *b* gene – meristic data – molecular phylogeny – polytypic species – species delimitation.

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INTRODUCTION

The delimitation of species boundaries is a major goal in solving systematic uncertainties using previously proposed systematic groups as hypotheses to be tested (Sites & Marshall, 2003, 2004). The choice of a single method for the evaluation of species boundaries may be problematic, especially in taxonomic groups with complex evolutionary histories. Even the use of a variety of methods with different statistical power may lead to conflicting answers when elucidating the same systematic issue (Hey *et al.*, 2003; Marshall *et al.*, 2006). These issues are enforced when cryptic species are suspected within a taxonomic group, and the comparison of different types of marker has been of great utility in herpetozoan studies (Godinho *et al.*, 2005; Camargo, De Sa & Heyer, 2006; Sanders, Malhotra & Thorpe, 2006; Pinho, Harris & Ferrand, 2007). Mitochondrial DNA (mtDNA) has been proven to be highly useful in systematic studies (Wiens & Penkrot, 2002), and has been widely used in species delimitation of vertebrate groups. Isozyme electrophoresis has traditionally been used as a tool to examine divergence at the specific level when morphological inference is inconclusive (Buth & Murphy, 1999). However, traditional morphology-based methods are still widely used in systematic studies, providing several advantages when molecular methods cannot be applied (Hillis & Wiens, 2000). An integrative approach to the delineation of species using multiple complementary methods is expected to produce robust results of a specific systematic study (Dayrat, 2005; Sanders *et al.*, 2006).

The lizard genus *Liolaemus* is widely distributed in southern South America, ranging from arid Patagonian to high-altitude Andean environments, including valley and coastal ranges. The adaptive radiation of *Liolaemus* has produced interesting patterns of developmental (Lobo & Espinoza, 1999, 2004), genetic (Morando *et al.*, 2004; Avila, Morando & Sites, 2006) and morphological (Harmon *et al.*, 2003; Schulte *et al.*, 2004) variation, as well as contrasting rates of molecular evolution (Schulte *et al.*, 2000). Some of these recently described phenomena have produced, as an indirect outcome, revised species taxonomy. In this article, we examine an interesting example of *Liolaemus* diversification and its implications for species classification and evolutionary relationships in the genus.

Liolaemus monticola (Müller & Hellmich, 1932) is an endemic lizard species distributed along the Andes between latitudes 32° and 41°S and at 600 to 2300 m (Donoso-Barros, 1966; Lamborot *et al.*, 1981). Currently, it is composed of four subspecies (morphotypes) based on morphological and distributional patterns. The nominal subspecies, *Liolaemus monticola monticola*, has been described in the San Francisco valley in the Santiago de Chile mountains (32°22′S, 70°25′W) at 1700 m (Fig. 1). It has also been reported in coastal and transversal mountain ranges (33°S) between 600 and 1800 m in central Chile (Lamborot & Alvarez-Sarret, 1993; Lamborot & Eaton, 1997). Two additional subspecies have been described from the same region ('Cordillera de Chillán', 36°52′S), albeit at a different elevation: *Liolaemus monticola chillanensis* inhabits environments at *c.* 1700 m in Termas de Chillán (Fig. 1), whereas the unnamed *Liolaemus monticola* ssp. occupies habitats above 2300 m (on 'Lavas del Volcán Chillán', VIII Region, Chile). However, *L. m. chillanensis* and *L. monticola* ssp. have recently been found in syntopy, showing no evidence of hybridization or genetic introgression (Torres-Pérez *et al.*, 2003). The latter subspecies has remained unnamed from its description for more than 70 years. The southernmost subspecies of the group, *Liolaemus monticola villaricensis*, inhabits the lava fields of the Villarrica Volcano (41°S) at 1400 m.

Liolaemus monticola shows a remarkable characteristic within *Liolaemus*. There is extensive variation in the chromosomal number from north to south. The nominal subspecies (*L. m. monticola*) exhibits high chromosomal polymorphism ($2n = 34$–44), separated by chromosomal races apparently maintained by riverine barriers (Lamborot, 1991, 1998b), whereas the southern *L. m. chillanensis* and *L. m. villaricensis* show $2n = 32$. Males of *L. m. villaricensis* and *L. monticola* ssp. do not have anal pores, a trait considered to be rare in the genus *Liolaemus* (Donoso-Barros, 1966; Videla & Cei, 1998). Remarkably, anal pores are present in males of *L. m. monticola* and *L. m. chillanensis*. Phylogenetic analyses involving some of the taxa of the *L. monticola* group have been performed using allozymes (Young-Downey, 1998) and morphological characters (Lobo, 2001, 2005). The morphological approach showed first monophyly (Lobo, 2001) and then paraphyly (Lobo, 2005) of the group. A complete systematic study involving all four cited subspecies is essential to elucidate the status of each current subspecies in the *L. monticola* group. In addition, we aim to determine the evolutionary relationships among taxa in this group using molecular sequence data, and to test the monophyly/paraphyly of *L. monticola* as proposed by previous morphological phylogenetic analyses.

We therefore test the following hypotheses: (1) all current subspecies of the *L. monticola* group represent different taxa at the species level; and (2) *L. monticola* constitutes a paraphyletic group. Species boundaries were studied using nuclear

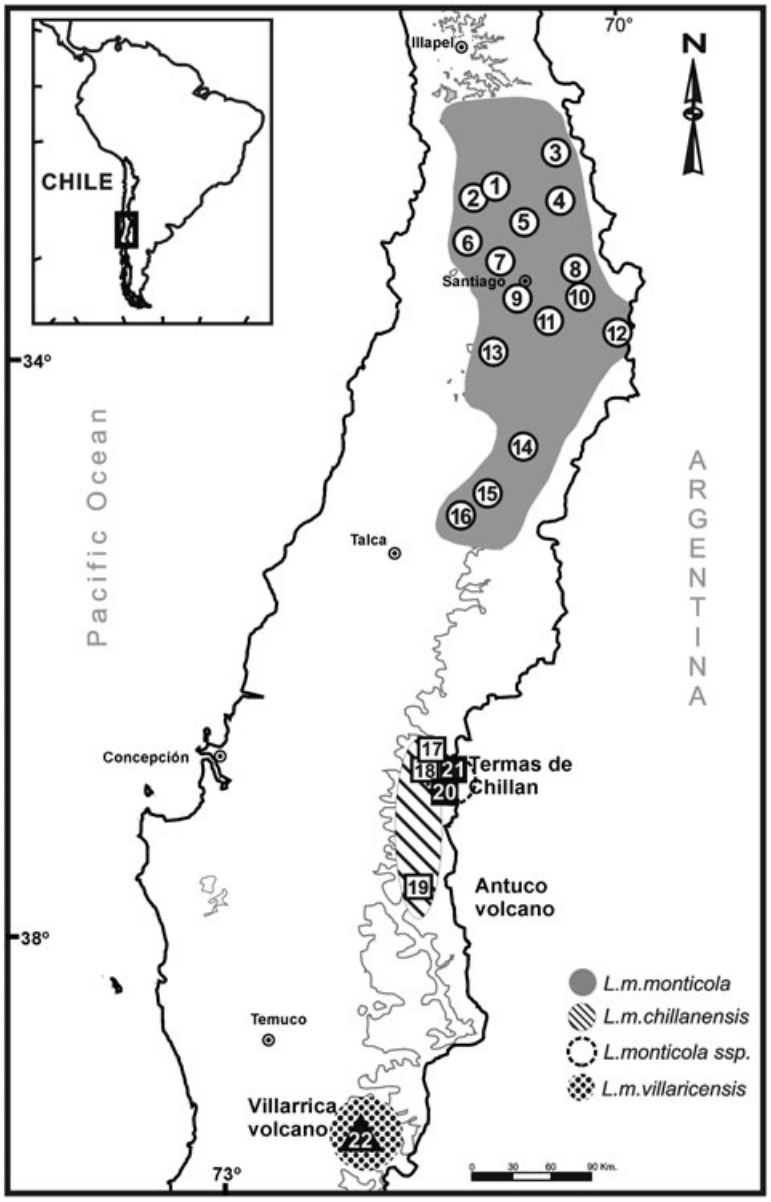

Figure 1. Map of the sampling localities and currently known geographical distributions of the *Liolaemus monticola* group. Numbers correspond to the localities sampled for the four *L. monticola* subspecies: open circles, *L. m. monticola*; open squares, *L. m. chillanensis*; filled squares, *L. monticola* ssp.; filled triangle, *L. m. villaricensis*. See Appendix S1 for specific sampled localities.

molecular markers (allozymes) and by performing multivariate analyses for the assessment of morphological divergence. Diagnostic characters using allozymes and/or significant morphometric differences among putative species will allow us to postulate distinctive evolutionary lineages in the *L. monticola* group. This information is complemented by phylogenetic reconstructions of taxa using sequencing data from the mitochondrial cytochrome *b* (cyt-*b*) gene.

MATERIAL AND METHODS

MORPHOMETRIC DATA ANALYSES

We sampled 140 adult specimens (Appendix S1, see Supporting Information) belonging to the four morphotypes of the *L. monticola* group from north to south: *L. m. monticola*, $N = 18$ males and $N = 14$ females, from the Santiago mountains (33°20′S, 70°19′W); *L. m. chillanensis*, $N = 16$ males and $N = 14$ females, and *L. monticola* ssp., $N = 26$ males

and $N = 26$ females, from the Chillán mountains (36°52′S, 71°28′W) between 1500 and 2000 m; *L. m. villaricensis*, $N = 19$ males and $N = 7$ females, from the foothills of the Villarrica volcano (39°35′S; 71°45′W; Fig. 1). Thirteen morphometric variables were measured for 140 adult specimens: (1) snout to vent length (SVL); (2) head length (HL); (3) head width (HW); (4) head height (HH); (5) interorbital distance (IOD); (6) snout eye distance (SED); (7) eye length (EL); (8) eye tympanum distance (ETD); (9) snout length (SL); (10) parietal snout distance (PSD); (11) armpit groin distance (AGD); (12) fore limb length (FLL); (13) hind limb foot length (HLL). Anatomical measures were taken as described previously (Victoriano, 1993; Abdala, 2007). In order to extract the influence of body size in the characters measured, all variables were regressed against SVL. Residuals of each regression were used thereafter. Principal components analysis (PCA) was conducted using a variance–covariance matrix of all measured characters to identify the most important characters for the differentiation of individuals (Jollife, 2002). *A priori* taxonomic boundaries were also studied through discriminant function analyses (DFAs). We used a jackknife method in order to estimate the misclassification rates of the discriminant functions. Both males and females were separately considered in PCA and DFA. All multivariate analyses were performed using the SYSTAT 11.0 program.

Meristic data analyses

Thirteen variables were used for meristic analyses in all 140 individuals as described above: (1) scales around the midbody (SAB); (2) dorsal sales from occiput to the middle line anterior to the hind limbs (DS); (3) number of scales between the last supralabial and the ear (SSUPE); (4) supralabial scales (SUPS); (5) lorilabial scales, scales in the loreal region between canthals, supralabials and subocular (LORS); (6) infralabial scales (INFS); (7) superciliaries, row of enlarged scales lying in the border of the upper eyelid ciliary scales (SCI); (8) supraorbital scales (SORS); (9) number of scales between the supraorbitals and the oculars (SSOROC); (10) temporal scales, number of scales between the posterior margin of the eye and the middle of the ear (TEMS); (11) number of scales in contact with the interparietal scale (SCIP); (12) number of infradigital scales on the fourth finger (SINF); (13) number of infradigital scales on the fourth toe (SINT). Meristic variables were taken as described previously (Lamborot, Eaton & Carrasco, 2003; Abdala, 2007). Logarithmic transformation (ln + 1) was used on the 13 meristic characters to normalize the data. PCA and DFA were performed for males and females separately, as explained above.

Allozyme analyses

Seventy individuals (Appendix S1) were used for the screening of allozyme polymorphisms: *L. m. monticola*, $N = 23$; *L. m. chillanensis*, $N = 9$; *L. monticola* ssp., $N = 20$; *L. m. villaricensis*, $N = 18$. Samples of liver and skeletal muscle were extracted from freshly killed specimens and stored at –80 °C. Small pieces of tissue were manually ground and diluted 1 : 1 with distilled water. The homogenate was centrifuged at 2000 ***g*** for 5 min and lysates were run in starch gels at 12% (w/v) concentration. Electrophoretic conditions and staining protocols followed Murphy *et al.* (1996) and Torres-Pérez *et al.* (2003).

Gene products for the following 24 presumptive enzyme loci were analysed: alcohol dehydrogenase (Ec 1.1.1.1, Adh-1 and Adh-2); aspartate aminotransferase (Ec 2.6.1.1, Aat-1 and Aat-2); cytosol aminopeptidase (Ec 3.4.11.1, CAP); esterase (naphthyl acetate) (Ec 3.1.1.-, Est-1 and Est-2); glucose-6-phosphate isomerase (Ec 5.3.1.9, Gpi); glycerol-3-phosphate dehydrogenase (Ec 1.1.1.8, G3pdh-1 and G3pdh-2); iditol dehydrogenase (Ec 1.1.1.14, Iddh); isocitrate dehydrogenase (Ec 1.1.1.42, Idh-1 and Idh-2); L-lactate dehydrogenase (Ec 1.1.1.27, Ldh-1 and Ldh-2); lactoylglutathione lyase (Ec 4.4.1.5, Lgl); malate dehydrogenase $NADP^+$ (Ec 1.1.1.40, Mdhp-1); malate dehydrogenase NAD^+ (Ec 1.1.1.37, Mdh-1 and Mdh-2); phosphoglucomutase (Ec 5.4.2.2, Pgm); phosphogluconate dehydrogenase (Ec 1.1.1.44, Pgdh-1); superoxide dismutase (Ec 1.15.1.1, Sod-1 and Sod-2); general proteins (Gp).

Genetic analyses of allozyme polymorphisms included the calculation of genotypic frequencies, proportion of polymorphic loci (P_l), average observed heterozygosity ($H_{obs.}$) and number of alleles per locus ($N_{Al.}$). Genetic homogeneity among morphotypes was evaluated by Fisher's exact test, and confidence levels were assessed using 1000 Markov chain iterations. All analyses were performed using GENEPOP version 3.4 (Raymond & Rousset, 1995). For the detection of dissimilar patterns among morphotypes, a non-metric multidimensional scaling (MDS) analysis was performed based on a genetic distance matrix (Cavalli-Sforza & Edwards, 1965). Finally, we used the frequency cut-off method (Wiens & Servedio, 2000) to evaluate the strength of the evidence for morphotype distinctiveness. This method was developed to allow a certain level (for example, 5–10%) of polymorphism in the presumptive diagnostic characters, given that distinguishing between polymorphic and truly fixed characters with certainty may be impossible with limited sample sizes. We performed the Wiens and Servedio

test by setting a frequency cut-off of 10% ($P = 0.10$; see Torres-Pérez *et al.*, 2003; Marshall *et al.*, 2006) and a 5% confidence interval ($\alpha = 0.05$) as criteria to delimit putative species with allozyme data.

mtDNA sequencing and phylogenetic analysis

Total genomic DNA was extracted from the liver of specimens preserved in 96% ethanol. A fragment of the mtDNA region encoding the cyt-*b* gene was amplified via polymerase chain reaction (PCR) from 6–16 ($N = 42$) individuals for each morphotype of the *L. monticola* group. Samples are representative of all types and known localities of the species (Fig. 1). For all populations, a fragment of 700 bp was amplified using the light strand primer GLUDG (5′-TGACT TGAARAACCAYCGTTG-3′) and the heavy strand primer CB3 (5′-GGCAAATAGGAARTATCATTC-3′) (Palumbi, 1996). These primers have been successfully used to amplify several other *Liolaemus* species (Morando, Avila & Sites, 2003; Morando *et al.*, 2004, 2007; Avila *et al.*, 2006). The following thermocycling conditions were used to amplify the cyt-*b* gene: 94 °C denaturation for 1 min 30 s, 52–54 °C annealing for 30 s and 72 °C extension for 1 min 10 s, for 35 cycles. Amplification reactions were carried out in 25 μL of reaction mixture containing 1–10 ng of template DNA, 10× PCR buffer, 25 mM $MgCl_2$, 10 mM of deoxynucleoside triphosphate (dNTP) mix, 10 μM of each primer and 5 U mL^{-1} Taq polymerase. PCR-amplified products were checked by electrophoresis on a 1.2% agarose gel in 1× Tris-Borate-EDTA (TBE) TBE running buffer. Double-stranded PCR products were purified with the Wizards PCR Preps (Promega) and QIAquik (Qiagen) methods. Sequencing was conducted through cycle sequencing on an ABI Prism 3100 automated sequencer, using the same primers as employed for PCR amplification, but diluted at 1 μM. Conditions for cycle sequencing reactions were 96 °C denaturation for 10 s, 50 °C annealing for 5 s and 60 °C extension for 4 min, for 25 cycles.

We also included ten *Liolaemus* species and *Phymaturus indistinctus* in phylogenetic analyses. This large number of *Liolaemus* species was included as an 'extended ingroup' to more accurately test the monophyly/paraphyly of the *L. monticola* group. Six of these species were sequenced in this study (*L. nigromaculatus*, *L. nigroviridis*, *L. nitidus*, *L. pseudolemniscatus*, *L. platei*, *L. tenuis)*, whereas sequences for the remaining five species were extracted from GenBank (see Appendix S1). Two species (*L. tenuis* and *L. neuquensis*) were included from the *tenuis* group, which appears sister to the *chillanensis–villaricensis* clade (Lobo, 2005). A member of the *pictus* group (*L. pictus*) was used as a sister taxon to the clade containing *chillanensis–villaricensis* plus the *tenuis* group (Lobo, 2005). Lobo (2005) recovered *L. monticola* within the *nigromaculatus* group; thus, we included three species from this group (*L. nigromaculatus*, *L. nigroviridis*, *L. platei*). Schulte *et al.* (2000) recovered *L. nitidus* as sister to *L. monticola* and *L. nigroviridis* as part of a clade closely related to *L. monticola*. *Liolaemus kriegi* and *L. elongatus* were included as these species are distributed in latitudes and habitats similar to those in which our morphotypes occur, although mostly on the east side of the Andes (Cei, 1986; Morando *et al.*, 2003). We used the published sequence of *P. indistinctus* (Morando *et al.*, 2003), reported as the sister genus of *Liolaemus*, as the most distant outgroup (Etheridge, 1995; Schulte *et al.*, 2000) to root the trees. Sequences were edited and aligned using the BioEdit program (Hall, 1999).

Maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were employed for phylogenetic reconstruction using PAUP* (Swofford, 2002), considering all characters as unordered with four possible states (A, C, G, T). Heuristic searches were performed for both optimality criteria. We employed 100 (MP) and 10 (ML) random stepwise additions of sequences and tree bisection–reconnection (TBR) branch swapping. Node support was evaluated with 5000 non-parametric bootstrap pseudoreplicates for MP (Felsenstein, 1985), using the same searching conditions as described above. Non-parametric bootstrap for ML analyses was conducted using GARLI v0.95 (Zwickl, 2006), and consisted of 1000 pseudoreplicates using the settings obtained from MODELTEST (see below). Bootstrap values above 70% were considered as strong support for a clade (but see caveats in Hillis & Bull, 1993). MODELTEST v. 3.7 (Posada & Crandall, 1998) identified the most probable model of sequence evolution, using the Akaike-corrected (AICc) option, as a general time-reversible model with a proportion of invariable sites with a discrete gamma distribution (GTR + I + Γ; Tavare, 1986).

Bayesian analyses were performed using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001), based on the selected nucleotide substitution model obtained for ML searches. To more thoroughly explore the parameter space, we ran Metropolis-coupled Markov chain Monte Carlo simulations (MCMCMC) with six incrementally heated chains. From random starting trees, six independent runs (three runs of two simultaneous, independent runs each) of 1×10^7 generations each were performed, with the resulting trees sampled every 1000 generations. We determined when a stationary state was reached (to discard the burn-in samples; 1000 trees) by plotting the logarithmic likelihood scores of sample points against generations. The last 9000 trees were used to compute a 50% majority rule consensus tree. The percentage of

samples that recover any particular clade on this tree represents that clade's posterior probability; we considered $P \geq 95\%$ as evidence for significant support (Alfaro, Zoller & Lutzoni, 2003).

To test the monophyly of the *L. monticola* group, we conducted the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) as implemented in PAUP* (Swofford, 2002). We enforced the monophyly of the *L. monticola* morphotypes and compared the constrained likelihood score to the tree with the best ML score (unconstrained search). The significance of the differences among the likelihood scores was determined with resampling of estimated log-likelihoods (RELL) bootstrap (one-tailed test), using 1000 permutations of the data. Wilcoxon signed-rank tests (Templeton, 1983), as implemented in PAUP*, were also used to determine whether the most parsimonious tree was significantly shorter than each alternative (constrained) tree.

Finally, using a non-metric MDS analysis, cyt-*b* gene sequences were also employed to detect dissimilar patterns among *L. monticola* morphotypes. This analysis was performed on the basis of a $1 - F_{st}$ matrix obtained in Arlequin version 3.1 (Excoffier, Laval & Schneider, 2005).

RESULTS

Morphometric data analyses

The PCA performed in males showed that the first three components explained 64.16% of the variance (first component, 36.23%; second component, 15.03%; third component, 12.90%; Fig. 2A). High positive loadings of HL (0.921), HW (0.742), SED (0.783), ETD (0.740) and SL (0.869) on PC1, coupled with positive EL (0.564) and PSD (0.638) and negative IOD (–0.546) loadings on PC2, indicated the strong influence of head proportions. When the first two PCs were

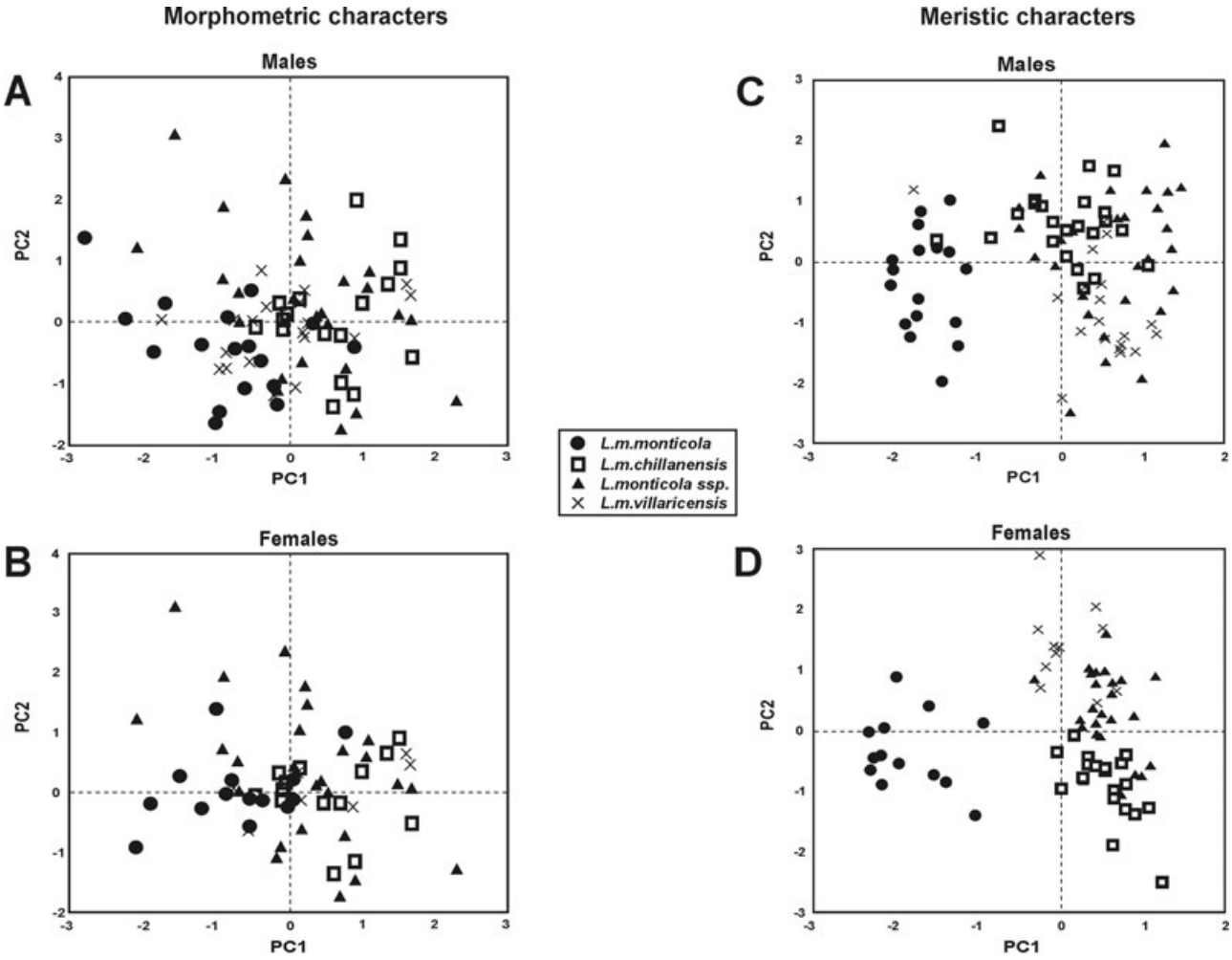

Figure 2. Principal component score plots based on morphometric measures for males (A) and females (B), and meristic data for males (C) and females (D), for the *Liolaemus monticola* morphotypes.

Table 1. Discriminant function analysis classification matrix for females (F) and males (M) of the four subspecies of the *Liolaemus monticola* group using morphometric and meristic characters

Morphometric characters

	L. m. monticola		*L. m. chillanensis*		*L. monticola* ssp.		*L. m. villaricensis*		Correct (%)	
	F	M	F	M	F	M	F	M	F	M
L. m. monticola	5	12	2	1	4	3	3	2	36	67
L. m. chillanensis	2	2	12	15	1	2	1	0	75	79
L. monticola ssp.	5	3	4	6	10	15	7	4	38	54
L. m. villaricensis	2	1	0	0	1	1	7	17	70	89
Total	14	18	18	22	16	21	18	23	52	70

Meristic characters

	L. m. monticola		*L. m. chillanensis*		*L. monticola* ssp.		*L. m. villaricensis*		Correct (%)	
	F	M	F	M	F	M	F	M	F	M
L. m. monticola	14	18	0	0	0	0	0	0	100	100
L. m. chillanensis	0	0	17	22	2	1	0	0	89	96
L. monticola ssp.	0	0	3	3	20	16	2	8	80	59
L. m. villaricensis	0	0	0	0	3	3	8	17	73	85
Total	14	18	20	25	25	20	10	25	86	83

used to summarize the morphometric variation, all four taxa overlapped in multivariate space (Fig. 2A), although a discrete differentiation was observed between *L. m. monticola* and *L. m. chillanensis*. This pattern suggests a lack of major differences in head proportions among these taxa. A DFA performed among males using eigenvalues extracted from PCA showed that three of the four morphotypes were significantly different (Wilks' lambda = 0.173, $F_{(21,213)} = 8.547$, $P < 0.0001$; Table 1): 67% of *L. m. monticola*, 79% of *L. m. chillanensis* and 89% of *L. m. villaricensis* males were correctly classified, whereas only 54% of *L. monticola* ssp. males were correctly classified. In females, PCA showed that the first three components explained 61.23% of the variance (first component, 32.72%; second component, 16.01%; third component, 12.25%; Fig. 2B). High positive loadings of HL (0.912), SED (0.795), SL (0.848) and FLL (0.784) on PC1, coupled with positive EL (0.615) and HLL (0.458) and negative IOD (–0.601) loadings on PC2, revealed differences among females. The same pattern as found in males was discovered in females when all four taxa were collapsed into a single group in multivariate space (Fig. 2B), with discrete differentiation only between *L. m. monticola* and *L. m. chillanensis*. When a DFA was performed for females, lower scores than those found for males were obtained for correctly classified individuals [*L. m. monticola* (36%), *L. m. chillanensis* (75%), *L. monticola* ssp. (38%) and *L. m. villaricensis* (70%)], although with statistical significance for distinctiveness (Wilks' lambda = 0.308, $F_{(21,161)} = 3.896$, $P < 0.0001$; Table 1).

Meristic data analyses

The PCA performed in males showed that the first three components explained 62.59% of the variance (first component, 26.19%; second component, 21.5%; third component, 14.9%). The highest loadings were found in the variables SAB (0.123), DS (0.102) and SSOROC (–0.112) on PC1, coupled with positive SSOROC (0.139) and negative SAB (–0.09) loadings on PC2. When the first two PCs were used to summarize the meristic variation, *L. m. monticola* appeared to be differentiated from the other three morphotypes, which overlapped in multivariate space (Fig. 2C). A DFA performed among males showed that three of the four morphotypes were significantly different (Wilks' lambda = 0.009, $F_{(39,213)} = 21.135$, $P < 0.001$; Table 1): 100% of *L. m. monticola*, 96% of *L. m. chillanensis* and 85% of *L. m. villaricensis* were correctly classified, whereas only 59% of *L. monticola*

ssp. were correctly classified. In females, PCA showed that the first three components explained 61.44% of the variance (first component, 26.48%; second component, 19.76%; third component, 15.2%). High positive loadings of SAB (0.138), DS (0.077), SSOROC (0.089) and SINF (0.076) on PC1, coupled with positive SSUPE (0.102) and negative SSOROC (–0.123) loadings on PC2, revealed differences among females. A different pattern from that found in males was discovered in females: *L. m. monticola*, *L. m. chillanensis* and *L. m. villaricensis* appeared to be differentiated in multivariate space (Fig. 2D), and *L. monticola* ssp. scores overlapped *L. m. chillanensis* and *L. m. villaricensis*. When a DFA was performed for females, similar scores to those found for males were obtained for correctly classified individuals [*L. m. monticola* (100%), *L. m. chillanensis* (89%), *L. monticola* ssp. (80%) and *L. m. villaricensis* (73%)], with statistical significance for distinctiveness (Wilks' lambda = 0.007, $F_{(39,157)} = 18.021$, $P < 0.001$; Table 1).

Allozyme analysis

Sixteen enzymatic systems, coded by 24 presumptive loci, were screened for the four morphotypes. The genotypic frequencies for the variable loci, percentage of polymorphic loci, observed and expected heterozygosity, and mean number of alleles by locus are shown in Table 2. Six loci were variable for *L. m. monticola* (Est-1, Est-2, G3pdh-1, G3pdh-2, Idh-2, Pgm), three for *L. m. chillanensis* (Est-1, Est-2, Pgm), six for *L. monticola* ssp. (Aat-1, Est-1, Est-2, G3pdh-1, Idh-2, Gpi) and four for *L. m. villaricensis* (Aat-2, Est-1, Idh-2, Pgm). *Liolaemus monticola chillanensis* showed the lowest values of genetic variability ($P_l = 12.5$, $N_{Al.} = 1.1667$, $H_{obs.} = 0.0231$), and *L. monticola* ssp. the highest ($P_l = 25.1\%$, $N_{Al.} = 1.2917$, $H_{obs.} = 0.0685$; Table 2).

Alternative electromorphs were present in eight loci and were considered to be diagnostic. The Est-1 locus, although polymorphic, was shown to be diagnostic in all morphotypes, and the Est-2 locus (also polymorphic) was diagnostic in the three northernmost morphotypes (*L. m. monticola*, *L. m. chillanensis*, *L. monticola* ssp.). The Aat-2 and Gpi loci were also variable and diagnostic: the Aat-2 locus was useful in separating *L. m. villaricensis* from the other morphotypes (Aat-2, bb and bc genotypes), and the Gpi locus was diagnostic for *L. m. monticola* (Gpi, bb). *Liolaemus monticola monticola* showed the largest number of diagnostic loci (six loci: Est-1, Est-2, Mdhp-1, Gpi, Sod-1 and Sod-2), similar to those in *L. monticola* ssp. (five loci: Est-1, Est-2, Mdhp-1, Mdh-1 and Adh-2) and *L. m. villaricensis* (four loci: Aat-2, Est-1, Est-2 and Mdhp-1). Only three allozyme loci helped in the diagnosis of *L. m. chillanensis* (Est-1, Est-2 and Mdhp-1). The monoallelic Mdhp-1 locus proved to be diagnostic among the four morphotypes (Table 2). Genotypic differentiation based on the exact test showed highly significant *P* values ($P < 0.0001$) in ten loci: Aat-2 (*L. m. villaricensis* vs. *L. m. monticola*, *L. m. chillanensis*, *L. monticola* ssp.); Adh-2 (among all morphotypes, but *L. m. chillanensis* vs. *L. m. villaricensis*); Est-1, Est-2 and Mdhp-1 (among all morphotypes); G3pdh-1, Gpi, Sod-1 and Sod-2 (*L. m. monticola* vs. *L. m. chillanensis*, *L. monticola* ssp., *L. m. villaricensis*); Mdh-1 (*L. monticola* ssp. vs. *L. m. monticola*, *L. m. chillanensis*, *L. m. villaricensis*). The exact test showed that, across all loci, pairwise comparisons among all four morphotypes were highly significant ($P < 0.0001$).

The Wiens and Servedio method is intended to be applied to one species at a time (Wiens & Servedio, 2000); therefore, the presumptive diagnostic differences among the four morphotypes were evaluated in each morphotype separately. We obtained significant values ($\alpha = 0.05$) using the frequency cut-off statistical analysis in *L. m. monticola* ($P < 0.001$), *L. monticola* ssp. ($P < 0.001$) and *L. m. villaricensis* ($P < 0.01$), with non-significance for *L. m. chillanensis* ($P = 0.721$), thus accepting a 10% polymorphism in the presumptive fixed characters.

The results of the non-hierarchical analysis (MDS) employing the genetic distances with allozyme data and a $1 - F_{st}$ matrix using cyt-*b* sequences are shown in Figure 3. Both analyses supported previous (meristic and allozyme) results, showing a strong dispersal of morphotypes in multidimensional space (stress < 0.0001).

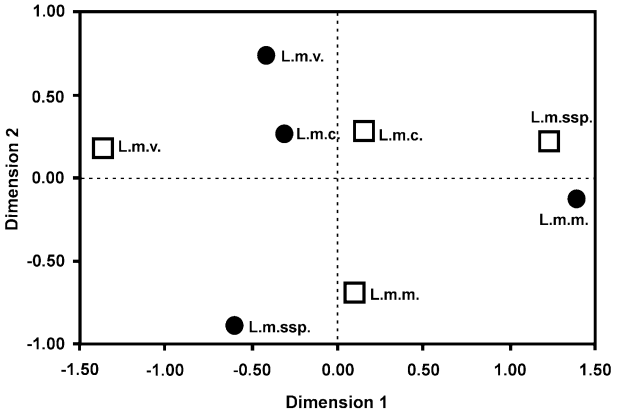

Figure 3. Bidimensional ordination for the four studied morphotypes of the *Liolaemus monticola* group using a non-metric multidimensional scaling method. Filled circles, analysis using allozymes (stress < 0.0001); open squares, analysis using cytochrome *b* gene (stress < 0.00001). L.m.c., *L. m. chillanensis*; L.m.m., *L. m. monticola*; L.m.ssp., *L. monticola* ssp.; L.m.v., *L. m. villaricensis*.

Table 2. Genotypic frequencies for polymorphic and monoallelic loci with differences in electrophoretic mobilities of the four morphotypes of the *Liolaemus monticola* group

		L. m. monticola	*L. m. chillanensis*	*L. monticola* ssp.	*L. m. villaricensis*
Locus	Genotype	$N = 23$	$N = 9$	$N = 20$	$N = 18$
Aat-1	aa	1.00	1.00	0.95	1.00
	ab			0.05	
Aat-2	aa	1.00	1.00	1.00	
	bb				0.89
	bc				0.11
Est-1	aa		0.89		
	ab		0.11		
	cc				0.89
	ec				0.11
	dd			0.05	
	df			0.15	
	ff			0.60	
	fg			0.15	
	gg			0.05	
	hh	0.78			
	hi	0.22			
Est-2	aa		0.56		
	ab		0.11		
	ac		0.22		
	bb		0.11		
	dd			0.10	
	de			0.40	
	ee			0.50	
	gg	0.61			
	gh	0.39			
	ff				1.00
G3pdh-1	aa	0.96	1.00	0.60	1.00
	ab	0.04			
	cc			0.05	
	ac			0.35	
G3pdh-2	aa	0.91	1.00	1.00	1.00
	ab	0.09			
Idh-2	aa	0.91	1.00	0.60	0.56
	ab	0.09		0.40	0.44
Pgm	aa	0.74	0.89	1.00	0.89
	ab	0.17	0.11		0.11
Mdhp-1	aa		1.00		
	bb	1.00			
	cc			1.00	
	dd				1.00
Mdh-1	aa	1.00	1.00		1.00
	bb			1.00	
Gpi	aa		1.00	0.6	1.00
	bb	1.00			
	ac			0.1	
Sod-1	aa		1.00	1.00	1.00
	bb	1.00			
Sod-2	aa		1.00	1.00	1.00
	bb	1.00			
Adh-2	aa	1.00	1.00		1.00
	bb			1.00	
P_l		25.0	12.5	25.0	16.67
$N_{Al.}$ (SD)		1.25	1.1667	1.2917	1.1667
		(0.4423)	(0.4815)	(0.5500)	(0.3807)
$H_{obs.}$ (SD)		0.0424	0.0231	0.0685	0.0324
		(0.0957)	(0.0731)	(0.1388)	(0.0954)

Parameters of genetic variability are summarized at the bottom: $H_{obs.}$, observed heterozygosity; $N_{Al.}$, average number of alleles by locus; P_l, percentage of polymorphic loci (95%); SD, standard deviation.

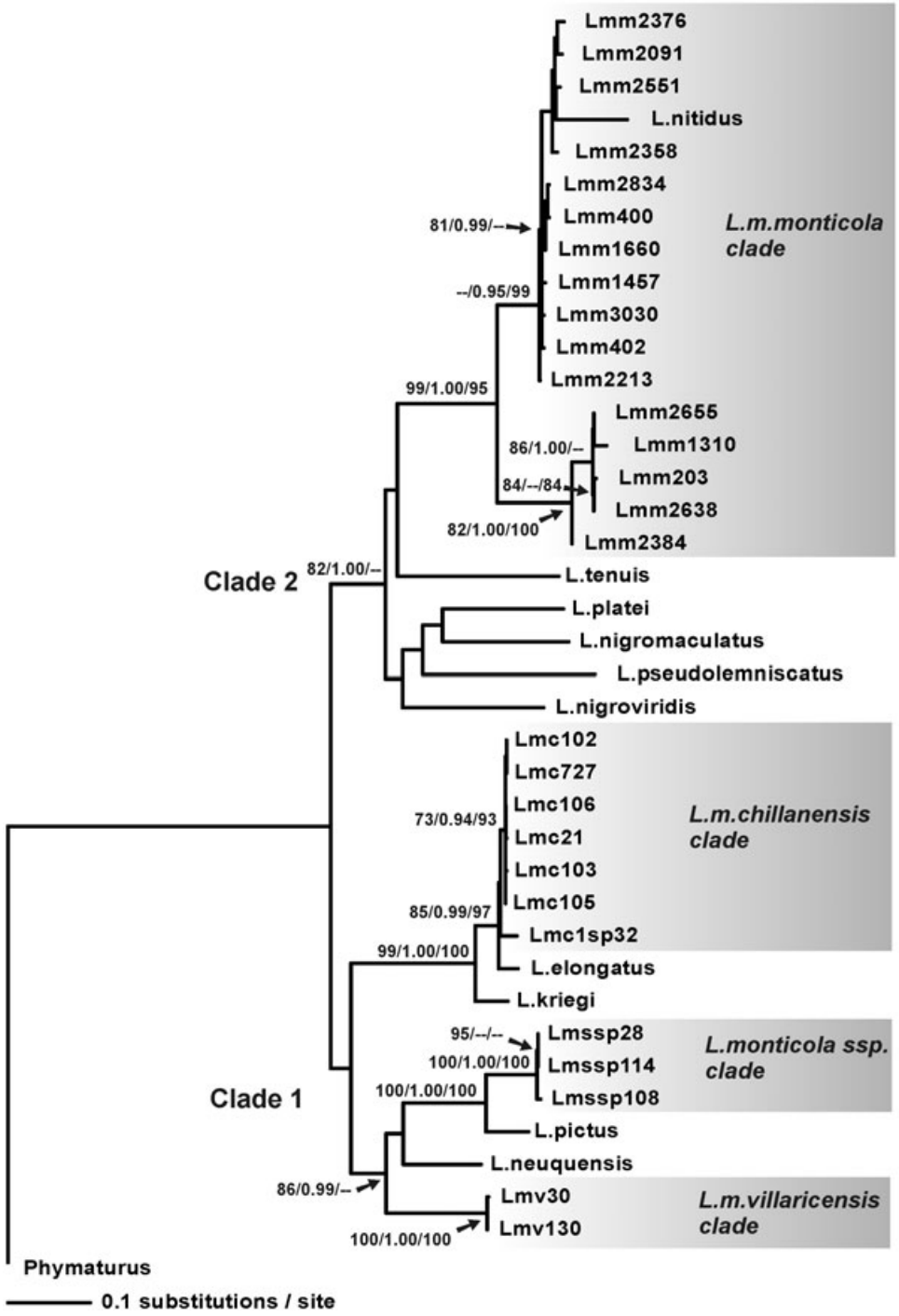

Figure 4. Maximum likelihood tree obtained from cytochrome *b* gene. Numbers above and below the branches indicate support based on the maximum likelihood (left) and maximum parsimony (right) bootstrap analyses, and posterior probabilities for Bayesian analyses (middle).

Phylogenetic analyses

Twenty-eight haplotypes were obtained from the 42 specimens of the four *L. monticola* morphotypes, and each of the selected outgroup species represented a unique haplotype. Figure 4 shows the ML haplotype tree. Bayesian topology differed from that of ML by not resolving *L. tenuis* and *L. nigroviridis* within Clade 2. The MP analysis recovered 240 most parsi-

monious trees [length, 959 steps; consistency index (CI), 0.447; retention index (RI), 0.779] based on 238 informative characters. MP strict consensus and 50% majority rule differed from Bayesian and ML topologies in the position of *L. nitidus* (see below), in grouping *L. platei* and *L. tenuis*, *L. pseudolemniscatus* and *L. nigroviridis*, and in grouping *L. m. villaricensis* haplotypes with *L. neuquensis*. Also, the *L. pictus–L. monticola* ssp. clade was recovered as sister to the *L. m. chillanensis–L. elongatus–L. kriegi* clade. Given that the major relationships of the *L. monticola* morphotypes (focal species of this study) were similar in the three phylogenetic analyses, we refer to the ML topology for a discussion of the results. All the analyses recovered two reciprocally monophyletic groups (Clades 1 and 2) with different levels of support. In Clade 1 (unsupported), three of the four *L. monticola* morphotypes were recovered. All *L. m. villaricensis* samples representing two haplotypes were grouped (*L. m. villaricensis* clade) with high likelihood (LB = 100) and parsimony (PB = 100) bootstrap values, as well as a high posterior probability (PP = 1.00) value. *Liolaemus monticola* ssp. terminals (*L. monticola* ssp. clade) were grouped in a supported clade (LB = 100, PB = 100, PP = 1.00) sister to *L. pictus* (LB = 100, PB = 100, PP = 1.00). Haplotypes of *L. m. chillanensis* were grouped in a clade, with those from Termas de Chillán supported (LB = 73, PB = 93, PP = 0.94), and the haplotype from Antuco volcano unsupported. The *L. m. chillanensis* clade was recovered in close association with *L. elongatus* (LB = 85, PB = 97, PP = 0.99), and this clade was sister to *L. kriegi* (LB = 99, PB = 100, PP = 1.00). Within the second major clade (Clade 2; LB = 82, PP = 1.00), *L. m. monticola* haplotypes (*L. m. monticola* clade) formed a monophyletic group (LB = 99, PB = 95, PP = 1.00), with two subgroups congruent with northern and southern chromosomal races (Torres-Pérez *et al.*, 2007). All three phylogenetic reconstruction criteria placed *L. nitidus* within the *L. m. monticola* haplotypes. MP differed from ML and Bayesian inference in placing *L. nitidus* in the middle of two *L. m. monticola* subclades. *Liolaemus tenuis* was sister and unsupported to the *L. m. monticola* clade, and this clade was sister (but unsupported) to a clade composed of *L. platei*, *L. nigromaculatus*, *L. pseudolemniscatus* and *L. nigroviridis*.

The results of the SH test showed that the unconstrained ($-\ln L = 4915.90966$) and constrained (monophyly of *L. monticola* morphotypes, $-\ln L = 8146.74004$) topologies were significantly different ($P < 0.001$). Wilcoxon signed-rank test showed that the constrained tree (length, 2728 steps) was significantly longer ($N = 213$, $Z = -12.662$, $P < 0.0001$) than the shortest unconstrained tree (length, 959 steps). Thus, both tests reject the monophyly of *L. monticola*.

DISCUSSION

The study of polytypic species has historically been a major focus in systematic biology (Mayr, 1942), with many examples coming from reptiles. However, the use of the subspecies concept in the denomination of taxa at the intraspecific level has been controversial (Wilson & Brown, 1953; Burbrink, Lawson & Slowinski, 2000), with several polytypic species being revalidated at the specific level. The genus *Liolaemus* provides several examples of this situation, with a number of polytypic species still waiting to be studied or validated (Cei, 1993; Pincheira-Donoso & Nuñez, 2005). Given that most of the polytypic species in *Liolaemus* have been traditionally postulated using morphological characters and only a few individuals, the addition of independent data sets, a better sampling scheme and new methodologies seems to be necessary to re-evaluate their taxonomic status.

In a recent phylogenetic analysis of *Liolaemus* species of the *chiliensis* group, Lobo (2001) found *L. monticola* subspecies to be monophyletic. A subsequent analysis with character re-evaluation and more extensive species sampling found the three *L. monticola* subspecies to be paraphyletic (Lobo, 2005). In the latter study, however, different character weighting schemes showed different affinities, although *L. m. chillanensis* and *L. m. villaricensis* were clustered in five of six approaches. In our phylogenetic analyses, *L. monticola* and all of its associated subspecies did not form a monophyletic group (Fig. 4). All reconstruction criteria (ML, MP, Bayesian inference) recovered each of the previously described subspecies related to different *Liolaemus* species; thus, *L. monticola* is polyphyletic. Six *Liolaemus* species appeared to be related to *L. m. monticola*, which is, for the most part, concordant with the topology obtained by Schulte *et al.* (2000) and Pincheira-Donoso, Scolaro & Schulte (2007) using mitochondrial genes. An unexpected result is the position of *L. nitidus* within the *L. monticola* clade (Fig. 4), both being reported previously as sister species (Schulte *et al.*, 2000). *Liolaemus nitidus* has a partially overlapping distribution with the *L. m. monticola* geographical range, and its position in this clade may be related to secondary contact or the introgression of mtDNA between these two species. Both species show strong differences in morphology (Donoso-Barros, 1966), chromosomes (Lamborot & Alvarez-Sarret, 1989; Lamborot, 1993), and some physiological and ecological traits (Escobar, Labra & Niemeyer, 2001; Pincheira-Donoso & Nuñez, 2005). mtDNA introgression has been reported previously between closely related *Liolaemus* species (from *L. darwinii* into *L. laurenti*) in Argentina (Morando *et al.*, 2004), and this process may also be taking place in this subclade of *L. m. monticola*.

Additional sampling and the sequencing of variable nuclear markers for *L. nitidus* and *L. m. monticola* may be necessary to clarify our findings.

Another interesting point of our analyses is that *L. m. chillanensis* is sister to *L. elongatus* and, in turn, related to *L. kriegi* (Fig. 4). *Liolaemus elongatus* is part of the *elongatus–kriegi* complex (Morando *et al.*, 2003); however, the *elongatus* group inhabits the southernmost range, and its distribution does not seem to currently overlap with that of *L. m. chillanensis*. A comprehensive evolutionary analysis of the *elongatus–kriegi* group, including *L. m. chillanensis*, is needed. Until then, we will consider *L. m. chillanensis* as a different taxon from *L. elongatus*, although related to the *elongatus–kriegi* complex. On the other hand, *L. monticola* ssp. is closely related to *L. pictus*. These two species are strongly divergent in terms of both the habitats in which they occur and in their morphological traits (Donoso-Barros, 1966; Vidal, Veloso & Méndez, 2006). Hence, we interpret the position of *L. monticola* ssp. and *L. pictus* as sister species as an artefact derived from the absence of other *Liolaemus* species. Indeed, Schulte *et al.* (2000) and Pincheira-Donoso *et al.* (2007) found *L. pictus* in a clade including other *Liolaemus* species (*L. chiliensis*, *L. cyanogaster*, *L. belli*), which were not included in our study. The polyphyly of *L. monticola* was also statistically corroborated when SH and Wilcoxon signed-rank tests showed significant differences between the unconstrained (Fig. 4) and constrained (monophyly of *L. monticola* morphotypes) trees. Several hypotheses have been proposed for the occurrence of polyphyletic groups (Funk & Omland, 2003), with imperfect taxonomy the most plausible for explaining the polyphyly of *L. monticola* morphotypes. Four *L. monticola* morphotypes show convergence in some morphological traits (for example, dark bands in flanks), which seems to be a product of independent adaptations rather than of a common ancestry (Wiens, Chippindale & Hillis, 2003).

In this study, the pattern of phenotypic variation is mostly congruent between the sexes, but does not reflect the phylogenetic divergence revealed by the cyt-*b* gene tree. Morphometric analyses could not discriminate among the four *L. monticola* morphotypes. In both males and females, PCA showed overlapping values in the first two components among the four morphotypes (Fig. 2A, B), suggesting that within-group variability largely exceeds between-group variability. Although DFAs recovered, on average, low values of correctly assigned individuals, males appeared to be more useful to discriminate among morphotypes (Table 1). Only *L. m. villaricensis* appeared to be relatively well assigned compared with the other morphotypes. Given that the four morphotypes occur in similar mountain habitats, the absence of differences using morphometric characters may be explained by similar ecological adaptations (Losos & Irschick, 1994; Losos, 2004; Sanders, Malhotra & Thorpe, 2004; Thorpe *et al.*, 2004) or by exaptation (Gould & Vrba, 1982; Revell *et al.*, 2007), leading to convergence in certain morphological traits. However, ecomorphological characteristics in *Liolaemus* show a different pattern, with no relationship between morphology (morphometrics) and habitat use (Schulte *et al.*, 2004). Therefore, our selected morphometric variables and/or the number of sampled individuals may be the reason for the lack of morphometric divergence in our analyses. On the other hand, meristic characters showed higher variability among groups, and were more useful in discriminating among the four *L. monticola* morphotypes than were morphometric analyses. Both males and females showed, on average, high percentages of correctly assigned individuals. *Liolaemus monticola monticola* was the most differentiated morphotype, with all individuals correctly assigned in males and females (100%), congruent with the large number of diagnostic loci detected using allozyme data. Independent evidence at the chromosomal level has shown that the northernmost and more widely distributed morphotype *L. m. monticola* exhibits high chromosomal polymorphism, with variable chromosome numbers ranging from $2n = 34$ to $2n = 44$ (Lamborot, 1991, 1998a; Lamborot & Eaton, 1997). This variation is also congruent with morphological (meristic), allozyme and cyt-*b* analyses, with past fragmentation processes among populations mediated by riverine barriers (Lamborot & Eaton, 1992; Lamborot *et al.*, 2003; Torres-Pérez *et al.*, 2007; Vásquez, Torres-Pérez & Lamborot, 2007). This chromosomal pattern is also divergent from the southern *L. m. chillanensis* and *L. m. villaricensis* morphotypes, which show a primitive chromosomal number of $2n = 32$ (Lamborot *et al.*, 1981).

Our allozyme results showed a strong genetic divergence among the four morphotypes, with at least one presumptive fixed locus below the cut-off frequency (10%), except in the *L. m. chillanensis* morphotype. The small number of sampled individuals of *L. m. chillanensis* in allozyme analyses could explain the absence of significance in the Wiens–Servedio method. Specifically, three allozyme markers (Est-1, Est-2 and Mdhp-1) independently diagnosed each morphotype (Table 2), indicating that allozymes are adequate markers for distinguishing taxa at the specific level in some *Liolaemus* species. The exact test of genotypic differentiation showed that genotype frequencies were not significantly homogeneous among the four morphotypes, and the MDS analysis showed high genetic distances (and F_{st} values using cyt-*b*), as

evaluated by a strong dispersal of plots in multidimensional space (Fig. 3).

The choice of species concept in a species delimitation study is not a trivial issue, as many species concepts have been proposed (for example, see the review in Hey, 2001), and several operational criteria are applicable to each depending on the data used (Sites & Marshall, 2004). However, a consensus among evolutionary biologists is that species are real and represent distinct evolutionary lineages (Wiens & Penkrot, 2002; de Queiroz, 2005). Our phylogenetic reconstructions based on molecular sequence data are congruent with allozyme and meristic data, showing that what was once thought to represent different subspecies of a single species, *L. monticola*, constitutes multiple lineages (species). This result is supported by the congruence between analyses of independent sources of data (Wiens & Penkrot, 2002; Morando *et al.*, 2003). The lineages in our study probably diverged a long time ago, as these forms not only had enough evolutionary time to achieve reciprocal monophyly, but are also phylogenetically related to different groups of *Liolaemus*. Therefore, we propose that the subspecifically named focal taxa of this study should be henceforth referred to as *Liolaemus monticola*, *Liolaemus chillanensis* and *Liolaemus villaricensis*. The nomenclatural status of the unnamed *L. monticola* ssp. has remained unresolved for more than 70 years. Preliminary morphological analyses show that *L. monticola* ssp. cannot be differentiated (Torres-Pérez, 2004) from type museum specimens of *L. cristiani* (Nuñez, Navarro & Loyola, 1991; Navarro & Nuñez, 1992). The latter species was described from El Cerro El Peine (35°37′S, 71°02′W), around 170 km north of the locality in which *L. monticola* ssp. was originally reported (Müller & Hellmich, 1932). Both taxa lack anal pores in males, inhabit similar mountain environments and are similar in design and coloration patterns. These features can also be found in the Argentinean *L. thermarum* (Videla & Cei, 1996), although these authors documented subtle differences between *L. thermarum* and *L. cristiani* in squamate characters and dorsal coloration. Therefore, additional analyses including these three taxa are necessary to elucidate their taxonomic status. Our study and previous investigations (Morando *et al.*, 2003; Espinoza, Wiens & Tracy, 2004; Schulte *et al.*, 2004; Avila *et al.*, 2006; Pincheira-Donoso *et al.*, 2007) are shedding light on a number of unresolved systematic issues and the exceptional radiation and speciation patterns in the genus *Liolaemus*.

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SUPPORTING INFORMATION

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

Appendix S1. Material examined.

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APPENDIX 1

MATERIAL EXAMINED

Specimens are stored in the "Museo de Zoología de la Universidad de Concepción" (MZUC), the "Colección de la Universidad de Chile" (CUCH), the "Zoologische Staatssammlung München" (ZSM), and the "Museo Nacional de Historia Natural de Santiago" (MNHN). Voucher specimen collections, collection localities (numbers in parenthesis are the same as in Fig. 1), and GenBank accession numbers are as follow.

PHYLOGENIES

Liolaemus monticola monticola

CUCH-2358, Mina Cerrillos (1), AY851706; CUCH-2376, Cabrería (2), AY851708; CUCH-2091, Rocín (3), AY851710; CUCH-2551, Colorado Norte (4), AY851713; CUCH-2213, Cuesta Chacabuco (5), AY851718; CUCH-1457, Quebrada Alvarado (6), AY851726; MZUC-28604, Lampa (7), AY851720; CUCH-1660, Yerba Loca (8), AY850619; CUCH-3030, Maipú (9), AY851724; CUCH-2834, Alfalfal Norte (10), AY850616; CUCH-2655, Rio Clarillo (11), AY851737; MZUC-28601, Baños Morales (12), AY851727; CUCH-1310, Cantillana (13), AY851735; MZUC-28603, San Fernando (14), AY851738; CUCH-2638, Los Queñes (15), AY851739; CUCH-2384, Rio Lontué (16), AY851740

Liolaemus m. chillanensis

MZUC-28249, Termas de Chillán (18), AY730668; MZUC-28255, Termas de Chillán, (18), AY730673; MZUC-28254, Termas de Chillán, (18), AY850621; MZUC-28251, Termas de Chillán.(18), AY730669; MZUC-28252, Termas de Chillán (18), AY529901; MZUC-28253, Termas de Chillán (18), AY529902; MZUC-25707, San Fabián de Alico (17), AY850622; MZUC-29230, Volcán Antuco (19), AY850630; MZUC-29215, Volcán Antuco (19), MZUC-29209, Volcán Antuco (19); MZUC-29229, Volcán Antuco (19); MZUC-29187, Volcán Antuco (19).

Liolaemus monticola ssp.

MZUC-28258, Termas de Chillán (20), AY529903; MZUC-28263, Termas de Chillán (20), AY850623; MZUC-28264, Termas de Chillán (20), AY850624; MZUC-28257, Termas de Chillán (20), AY730670; MZUC-28259, Termas de Chillán (20), AY529904; MZUC-912, Cordillera de Ñuble (21), AY850625

Liolaemus. m. villaricensis

MZUC-28238, Volcán Villarrica (22), AY529906; MZUC-28245, Volcán Villarrica (22), AY730671; MZUC-28241, Volcán Villarrica (22), AY850629; MZUC-28332, Volcán Villarrica (22), AY730672; MZUC-28232, Volcán Villarrica (22), AY525905; MZUC-28234, Volcán Villarrica (22), AY850626; MZUC-28240, Volcán Villarrica (22), AY850628; MZUC-28237, Volcán Villarrica (22), AY850627

Outgroups

Liolaemus nitidus CUCH-3042, EU220835; *Liolaemus nigromaculatus* CUCH-3143, EU220834; *Liolaemus nigroviridis* MZUC-28600, AY850633; *Liolaemus platei* MZUC-30556, AY850634; *Liolaemus pseudolemniscatus* CUCH-1822, EU220833; *Liolaemus tenuis* CUCH-2656, AY851742. *Liolaemus pictus* AY173795, *Liolaemus kriegi* AY173856, *Liolaemus neuquensis* AY173828, *Liolaemus elongatus* AY173855, *Phymaturus indistictus* AY173541 (Morando *et al.* 2003).

MORPHOLOGY (AND ALLOZYMES)

Liolaemus monticola monticola

MZUC-0261, 0262, 0266, 0267, 0326, cn282-287, cn101-104, cn364, cn366, 28591-285598, 28585, 28604, 28605, MNHN-548-551.

Liolaemus m. chillanensis

ZSM-60/1931a, ZSM-60/1931b (holotypes), MZUC-25687, 25688, 25690-25703, 30602, 30603, 25704, 25705, 25708, 01517R, 01518R, 28266-28275, 28249, 28251, 28254, 28276-28278, MZUCJCO-727

Liolaemus monticola ssp.

MZUC-25711-25714, 25716, 25718, 25721, 25723, 01506R, 01507R, 01513R,01515R, 28256-28259, 28261-28265, 28280-28282, 28284-28286, 28288, 28291-28294, 28296-28298, 30604-30611, MNHN-1957-1961, MZUCJCO-912.

Liolaemus m. villaricensis

MZUC-28232-28238, 28242, 28245, 28299-28318, 28332, 4116.