Trabajo

Molecular phylogeny of the "salty" *Liolaemus* species (Reptilia: Squamata): *anomalus* group, endemic of the Argentina Monte Desert

Mariana Morando[1,3], Melisa Olave[2], Jack W. Sites, Jr.[4], Luciano Javier Avila[1]

[1] Instituto Patagónico para el Estudio de los Ecosistemas Continentales, Consejo Nacional de Investigaciones Científicas y Técnicas (IPEEC-CONICET), Boulevard Almirante Guillermo Brown 2915, 9120, Puerto Madryn, Chubut, Argentina.

[2] Instituto Argentino de Investigaciones de Zonas Áridas, Consejo Nacional de Investigaciones Científicas y Técnicas (IADIZA-CONICET), Mendoza, Argentina.

[3] Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Bv. Almte Brown 3051, U9120ACD Puerto Madryn, Chubut, Argentina.

[4] Department of Biology and M. L. Bean Life Science Museum, Brigham Young University, 4029 LSB, 84602, Provo, Utah, USA. Current address: Department of Biology, Austin Peay State University, Clarksville, Tennessee 37044, USA.

ABSTRACT

In Argentina, the Monte ecoregion extends for more than 2,000 km from NW Argentina to boreal Patagonia, and includes many salt-pans and barren flatlands colonized by the monophyletic *Liolaemus anomalus* group that includes seven species. Some of their external morphological characteristics and behaviors are very unusual for the genus, and this has led to a complex taxonomic history. The group is very poorly known except for a recent paper with descriptions of three species and morphological-based phylogenetic hypotheses. Of the four recognized species, two are threatened and one is vulnerable, and while the conservation status of the three recently described species is unknown, they are suspected to also be under some degree of threat. We reviewed all georeferenced localities known to produce a distribution map, and sequenced two mitochondrial and four nuclear genes, for representatives of all species. We inferred a time-calibrated species tree showing that the evolutionary history of this clade occurred entirely during the Pleistocene, with most of the divergences very recent. The climatic and geomorphological changes driving this divergence started during the Great Patagonian Glaciation, initially separating the two northwestern-most distributed species (*L. pipanaco + L. pseudoanomalus*) from the rest. Given the very recent evolutionary history of the group and their unique and conserved morphology, incongruent topologies among datasets are expected; a detailed genome-wide dataset will be needed to fully assess and resolve their speciation history.

Key Words: Species Tree; Liolaemini; Liolaemidae; Pleistocene.

RESUMEN

En Argentina, la ecorregión de Monte se extiende por más de 2.000 km desde el noroeste de Argentina hasta la Patagonia boreal, e incluye muchas salinas y llanuras áridas colonizadas por el grupo monofilético *Liolaemus anomalus* que incluye siete especies. Algunas de sus características y comportamientos morfológicos externos son muy inusuales para el género y esto dio lugar a una compleja historia taxonómica. Recientemente se describieron tres nuevas especies y se propusieron hipótesis filogenéticas basadas en morfología. Desde el punto de vista de su conservación, solo cuatro están categorizadas, dos amenazadas y una vulnerable, y aunque se desconoce el estado de conservación de las tres últimas especies descritas, se sospecha que se encuentran bajo algún grado de amenaza. Revisamos todas las localidades georreferenciadas conocidas para producir un mapa de distribución, y secuenciamos dos genes mitocondriales y cuatro nucleares, para representantes de todas las especies. Inferimos un árbol de especies calibrado en el tiempo que muestra que la historia evolutiva de este clado ocurrió en su totalidad durante el Pleistoceno, siendo la mayoría de las divergencias muy recientes. Los cambios climáticos y geomorfológicos que impulsaron esta divergencia ocurrieron durante la Gran Glaciación Patagónica, inicialmente separando las dos especies distribuidas más al noroeste (*L. pipanaco + L. pseudoanomalus*) del resto. Dada la historia evolutiva muy reciente del grupo y su morfología única y conservada, se esperan topologías incongruentes entre los conjuntos de datos. Se necesitará un conjunto de datos detallado de todo el genoma para evaluar y resolver completamente su historia de especiación.

Palabras claves: Árboles de Especies; Liolaemini; Liolaemidae; Pleistoceno.

Author for correspondence: morando@cenpat-conicet.gob.ar

Introduction

Lizards of the genus *Liolaemus* represent one of the most extraordinary cases of evolutionary diversification among terrestrial vertebrates, now registering at least 273 described species (Uetz *et al.*, 2020). Most species-rich regions are in Argentina and Chile, and they are especially diverse in the Andes, Puna, Patagonian Steppe and Monte ecoregions. The Monte formation extends over 2,000 km from NW Argentina to central Patagonia, and includes many salt-pans and barren flatlands colonized by the monophyletic *Liolaemus anomalus* group (Abdala and Juárez Heredia, 2013; Olave *et al.*, 2014), that share a number of unique morphological and behavioral traits (Abdala and Juarez Heredia, 2013). This group is included in the *L. boulengeri* series and is strongly supported as sister to the *L. wiegmannii* group on the basis of both morphological (Abdala and Juárez Heredia, 2013) and genomic data (Morando *et al.*, 2020a).

Species of the *Liolaemus anomalus* group are difficult to observe in nature; the first photographs of live *L. lentus* were only published in 2011 (Perez *et al.*, 2011), they are very poorly represented in biological collections, and their geographic sampling is very limited (Avila *et al.*, 2003; Pérez and Avila, 2011; Abdala and Juárez Heredia, 2013; Avila *et al.*, 2015). Some of their external morphological characteristics and behaviors are more similar to some groups of Leiosaurini (*Diplolaemus* or *Leiosaurus*), than other *Liolaemus*. These include short stout bodies, prominent heads, short thin tails, lack of sexual dichromatism, and mimetic behavior. It is worth nothing that in the presence of a potential threat, they show a tendency to remain motionless, in contrast to the majority of species of the genus *Liolaemus* that tend to escape (Schulte *et al.*, 2004). This strategy, coupled with their irregular dorsal coloration, can make them very cryptic even at very short distances. Their sprinting behavior is fast and non-linear running and the body and tail are lifted on the legs, again, in contrast to most *Liolaemus* species (LJA Pers. Observ.). These features led early researchers to suggest several alternative relationships for the *L. anomalus* group with distant species groups until Etheridge (1995) recognized them all as *Liolaemus*. The complex nomenclatural history of this group was reviewed by Abdala and Juarez Heredia (2013).

The *Liolaemus anomalus* group includes seven species, mostly described based on morphological characters following a classical approach (descriptions based on general coloration, external scalation and/or body proportions). They are restricted to isolated salt pans (Fig. 1) and related barren areas where temperatures are usually so high that lizards have only a small "window" of time (2-3 hr/day) to forage, mate, and interact socially. They are likely vulnerable to rising temperatures that reduce this "activity window", thus they could be driven to extinction before we know their real diversity. Global warming is predicted to heavily impact lizard populations and requires accelerated species discovery coupled with mapping of species distributions (Sinervo *et al.*,
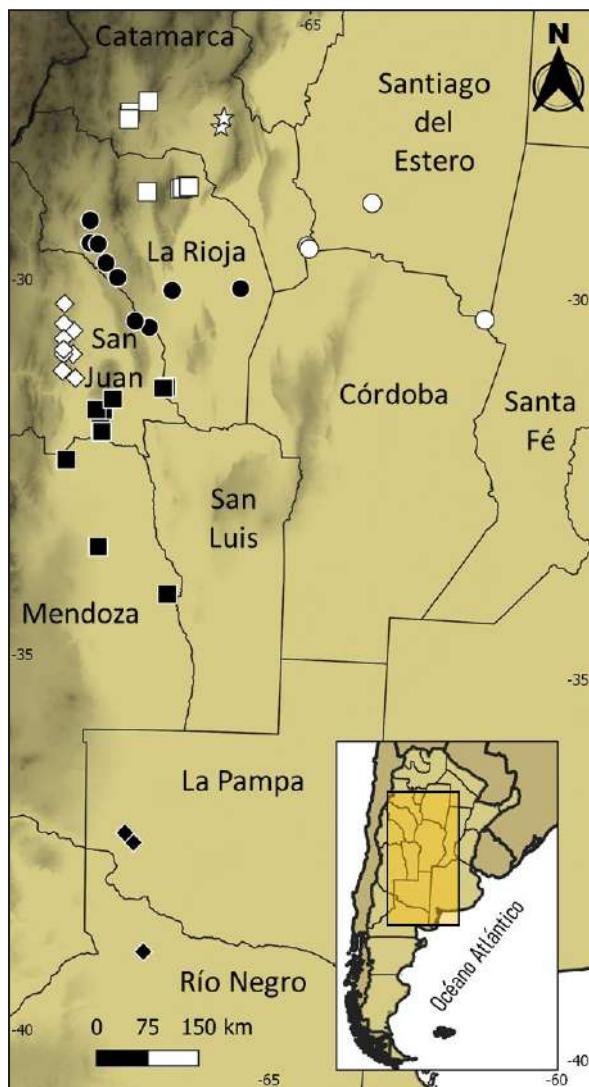
Figure 1. Distribution map with known georeferenced localities of species of the *Liolaemus anomalus* group. White stars: *L. pipanaco*; white squares: *L. pseudoanomalus*; Black circles, *L. anomalus*; white romboids: *L. acostai*; black squares: *L. millcayac*; white circles, *L. ditadai*; black romboids: *L. lentus*.

2010; Bernardo, 2011), as well as increased rigor in taxonomic descriptions.

Knowledge of this species group is limited; the majority of publications are the original species descriptions, geographic citations of new localities, or a few revisions or nomenclatural discussions. The single exception is (Abdala and Juárez Heredia, 2013); these authors described three new species and present morphology-based phylogenetic hypotheses for their relationships (alternative topologies depending on k values).

Almost all species seem to have low population densities, cryptic coloration, and very characteristic behaviors, rendering them difficult to find and study. They use abandoned mammal burrows and shrubs roots on ¨nebkas¨ or coppine dunes, salt crust plates, and even some road garbage for temporary or permanent shelters; they are oviparous, apparently insectivorous, and show limited sexual dimorphism (LJA Pers. Obs.). Four species have been assigned to three conservation categories: *Liolaemus ditadai* and *L. lentus* as threatened, *L. anomalus* as vulnerable, and only *L. pseudoanomalus* as least concern (Abdala *et al.* 2012). Here we reviewed georeferenced geographic data from various scientific collections to produce a map of their known localities, and we use mitochondrial and nuclear gene sequences collected for all described species, to infer their phylogenetic history using a colaescent-based species tree approach.

Materials and methods

Taxa: We used 38 individuals representing all the seven described species of the *anomalus* group: *L. acostai* (4), *L. anomalus* (7), *L. ditadai* (6), *L. lentus* (6), *L. millcayac* (8), *L. pipanaco* (1), *L. pseudoanomalus* (5); and 2 individuals of *L. scolaroi* (*L. lineomaculatus* series) as the outgroup. (For voucher numbers, localities and GB accession numbers see Suppl. Mat. Tables 1, 3).

Gene fragments: We collected sequences from two mitochondrial gene fragments: 1-cytochrome b (841 bp aligned matrix), using the light-strand primers Glu-DGL (Palumbi, 1996) and the heavy-strand primer Cytb 2 and Cytb 3 (Palumbi, 1996), and the Cyt.F.1 primers (Whiting *et al.*, 2003) were used as internal sequencing primers; 2-12S (956 bp), using the primers of Wiens *et al.* (1999). Mitochondrial PCR conditions followed Morando *et al.* (2003). We sequenced fragments of four protein-coding nuclear genes: 1 - dmX-like protein 1 (977 bp) (DMXL1; Werneck *et al.*, 2012); 2 - dynein axonemal heavy chain 3 (726 bp) (DNAH3; Townsend *et al.*, 2008); 3 – kinesin family member 24 (551 bp) (KIF24; Portik *et al.*, 2012); and 4 - prolactin receptor (508 bp) (PRLR; Townsend *et al.*, 2008).

Species tree and divergence time estimation: All nuclear genes were phased using the algorithm implemented at DnaSp v5 (Librado and Rozas, 2009). For each gene we selected the best-fitting model of evolution with JModelTest v0.1.1 (Posada, 2008), using the Bayesian Criterion Information (BIC, Table 1). A total of 6 loci and 75 individuals were included for species tree estimation using the coalescent based program *BEAST v1.8.3 (Heled and Drummond, 2010). Uncorrelated lognormal clocks modelled for each gene were used. We estimated divergence times based on available *Liolaemus* mutation rates (Olave *et al.*, 2015) to calibrate five of the loci in this study. The only exception is the missing mutation rate for the DMXL gene, which here was modeled with mean = 0. 001 site per million years and sd= 1.2; this gives a close value expected relative to other nuclear loci in *Liolaemus* (Olave *et al.*, 2015). All mutation rates are summarized in Table 1, for a lognormal hyperprior in real space. Standard deviations were set= 1.2 for all genes to insure a relatively large interval within

Table 1. Details of loci used for species tree estimation, substitution model selected and priors used for divergence time estimation.

Gene	Length (bp)	Model	Lognormal hyperprior media	Lognormal hyperprior s.d.
12S	898	HKY+I	0.00633900	1.2
cytb	841	HKY+G	0.01935500	1.2
DMXL	977	HKY	0.00100000	1.2
DNAH3	726	HKY+I	0.00076162	1.2
KIF24	551	HKY+I	0.00190210	1.2
PRLR	508	HKY+I	0.00132228	1.2

95% values of the curve. The tree root was also calibrated following Olave *et al.* (2015), using a normal hyperprior with mean = 15 my, standard deviation = 1 and offset values 10 – 20 my. We used a birth-death tree prior model run in two independent analyses for 100 x10^7 MCMC generations (burnin 10%), sampling every 100,000 intervals, and convergence was diagnosed when ESS values > 200.

Cytochrome-b pairwise genetic distances: We used the R function dist.gene() for the ingroup taxa. Individuals including missing data were removed to avoid calculation bias.

Computing gene tree – species tree discordance: We implemented the program *PhyParts v0.0.1* (Smith *et al.*, 2015) to calculate the level of gene tree – species discordance. This algorithm offers the advantage of estimating the level of observed gene tree discordance among all nodes of the species tree, instead of traditional estimates of a single value of discordance for the entire topology (e.g. Robinson-Foulds distance [Robinson and Foulds, 1981], and a single branch length score [Kuhner and Felsenstein, 1994]). *PhyParts* calculates the number of bi-partitions across gene trees that conflict with each node of a given species tree, using the algorithm introduced by Salichos *et al.* (2014). Under this approach, each edge of a tree is deconstructed to obtain the set of all bipartitions. Then each gene tree is examined to detect whether a given bipartition is in concordance (or in conflict) with the species tree. Given a set of rooted trees, a bipartition (h) is in conflict with a species tree (s) if (i) the ingroup of h contains any of the ingroup of s, (ii) the ingroup of h contains any of the outgroups of s, and (iii) the ingroup of s contains any of the outgroup of h. We summarized this information as follows (per node): number of bipartitions in concordance, number of bipartitions in concordance with a specific alternative topology, and remaining number of bipartitions in conflict. In addition, we applied a bootstrap filter where edges with low bootstrap values were ignored in further analyses. Specifically, bootstrap values lower than 50% were considered as polytomies. This prevents errors of inflating the level of concordance/conflict given a high uncertainty in gene tree estimation. The analysis was run using the species tree and all gene trees estimated by BEAST, as described above.

Results and Discussion

The phylogenetic history of the *Liolaemus anomalus* species group was inferred to be completely in the Pleistocene (Fig. 2). The first diversification occurred around 1.29 ma, separating the two northwestern-most distributed species (*L. pipanaco* and *L. pseudoanomalus*), from its sister clade. The second clade includes the other five species, which although they have radiated very recently, *L. ditadai* was inferred (PP = 1) as the first lineage that split from the rest. The other four species have moderate support values and they seem to have radiated simultaneously. The earliest split of the group is coincident with the Great Patagonian Glaciation (~1.68–1.02 my; Rabassa *et al.*, 2005), which is also similarly correlated with the first split within the sand dune specialist *L wiegmannii* complex (most of its central and western distribution overlaps with the *anomalus* group), separating its northwestern populations (sub-Andean sedimentary formations), from the "sand fields" populations in the Pampas and northern Patagonia (Villamil *et al.*, 2019).

Within the second clade of the *Liolaemus anomalus* group, the southernmost (*L. lentus*) and the easternmost (*L. ditadai*) distributed species, were inferred as slightly older splits than those with more central distributions, but it is important to highlight that most support values are moderate (PP= 0.7-0.9) and one is very low (PP= 0.53).

Most of gene trees (Suppl. Mat.) are concordant with the species tree (Fig. 3; blue proportion in pie charts), but the three most recent divergences had increased proportions of gene tree discordance (Fig. 3; nodes 3-5), as expected given their very low support values (Fig. 2). Increased gene tree discordance is expected in recent divergences if processes such incomplete lineage sorting play a role (see Maddison 1997). Alternatively, gene tree discordance could also be a product of interspecific hybridization, which has been suggested as an important process driving the evolution of many groups within *Liolaemus* (Olave *et al.*, 2018; all reviewed in Morando *et al.*, 2020b). If this is also true for the *L. anomalus* complex, then larger datasets are needed to provide sufficient statistical power to identify the roles of incomplete lineage sorting vs. hybridization in the evolution of this clade.

It has been common to report uncorrected pairwise cyt-b distances as a "raw" indicator of levels of divergence between closely related species of *Lio-*
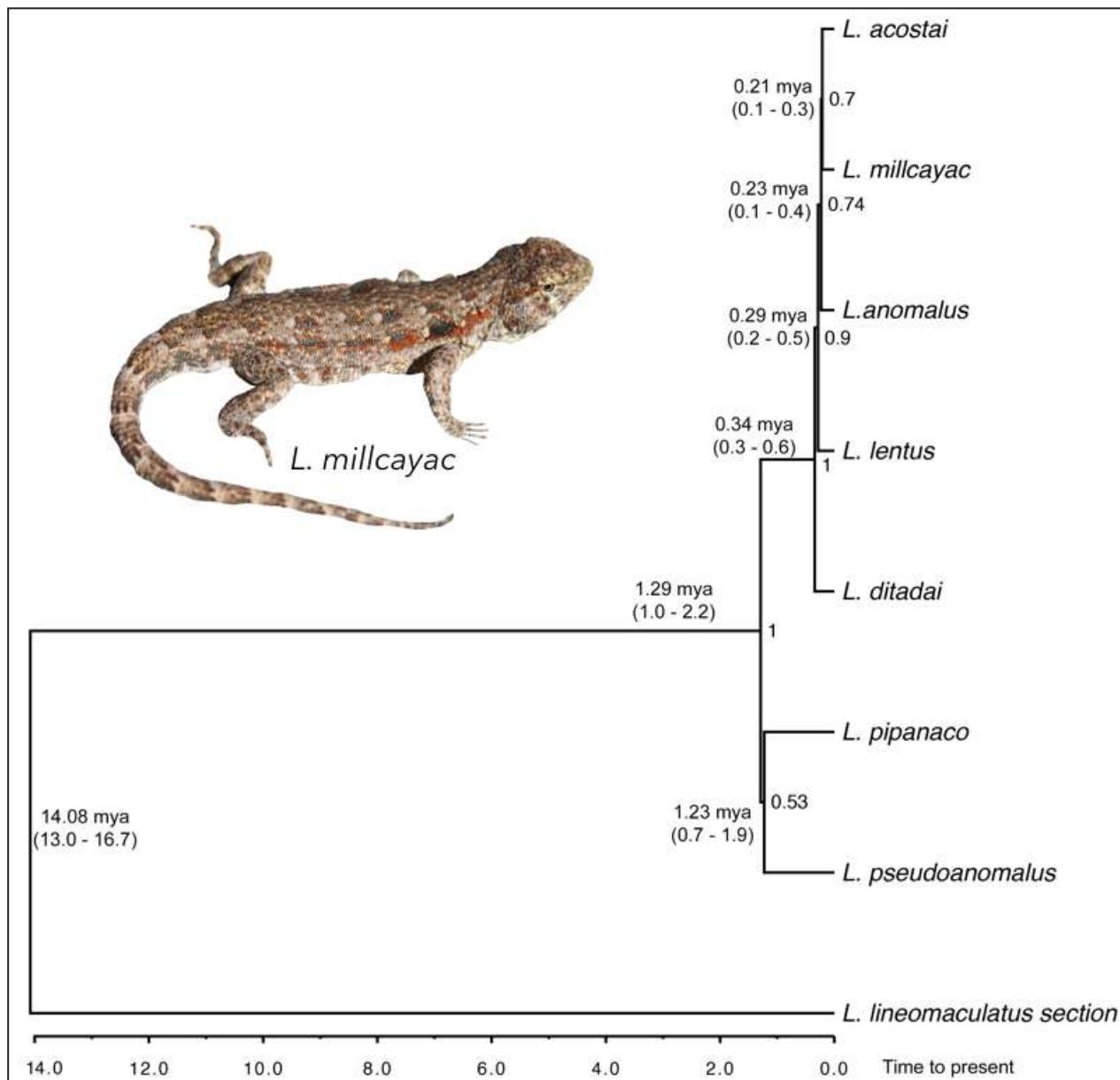
Figure 2. Species tree inferred with *BEAST based on two mitochondrial and X nuclear markers. Numbers right of nodes are posterior probabilities and on the left divergence estimation times in million years.

laemus, and in many/most studies the average threshold has been around 3% (Breitman *et al.*, 2012). Distances between *L. pipanaco* or *L. pseudoanomalus* and the other species are all > 5-6%, while between all the other five species they are < 3% and among *L. acostai* and *L. millcayac* this is 1.7-1.8% (Fig. 4; Suppl. Mat. Table 2). For other *Liolaemus* groups that include closely related species, some distances have been reported under 3% (*L. wiegmannii*, Avila et al., 2009; L. *lineomaculatus* section, Breitman et al., 2012, 2015). It is worth to highlight that the *L. wiegmannii* complex has a similar distributional pattern as the *L. anomalus* group, fragmented patches

of sand dune habitats that most possibly originated concomitant with the salty patches of the *L. anomalus* group as a result of similar geomorphological and climatic processes (Villamil et al. 2019).

This molecular phylogenetic hypothesis is different from the alternative morphologically-based topologies inferred with characters equally weighted, or with K=1/2 or K=3/20 that also differ among them (Abdala and Juárez Heredia, 2013). The only congruent inference with molecular and morphological data is the relationship between the geographically very close *L. acostai* and *L. millcayac*, although the morphological hypotheses (k=1/2 or
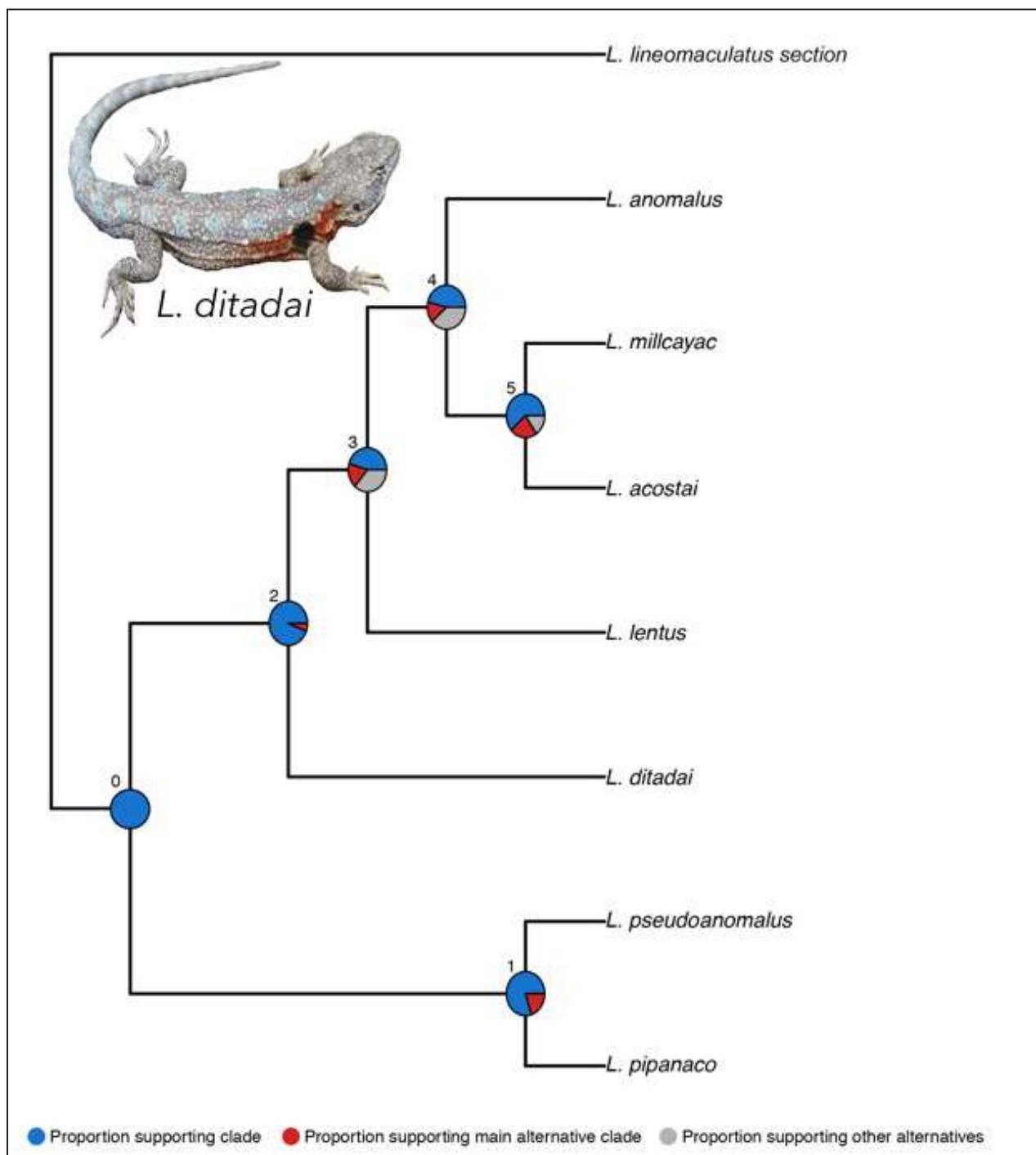
Figure 3. Gene tree – species tree discordance calculated using PhyParts program. Blue: proportion of gene trees supporting the clade (i.e. no gene tree discordance); red: proportion of gene trees supporting a main alternative topology; gray: proportion of gene trees supporting other alternatives. Nodes were numbered from 0 (root) to 5.

k=3-20) also include the more geographically distant *L. pipanaco* in this clade while the molecular one infers *L. pipanaco* as distantly related.

These lizards share some unique morphological features that most probably are adaptations for the extremely dry, low resource environments they inhabit. These could be due to phylogenetic constra-

ints, given their recent history of divergence, most probably coupled with stabilizing selection that has been inferred for other *Liolaemus* species inhabiting harsh environments (Olave *et al.*, 2017). This conserved morphology also presents a difficult challenge for morphological inferences about species limits and phylogenetic relationships; for example, there
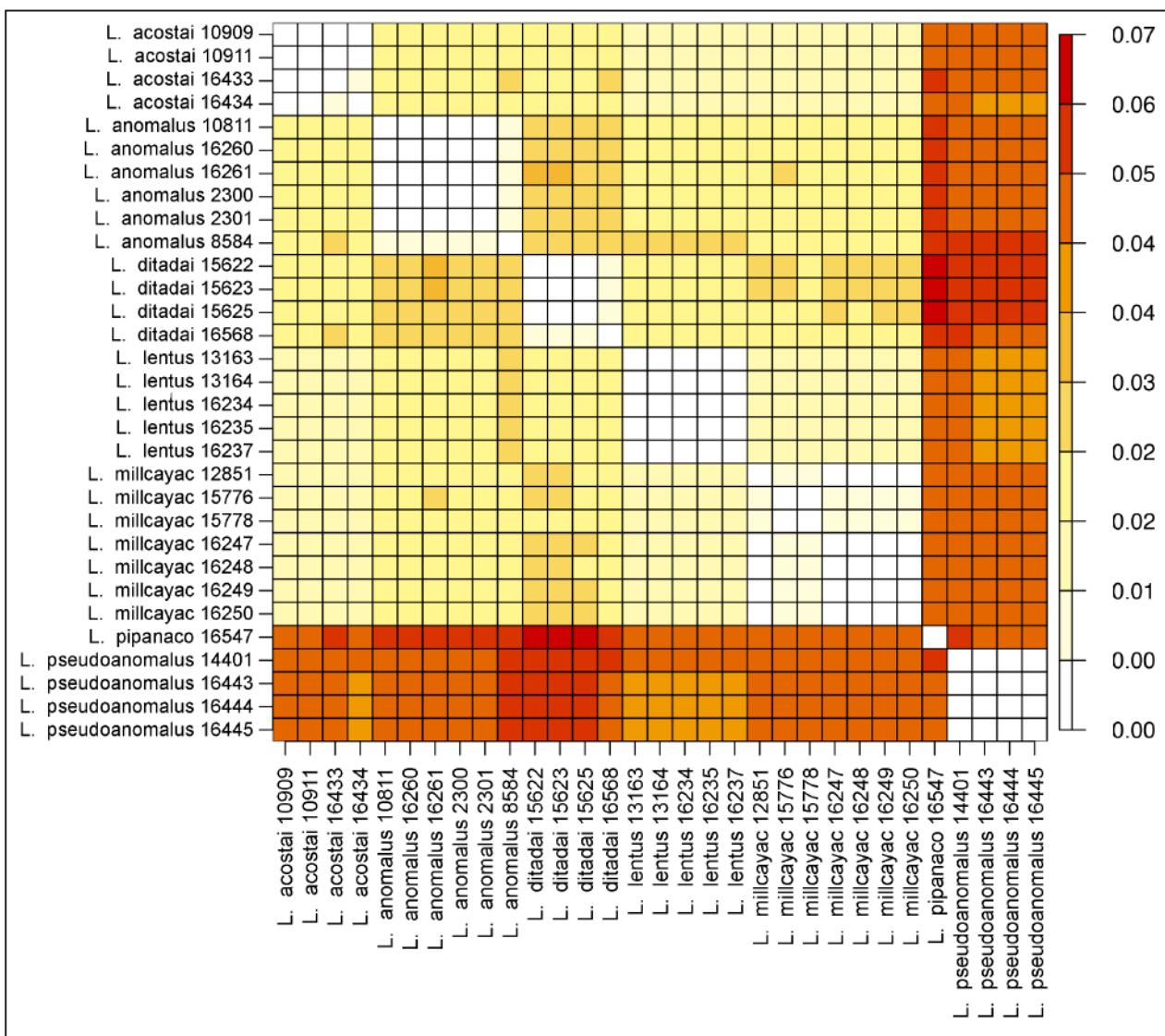
Figure 4. Proportion of cyt-b genetic distances for pairwise comparisons of the ingroup taxa expressed in color gradient as indicated by the bar color on the right.

is no exclusive morphological character to differentiate *L. acostai* (as pointed out by Abdala and Juárez Heredia, 2013), although additional characters and more thorough statistical analyses are warranted.

The very recent divergence history of this group coupled with its conserved morphology contributes to the incongruent morphological and molecular phylogenetic hypotheses. Most probably insufficient time has passed for these lineages to accumulate detectable morphological differences, but strong stabilizing selection and/or the near-simultaneous fragmentation/isolation of populations must also be considered. Genome wide SNP data coupled with an integrative morphological approach are needed in order to further understand the evo-lutionary history of this unique and endangered group of "salty" lizards, before they are "burned" to extinction by the exponentially increasing global temperatures (Sinervo *et al.*, 2010).

Literature cited

Abdala C.S. & Juárez Heredia V.I. 2013. Taxonomía y filogenia de un grupo de lagartos amenazados: el grupo de *Liolaemus anomalus* (Iguania, Liolaemidae). *Cuadernos de Herpetología* 27: 109–153.

Avila, L.J., Morando, M. & Belver, L.C. 2003. Natural history and geographic distribution of the desert lizard *Liolaemus pseudoanomalus* (Squamata: Iguania: Liolaemidae) from northern Monte region, Argentina. *Bulletin Chicago Herpetological Society* 38: 52–53.

Avila, L.J., Pérez, C.H.F. & Cacciali, P. 2015. New record of *Liolaemus ditadai* Cei, 1983 (Squamata, Iguania, Liolaemini) from Santiago del Estero province, Argentina. *Check List* 11: 1–3.

Breitman, M. F., Avila, L. J., Sites Jr., J. W., & Morando, M. 2012. How lizards survived blizzards: phylogeography of the *Liolaemus lineomaculatus* group (Liolaemidae) reveals multiple breaks and refugia in southern Patagonia and their concordance with other codistributed taxa. *Molecular Ecology* 21: 6068–6085.

Breitman, M.F., Bonino, M., Sites Jr., J.W., Avila, L.J. & Morando, M. 2015. Morphology, niche modeling and phylogeography of four species of lizards of the *Liolaemus lineomaculatus* section species (Liolaemini) from southern Patagonia. Herpetological Monographs. 29: 65–88.

Heled, J. & Drummond A.J. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* 27: 570–580.

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

Morando, M., Avila, L.J. & Sites, J.W., Jr. 2003. Sampling strategies for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus–kriegi* complex (Squamata: Liolaemidae) in Andean–Patagonian South America. *Systematic Biology* 52: 159–185.

Morando, M., Olave, M.; Avila, L.J.; Sites, J.W., Jr. & Leaché, A.D. 2020a. Phylogenomic data resolve higher-level relationships within South American *Liolaemus* lizards. *Molecular Phylogenetics and Evolution* 147: 106781.

Morando, M., C.D. Medina, I. Minoli, C.H.F. Pérez, J.W. Sites, Jr. & L.J.Avila. 2020b. Diversification and Evolutionary Histories of Patagonian Steppe Lizards: 217–254. *In*: M. Morando & L.J. Avila (Eds.). Lizards from Patagonia. Springer Nature.

Olave M., Avila, L.J., Sites Jr., J.W., & Morando, M. 2015. Model-based approach to test hard polytomies in the South American lizard genus *Liolaemus* (Liolaemini, Squamata). *Zoological Journal of the Linnean Society of London* 174: 169–184.

Olave M., Avila, L.J., Sites Jr., J.W. & Morando, M. 2017. Hidden diversity within the lizard genus *Liolaemus*: genetic vs morphological divergence in the *rothi* complex (Squamata:Liolaeminae). *Molecular Phylogenetics and Evolution* 107: 56–63.

Palumbi, S.R. 1996. Nucleic acids I: the polymerase chain reaction: 205–247. *In*: D. M. Hillis, C. Moritz & B. K. Mable (Eds.) Molecular Systematics. Sinauer, Sunderland, MA.

Perez, C.H.F. & Avila, L.J. 2011. First record of *Liolaemus lentus* Gallardo, 1966 (Squamata, Iguania, Liolaemini) in Río Negro province, Argentina. *Herpetology Notes* 4: 191–193.

Portik, D.M., Wood, P.L. Jr., Grismer, J.L., Stanley, E.L. & Jackman, T.R. 2012. Identification of 104 rapidly-evolving nuclear protein-coding markers for amplification across scaled reptiles using genomic resources. *Conservation Genetics Resources* 4: 1–10.

Schulte, J.A., Losos, J.B., Cruz, F.B. & Núñez, H. 2004. The relationship between morphology, escape behavior and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae:Tropidurinae: Liolaemini). *Journal of Evolutionary Biology* 17: 408–420.

Sinervo, B., Méndez-De la Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R. Martínez-Méndez, N., Calderón-Espinosa, M.L. , Nelsi Mesa-Lázaro, R., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Victoriano Sepúlveda, P., Duarte Rocha, C.F., Ibargüengoytía, N., Aguilar Puntriano, C., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D., Bauer, A., Branch, W., Clobert, J. & Sites, Jr., J.W. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328: 894–899. Townsend, T.M., Alegre, R.E., Kelley, S.T., Wiens, J.J. & Reeder, T.W. 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Molecular Phylogenetics and Evolution* 47: 129–142.

Uetz P., Freed, P. & Hosek, J. eds. 2020. The Reptile Database. http://www.reptile-database.org. Accessed August 24, 2020.

Villamil, J., Avila, L.J., Morando, M., Camargo, A., Leache, A. , Maneyro R. & Sites Jr., J.W. 2019. Coalescent-based species delimitation in the sand lizards of the *Liolaemus wiegmannii* complex (Squamata: Liolaemidae). *Molecular Phylogenetics and Evolution* 138: 89–101.

Werneck, F.P., Gamble, T., Colli, G.R., Rodrigues, M.T. & Sites, Jr., J.W. 2012. Deep diversification and long-term persistence in the South American "Dry Diagonal". Integrating continent-wide phylogeography and distribution modeling of geckos. *Evolution* 66: 3014–3034.

Whiting, A.S., Bauer, A.M. & Sites, Jr., J.W. 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). *Molecular Phylogenetics and Evolution* 29: 582–598.

Wiens, J.J., Reeder, T.W. & Nieto Montes de Oca, A. 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovii*). *Evolution* 53: 1884–1897.

Appendix I.

Liolaemus anomalus: **La Rioja**: Dto. Felipe Varela: Ruta Provincial 26, 3 Km N Pagancillo (-29,5155 S, -68,2394 W): LJAMM-CNP 2300/2540/BYU 47181. Ruta Nacional 76, 23.8 km S cruce Ruta Nacional 40, 2 km N Pagancillo (-29,5278 S, -68,1167 W): LJAMM-CNP 10811. Ruta Provincial 18, 5 km NE Pagancillo, entre Pagancillo y Puerto Alegre (-29,5278 S, -68,1167 W): LJAMM-CNP 16260/1. Ruta Nacional 76, 57.5 km SE Pagancillo, Parque Nacional Talampaya (-29,9716 S, -67,8152 W.): LJAMM-CNP 8584. Talampaya (-29,7798 S, -67,9933 W): FML 02740. Dto. Independencia: Guayapa, Patquia (-30,1349 S, -66,9772 W): MCZ 254200. Dto. General Lamadrid: Ruta Nacional 76, 9.86 km N Villa Unión, cerca de Río Colorado, 1 km S río La Calera (-29,2136 S, -68,2394 W): LJAMM-CNP 8579.

San Juan: Dto. Valle Fértil: Agua de la Peña: Ischigualasto (-30,0899 S, -65,9312 W): SDSU 1676/MCZ 58120/22. Barreales camino a San Ramón (-30,6294 S, -67,3181 W):

IMCN-UNSJ 112. Camino a Jaled, norte de Usno (-30,5512-67,5372 W): IMCN-UNSJ 58

Liolaemus acostai: **San Juan**: Dto. Albardón: Camino entre Baños La Laja y Baños del Salado, 0.5 km S Baños del Salado. (-31,3169 S, -68,4541 W): LJAMM-CNP 16433/4, IMCN UNSJ 232/3/5/42/62/453. Dto. Jáchal: Ruta entre Mogna y Ruta Nacional 40, 16 km W Mogna, W Quebrada de Mogna, Sierra de Morado (-30,6848 S, -68,4844 W): LJAMM-CNP 10911. Posta El Balde: Ruta Nacional 40, Km 241 (-30,5921 S, -68,6362 W): FML 03734. Río Las Lajas, Ruta Provincial 82, 24.9 km NE empalme Ruta Nacional 40, camino a Mogna (-30,9998 S, -68,4918 W): LJAMM-CNP 10909. 7 km S Adán Quiroga (-30,7916 S, -68,638 W): LJAMM-CNP 8523. 13 km N Talacasto (-31,00158 S, -68,63958 W): MVZ 126894. Ingeniero Matias G. Sanchez (-30,9362 S, -68,63801 W): MVZ 246231. 10 km S Adan Quiroga (-30,3282 S, -68,625' W): MVZ 92952. 14 km S Talacasto (-31,2200 S, -68,6539 W): MVZ 246235. Dto. Ullum: Loma de las Tapias, frente a complejo UNSJ (-37,3676 S, -67,5845 W): IMCN UNSJ 419. Matagusanos. (-3124401 S, -6862368 W): MVZ 137750.

Liolaemus ditadai: **Catamarca**: Dto. La Paz: Ruta Nacional 157, 2 km NW empalme Ruta Nacional 60, Salinas Grandes (-29,4958 S, -64,9320 W): LJAMM-CNP 15622/3/4/5. **Santiago del Estero**: Dto.Ojo de Agua: Salinas de Ambargasta, 0.5 km S Río Saladillo Ruta Nacional 9: (-28,8948 S, -63,9740 W): LJAMM-CNP 16568/9. Dto. Rivadavia: Colonia Mackinlay (-30,365 S, -62,1766 W): MACN-H 3938.

Liolaemus lentus: **La Pampa**: Dto. Puelén: 25 km SE Puelén (-37,4935 S, -67,4446 W): LJAMM-CNP 11850.
Dto. Puelén: Salar 4.9 km SE de Puelén (-37,3688 S, -67,5877 W): LJAMM-CNP 16234/5/6/7. **Rio Negro**: Dto. General Roca: Salitral a 21.37 km NW de Ingeniero Huergo, a la altura de Mainque (-38,9452 S, -67,2425 W): LJAMM-CNP 13163/4.

Liolaemus millcayac: **Mendoza**: Dto. La Paz: Ruta Nacional 146, Km 247, 92.7 km NE empalme Ruta Provincial 153 (-34,1794 S, -66,9620 W): LJAMM-CNP 17299/300.

Dto. Lavalle: Ruta Nacional 40, Km 3360, 21 km N Jocoli (-32,4058 S, -68,5919 W): LJAMM-CNP 16247/8/8/50/1/2/29/39/31/35/JMC-DC1225/6. Dto. Santa Rosa: Ruta Provincial 153, 30 km S Las Catitas (-33,5599 S, -68,0745 W): LJAMM-CNP 12851. **La Rioja**: Dto. Rosario Vera Peñaloza: Ruta Nacional 141, Salinas de Mascasin, 10 km W Mascasin (-31,4233 S, -67,0392 W): LJAMM-CNP 15776/7/8/9/80/81. San Juan: Dto. Caucete: 32 km E Caucete (-31,7399 S, -68,0146 W): SDSU 1674/5. 20 km de Caucete (-31,7354 S, -68,1223 W): FML 00690. Ruta Nacional 20, 4 km W Nikizanga (-31,5930 S, -67,8699 W): LJAMM-CNP 8524. Salinas de Mascasín (-31,4382 S, -67,0865 W): IMCN 154/466/8. 35 km W Encon (-31,9909 S, -68,0546 W): JMC-DC 3. 30 km W Encon (-32,0269 S, -68,0354 W): JMC-DC 89.

Liolaemus pipanaco: **Catamarca**: Dto. Andalgalá: Pasando Puesto Blanco, al oeste al Salar de Pipanaco (-27,8233 S, -66,2439 W): FML 18396. Dto. Pomán: Salar de Pipanaco, 9 km W Ruta Provincial 46, Establecimiento Resurgir del Algarrobal (-27,9502 S, -66,2866 W): LJAMM-CNP 16547.

Liolaemus pseudoanomalus: **La Rioja**: Dto. Castro Barros: 15 Km NE Anillaco (-28,7402 S, -66,7877W): LJAMM-CNP 131. Ruta Provincial 7, 16 Km E Anillaco (-27,8452 S, -67,6677 W): LJAMM-CNP 316/364/579/799/400/1/2/7/8/9/13/FML 08189. Ruta Provincial 7, 4 Km E Anillaco (-28,7836 S, -66,8816 W: LJAMM-CNP 414/1823/24/25. Ruta Provincial 7, 10 Km E Anillaco (-28,7663 S, -66,8483 W): LJAMM-CNP 1711/1815/6. 20 km al E de Anillaco - Ruta (-28,7481 S, -66,7495 W): FML 08440.Dto. Famatina: Campo de Loma Larga, Antinaco (-28,8255 S, -67,3801 W): FML 02087. **Catamarca**: Dto. Tinogasta: Ruta Nacional 60, 17.9 km S Fiambalá, Km 1357 (-27,8452 S, -67,6677 W): LJAMM-CNP 14401. Ruta Nacional 60, 5.2 km S Fiambalá, 13 km N del río de la Troya (-27,7719 S, -67,6538): LJAMM-CNP 15708. Quebrada La Troya y Ruta Nacional 60, 11 km N Anillaco, entre Tinogasta y Fiambalá (-27,8597 S, -67,6672 W): LJAMM-CNP 16443/4/5. 8,2 km N de Fiambalá, sobre ruta (-27,6207 S, -67,3801 W): FML 03004.

Suppl. Table 1. Localities for all sequenced individuals.

Samples	Locality
Liolaemus acostai	
LJAMM-CNP 10909	Río Las Lajas, Ruta Prov. 82, 24.9 km NE empalme Ruta Nacional 40, camino a Mogna. Dep. Jachal Prov. San Juan.
LJAMM-CNP 10911	Camino entre Mogna y Ruta Nac. 40, 16 km W Mogna, W Quebrada de Mogna, Sierra de Morado. Dep. Jachal Prov. San Juan.
LJAMM-CNP 16433/4	Camino entre Baños La Laja y Baños del Salado, 0.5 km S Baños del Salado. Dep. Albardón. Prov. San Juan.
Liolaemus anomalus	
LJAMM-CNP 10811	Ruta Nac. 76, 23.8 km S cruce Ruta Nac. 40, 2 km N Pagancillo. Dep. Felipe Varela. Prov.La Rioja.
LJAMM-CNP 16260/1	Ruta Prov. 18, 5 km NE Pangancillo, entre Pangancillo y Puerto Alegre. Dep. Felipe Varela. Prov. La Rioja.
LJAMM-CNP 2300/BYU 47181	Ruta Prov. 26, 3 Km N Pagancillo. Dep. Felipe Varela. Prov.La Rioja.
LJAMM-CNP 8579	Ruta Nac. 76, 9.86 km N Villa Uníon, cerca de Río Colorado, 1 km S río La Calera. Dep. Gral. Lamadrid. Prov. La Rioja.

LJAMM-CNP 8584	Ruta Nacional 76, 57.5 km SE Pagancillo, Parque Nac. Talampaya. Dep. Felipe Varela. Prov.La Rioja.
Liolaemus ditadai	
LJAMM-CNP 15622/3/4/5	Ruta Nac. 157, 2 km NW empalme Ruta Nac. 60, Salinas Grandes. Dep. La Paz. Prov. Catamarca.
LJAMM-CNP 16568/9	Salinas de Ambargasta, 0.5 km S Río Saladillo Ruta Nac. 9. Dep. Ojo de Agua. Prov. Santiago del Estero.
Liolaemus lentus	
LJAMM-CNP 13163/4	Salitral a 21.37 km NW de Ingeniero Huergo, a la altura de Mainque. Dep. General Roca. Prov. Rio Negro.
LJAMM-CNP 16234/5/6/7	Salar 4.9 km SE de Puelén. Dep. Puelén. Prov. La Pampa.
Liolaemus millcayac	
LJAMM-CNP 12851	Ruta Prov. 153, 30 km S Las Catitas. Santa Rosa. Mendoza.
LJAMM-CNP 15776/7/8	Ruta Nac. 141, Salinas de Mascasin, 10 km W Mascasin. Dep. Rosario Vera Peñaloza. Prov. La Rioja.
LJAMM-CNP 16247/8/9/50/435	Ruta Nac. 40, Km 3360, 21 km N Jocoli. Dep. Lavalle. Prov. Mendoza.
Liolaemus pipanaco	
LJAMM-CNP 16547	Salar de Pipanaco, 9 km W Ruta Prov. 46, Establecimiento Resurgir del Algarrobal. Dep. Pomán. Prov. Catamarca.
Liolaemus pseudoanomalus	
LJAMM-CNP 14401	Ruta Nac. 60, 17.9 km S Fiambala, Km 1357. Dep. Tinogasta. Prov. Catamarca.
LJAMM-CNP 16443/4/5	Quebrada La Troya y Ruta Nac. 60, 11 km N Anillaco, entre Tinogasta y Fiambala. Dep. Tinogasta. Prov. Catamarca.
LJAMM-CNP 15708	Ruta Nac. 60, 5.2 km S Fiambalá, 13 km N río de la Troya. Dep. Tinogasta. Prov.Catamarca.
Outgroup	
Liolaemus scolaroi	
LJAMM-CNP 13154/5	Camino a Reserva Jeinimeni, 49 km SW empalme camino Los Antiguos - Chile Chico, 4 km NE entrada a Reserva Jeinimeni, 1 km NE Río Jeinimeni. XI Región. Chile Chico. Chile.

Suppl. Table 2. cyb-distances. (request to Cuadernos de Herpetología editor)

Suppl. Table 3. GenBank accession numbers for each sequenced gene fragment per species. Voucher numbers correspond to LJAMM-CNP Herpetological Collection.

	Cyt-b	12S	DMXL1	DNAH3	KIF24	PRLR
L. acostai						
10909	10909/MW074925	10909/MW151831	10909/MW119283	10909/MW116084	10909/MW116106	10909/MW116128
10911	10911/MW074926	10911/MW151832	10911/MW119284	10911/MW116085	10911/MW116107	10911/MW116129
16433	16433/MW074927	16433/MW151833	16433/MW119296	16433/MW116096	16433/MW116118	16433/MW116142
16434	16434/MW074928	16434/MW151834	16434/MW119297	16434/MW116097	16434/MW116119	16434/MW116143
L. anomalus						
10811	10811/MW074929	10811/MW151835	10811/MW119282	10811/MW116083		10811
16260	16260/MW074930	16260/MW151836	16260/MW119295	16260/MW116095	16260/MW116117	16260/MW116140
16261	16261/MW074931					16261/MW116141
2300	2300/MW074932	2300/MW151837	2300/MW119281	2300/MW116102	2300/MW116123	2300/MW116147
2301	2301/MW074933		2301/MW119301	2301/MW116103	2301/MW116124	
8579	8579/MW074934	8579/MW151838	8579/MW119303			8579/MW116148
8584	8584/MW074935	8584/MW151839	8584/MW119304	8584/MW116105	8584/MW116126	8584/MW116156

L. ditadai

15622	15622/MW074936	15622/MW151840	15622/MW119278	15622/MW116091		15622/MW116134
15623	15623/MW074937	15623/MW151841			15623/MW116113	
15624	15624/MW074958					
15625	15625/MW074938					
16568	16568/MW074939	16568/MW151842	16568/MW119279			16568/MW116145
16569	16569/MW074940	16569/MW151843	16569/MW119280	16569/MW116101	16569/MW116122	16569/MW116146

L. lentus

13163	13163/MW074941	13163/MW151844	13163/MW119288	13163/MW116089	13163/MW116111	13163/MW116132
13164	13164/MW074942	13164/MW151845		13164/MW116090	13164/MW116112	13164/MW116133
16234	16234/MW074943	16234/MW151846	16234/MW119292			16234/MW116138
16235	16235/MW074944	16235/MW151847	16235/MW119293			16235/MW116139
16236	16236/MW074959				16236/MW116115	
16237	16237/MW074945					

L. millcayac

12851	12851/MW074946	12851/MW151848	12851/MW119285	12851/MW116086	12851/MW116108	12851/MW116151
15776	15776/MW074947	15776/MW151849	15776/MW119290	15776/MW116093		15776/MW116136
15777	15777/MW074960	15777/MW151850	15777/MW119291		15777/MW116114	
15778	15778/MW074948					15778/MW116137
16247	16247/MW074949	16247/MW151851	16247/MW119294			
16248	16248/MW074950	16248/MW151852		16248/MW116094	16248/MW116116	16248/MW116149
16249	16249/MW074951					
16250	16250/MW074952					
				16435/MW116098	16435/MW116120	16435/MW116144

L. pipanaco

16547	16547/MW074953	16547/MW151854	16547/MW119300	16547/MW116100	16547/MW116121	16547/MW116154

L. pseudoanomalus

641	641	641	641/MW119302	641/MW116104	641/MW116125	641/MW116155
14401	14401/MW074954	14401/MW151855			14401/MW116127	
16443	16443/MW074955	16443/MW151857	16443/MW119298	16443/MW116099	16443/MW152151	16443/MW116152
16444	16444/MW074956	16444/MW151858	16444/MW119299			16444/MW116153
16445	16445/MW074957				16445/ W152152	
15708		15708/MW151856	15708/MW119289	15708/MW116092		15708/MW116135

Outgroup

L. scolaroi

13154	13154/MW152149	13155/W151853	13154/MW119286	13154/MW116087	13154/MW116109	13154/MW116130
13155	13155/MW152150		13155/MW119287	13155/MW116088	13155/MW116110	13155/MW116131