Phylogeography between valleys and mountains: the history of populations of *Liolaemus koslowskyi* (Squamata, Liolaemini)

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The lizard genus *Liolaemus* is endemic to temperate South America and includes approximately 200 species. *Liolaemus koslowskyi* occurs in north-western Argentina, where it is confined to a system of interior basins and valleys. This topographically complex region is now viewed as different enough that it has been suggested for recognition as a separate zoogeographical region: The Monte Desert of Mountains and Isolated Valleys. Here we use the mtDNA cytochrome b sequence data to investigate the phylogeographical pattern of *L. koslowskyi* and its relationships with other species of the *darwinii* group. *Liolaemus koslowskyi* is monophyletic with respect to all the species of the *darwinii* group included in this analysis. Three main clades were recovered within *L. koslowskyi* and we hypothesized that at least one of these, which shows 7% genetic divergence, is a candidate species. We discuss the phylogeographical patterns in association with the geological history of the region. The highly structured *L. koslowskyi* clade suggests that it has a relatively ancient history in a topographically rich, terrestrial archipelago of habitat and tectonic islands that are themselves relictual mountains and valleys.

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Introduction

The lizard genus *Liolaemus* is endemic to temperate South America, where it is distributed over a wide geographical region and occupies a large range of latitudinal (14 30′ to 52 30′S), altitudinal (0–4500 m), and climatic regimes; *Liolaemus* range from the extremely arid Atacama desert to temperate *Nothofagus* rainforest (Donoso Barros 1966; Cei 1986, 1993; Etheridge & de Queiroz 1988; Lobo 2001). This genus is characterized by a rapid rate of discovery of new species (see recent examples in Avila *et al*. 2004, 2008; Abdala 2005; Abdala & Lobo 2006; Cabrera & Monguillot 2006; Monguillot *et al*. 2006; Vega *et al*. 2008); approximately 200 species have been described and Morando *et al*. (2003) recently estimated that the actual number of *Liolaemus* species could be as high as twice the number currently recognized.

Different taxonomic series, groups and complexes have been proposed within this genus (Cei 1979, 1986; Laurent 1983, 1985; Etheridge 1995; Schulte *et al*. 2000; Morando 2004; Avila *et al*. 2006; Abadala 2007; Morando *et al*. 2007). One of the more distinguishable groups is the *boulengeri* or 'patch group' (Etheridge 1992), diagnosed by the presence of a patch of enlarged spinose scales on the posteromedial surface of the thigh. A clade within the *boulengeri* group is the *darwinii* complex of species (Etheridge 1993), named as the *darwinii* group by Morando *et al*. (2004) and as the *laurenti* group by Abdala (2005), which is diagnosed by strong sexual dichromatism and moderately cusped, straight-sided to slightly expanded crowns of the posterior marginal teeth. Etheridge (1993) did not have evidence for monophyly of the group, and due to the high degree of intraspecific variation in the colour patterns of both male and female adults; it is difficult to make general comparisons within the group. In addition to their rather generalized morphology, most of the *darwinii* group species occupy a variety of habitats within their ranges. However, monophyly is supported by Morando (2004), Cruz *et al*. (2005) and Abdala (2007), and the *darwinii* group includes 18 species, most of them described in the 1990s and four recently described (Abdala & Diaz Gomez

2006; Abdala & Lobo 2006; Cabrera & Monguillot 2006; Monguillot *et al*. 2006).

Etheridge (1992, 1993) described five species that previously were included under the name *Liolaemus darwinii* (*L. laurenti*, *L. quilmes*, *L. abaucan*, *L. olongasta*, *L. koslowskyi*) from northern Argentina, in the provinces of Salta, Tucumán, Catamarca, La Rioja and San Juan. *Liolaemus koslowskyi* occurs in north-western Argentina (the Monte Phytogeographical Province) and ranges from south-central Catamarca Province to north-central La Rioja Province, where it is confined to a system of interior basins formed by the Campo de Belén and Salar de Pipanaco, the valleys of the Rio Belén and Rio Colorado-Rio Salado that drain into them, and the contiguous Bajo de Santa Elena and lower Rio Vinchina valley in western and central La Rioja Province. The topographically complex region is now viewed as different enough in its biological features that it has been suggested for recognition as a separate zoogeographical region: The Monte Desert of Mountains and Isolated Valleys (Burkart *et al*. 1999). In Catamarca Province *L. koslowskyi* extends northward in the valley of the Rio Abaucan, an upper tributary of the Rio Colorado, to Fiambala, where it apparently overlaps the southern range of *L. abaucan*. In the north-east it reaches Sierra de las Cuevas that contributes to the western border of the Campo Arenal, a flat sandy plain occupied by *L. quilmes* (Etheridge 1993). This landscape is heterogeneous and includes a series of interdigitating mountains and valleys which probably experienced a geologically dynamic past. Within this region *L. koslowskyi* occupies a variety of habitats including rocky flats and hillsides, sand flats and dune edges (Etheridge 1993).

Schulte *et al*. (2000) completed a mitochondrial DNA-based phylogenetic study of a large number of the species of the genus (n = 60), and recovered a sister relationship between *L. koslowskyi* and *L. quilmes*; these are diagnosed mainly by colouration pattern. Morando *et al*. (2004) included four different populations of *L. quilmes* in a phylogeographical study and found the same relationship, but Abdala (2005) recovered *L. koslowskyi* as one of the basal taxa of the *darwinii* group and *L. quilmes* as the sister taxa of *L. espinozai*. Geographically *L. quilmes* is confined to the valley of a complex interior drainage system that includes the southern Salta Province and extreme north-western Tucumán and north-eastern Catamarca Provinces. This distributional range is located north of the distribution of *L. koslowskyi*. In this study we explore the population history of *L. koslowskyi* and its phylogenetic relationships with most of the taxa included in the *darwinii* group.

Materials and methods

Taxon sampling

Previous experience (Morando *et al*. 2003, 2004, 2007; Avila *et al*. 2006) suggested that the mtDNA cytochrome b (cyt-b) gene was sufficiently variable for intraspecific phylogeographical studies in *Liolaemus*, and sequence data were collected from this gene from a total of 144 lizards from 43 localities (Table 1, Fig. 1, Appendix I) representing populations under the names *L. koslowskyi* (Bell 1843). *Liolaemus abaucan* Etheridge (1993), *L. albiceps* Lobo & Laurent (1995), *L. darwinii* N1, *L.* cf. *quilmes* 1, *L.* cf. *quilmes* 2, *L.* cf. *quilmes* 3, *L.* cf. *quilmes* 4, *L.* cf. *quilmes* 5, *L.* cf. *quilmes* 6, *L.* cf. *olongasta*, *L. chacoenis* Shreve 1948; *L. espinozai* Abdala (2005); *L. irregularis* Laurent 1986, *L. laurenti* Etheridge (1992); *L. lavillai* Abdala & Lobo (2006); *L. olongasta* Etheridge (1993), *L. quilmes* Etheridge (1993), and *L. uspallatensis* Macola & Castro 1982. All members of the *darwinii* group were used as nonfocal species (Wiens & Penkrot 2002; n = 1 each, Table 1, Appendix I), and *L. inacayali* Abdala 2003 and *L.* cf. *boulengeri* (members of another *Liolaemus* clade) were used to root the trees, thus allowing the position of the nonfocal species to remain unconstrained with respect to *L. koslowskyi*.

Table 1 Number of individuals of all ingroup and outgroup taxa, by locality; locality numbers (in parentheses) match those in Fig. 1 and Appendices I and II (which provides museum numbers for all specimens and haplotypes numbers). Numbers under the *N* column give the number of lizards sequenced from each locality.

Province department	Locality	*N*	Coordinates
Liolaemus koslowskyi (focal taxon)			
La Rioja			
Castro Barros	(1) 7 km E Villa Servil. Aimogasta Road	1	28 37′S 66 39′W
	(3) Anillaco	1	28 49′S 66 57′W
Felipe Varela	(2) R. Nac. 40, Cuesta Las Trancas	1	29 22′S 67 47′W
	(5) R. Pcial. 18, 10 km NE Pagancillo	1	29 28′S 68 01′W
Chilecito	(6) 10 km E Anguinan, Velazco Road	1	29 13′S 67 21′W
	(15) La Puerta	7	29 20′S 67 26′W
Famatina	(7) R. Nac. 40, km 657, 9 km E Pituil	2	28 32′S 67 22′W
	(11) Capayan Ruins, 14.8 km N Chilecito	4	29 03′S 67 26′W
	(14) Antinaco entry, 3.8 km E R. Nac. 40	5	28 50′S 67 24′W
	(36) Road between Chañarmuyo y Campanas, 3 km N Chañarmuyo. Las Talas River	7	28 34′S 67 35′W

Table 1 *Continued.*

Province department	Locality	*N*	Coordinates
Arauco	(12) 1 km S Bañado de los Pantanos	2	28 21′S 66 50′W
	(16) R. Pcial. 7, 28 km E Anillaco	3	28 48′S 66 40′W
	(25) Aimogasta	2	28 32′S 66 45′W
Capital	(17) R. Pcial. 10, 1 km N access R. Pcial. 9	2	28 56′S 66 37′W
San Blas de los Sauces	(19) R. Nac. 60, 2.1 km W Alpasinche	5	28 17′S 67 04′W
Gral. Lavalle	(32) R. Nac. 40, 20 km E Villa Unión	7	29 22′S 68 02′W
Catamarca			
Tinogasta	(4) R. Nac. 60 y La Puerta river, km 1298	2	28 14′S 67 27′W
	(8) Road to Tinogasta-Campanas, 22 km S Tinogasta	1	28 15′S 67 38′W
	(9) R. Nac. 60, 4 km W Salado	2	28 18′S 67 18′W
	(10) R. Nac. 60, 11.8 km N Tinogasta	1	27 58′S 67 38′W.
	(13) R. Nac. 60, 5 km W Tinogasta	3	28 01′S 67 37′W
	(33) 4 km S La Cuadra, Road to Campanas to Tinogasta	1	28 26′S 67 39′W
	(35) 4 km N Catamarca-La rioja border, Road to Campanas to Tinogasta	6	28 21′S 67 39′W
Belén	(18) R. Pcial. 46, 11 km E Belen	2	27 42′S 66 56′W
	(20) R. Pcial. 43, Puerta de Corral	6	27 14′S 66 54′W
	(23) R. Nac. 40, 30.3 km N Cerro Negro	3	28 02′S 67 11′W
	(24) R. Nac. 40, km 773, 7.5 km S Londres	4	27 46′S 67 10′W
	(26) R. Nac. 40, 5 km E Los Nacimientos	5	27 08′S 66 40′W
	(27) R. Nac. 40, 4 km S Los Nacimientos	6	27 10′S 66 45′W
	(30) 10 km N Cerro Negro	2	28 11′S 67 08′W
	(31) R. Nac. 40, 5.3 km N San Fernando Sur	3	27 17′S 66 53′W
	(38) R. Nac. 40, 6.5 km N La Cienaga	6	27 28′S 66 58′W
	(39) R. Nac. 40, 20 km S El Eje	2	27 25′S 66 57′W
	(40) R. Pcial. 17, 8 km N Barranca Larga	3	26 54′S 66 44′W
Andalgalá	(21) R. Pcial. 48, 20.4 km W Agua de las Palomas	1	27 38′S 66 12′W
	(22) R. Pcial. 46, 5 km S Andalgala	4	27 39′S 66 17′W
	(34) R. Pcial. 46, 39 km E Belen km 172	6	27 43′S 66 42′W
	(37) R. Pcial. 46, 66 km E Belen	8	27 36′S 66 30′W
	(41) R. Pcial. 46, Estacion Colpes	5	28 03′S 66 12′W
	(43) R. Pcial. 46, 31.2 km S Andalgala	4	27 50′S 66 10′W
Santa Maria	(28) R. Nac. 40, 46 km W Punta de Balasto	1	27 04′S 66 34′W
	(29) R. Nac. 40, 14.3 km E Los Nacimientos	4	27 05′S 66 37′W
Pomán	(42) R. Pcial. 46, 9 km N Sijan	2	28 11′S 66 13′W
Nonfocal taxa			
Liolaemus abaucan	Catamarca. Tinogasta. R. Pcial. 36, 16 km S Palo Blanco	1	27 26′S 67 40′W
L. albiceps	Salta. Rosario de Lerma. Sta. Rosa de Tastil	1	24 27′S 65 56′W
L. cf. *quilmes* 1	Salta. Guachipas. R. Nac. 68, 44.1 km NE Cafayate	1	25 52′S 65 42′W
L. cf. *quilmes* 2	Tucumán. Taf' del Valle. R. Pcial. 307, 21.7 km E Amaicha del Valle	1	26 40′S 65 48′W
L. cf. *quilmes* 3	Tucumán. Taf' del Valle. R. Nac. 40, 9.2 km N access road R. Nac. 307	1	26 25′S 65 69′W
L. cf. *quilmes* 4	Catamarca. Santa Maria. Santa Maria	1	26 40′S 66 02′W
L. cf. *quilmes* 5	Catamarca. Santa Maria. R. Nac. 40, 6 km W Punta de Balasto	1	26 59′S 66 14′W
L. cf. *quilmes* 6	Catamarca. Santa Maria. R. Nac. 40, 5 km SW Campo Los Pozuelos	1	27 01′S 66 29′W
L. cf. *olongasta*	San Juan. Ullum. R. Pcial. 436, 6 km E La Cienaga	1	30 52′S 68 53′W
L. chacoensis	La Rioja. Capital. R. Pcial. 9, 37.3 km E Anillaco. Sierra de Mazan	1	28 52′S 66 38′W
L. darwinii N1	San Juan. Caucete. R. Nac. 141, 15 km E Caucete	1	31 41′S 68 09′W
L. espinozai	Catamarca. Santa Maria. R. Pcial. 47, 20 km S Pta. Balasto. Campo Arenal	1	27 07′S 66 13′W
L. irregularis	Salta. Los Andes. 5 km NW San Antonio de los Cobres. Paraje Pompeya	1	24 14′S 66 19′W
L. laurenti	La Rioja. Famatina. R. Nac. 40, km 657, 9 km E Pituil	1	28 32′S 67 22′W
L. lavillai	Salta. La Poma. R. Nac. 40, 2 km N La Poma	1	24 41′S 66 11′W
L. olongasta	La Rioja. Felipe Varela. R. Nac. 40, 2 km S Guandacol	1	29 34′S 68 31′W
L. quilmes	Salta. Cachi. R. Nac. 40, 6.7 km N Cachi	1	25 04′S 66 07′W
L. uspallatensis	Mendoza. Las Heras. R. Nac. 7, 4 km W Uspallata	1	32 36′S 69 24′W
Outgroup taxa			
Liolaemus cf. *boulengeri*	Rio Negro. Ñorquinco. R. Pcial. 6, 31 km N Ñorquinco	1	47 46′S 70 37′W
L. inacayali	Rio Negro. 25 de Mayo. 40 km SE Maquinchao, road to El Cain	1	41 30′S 68 33′W

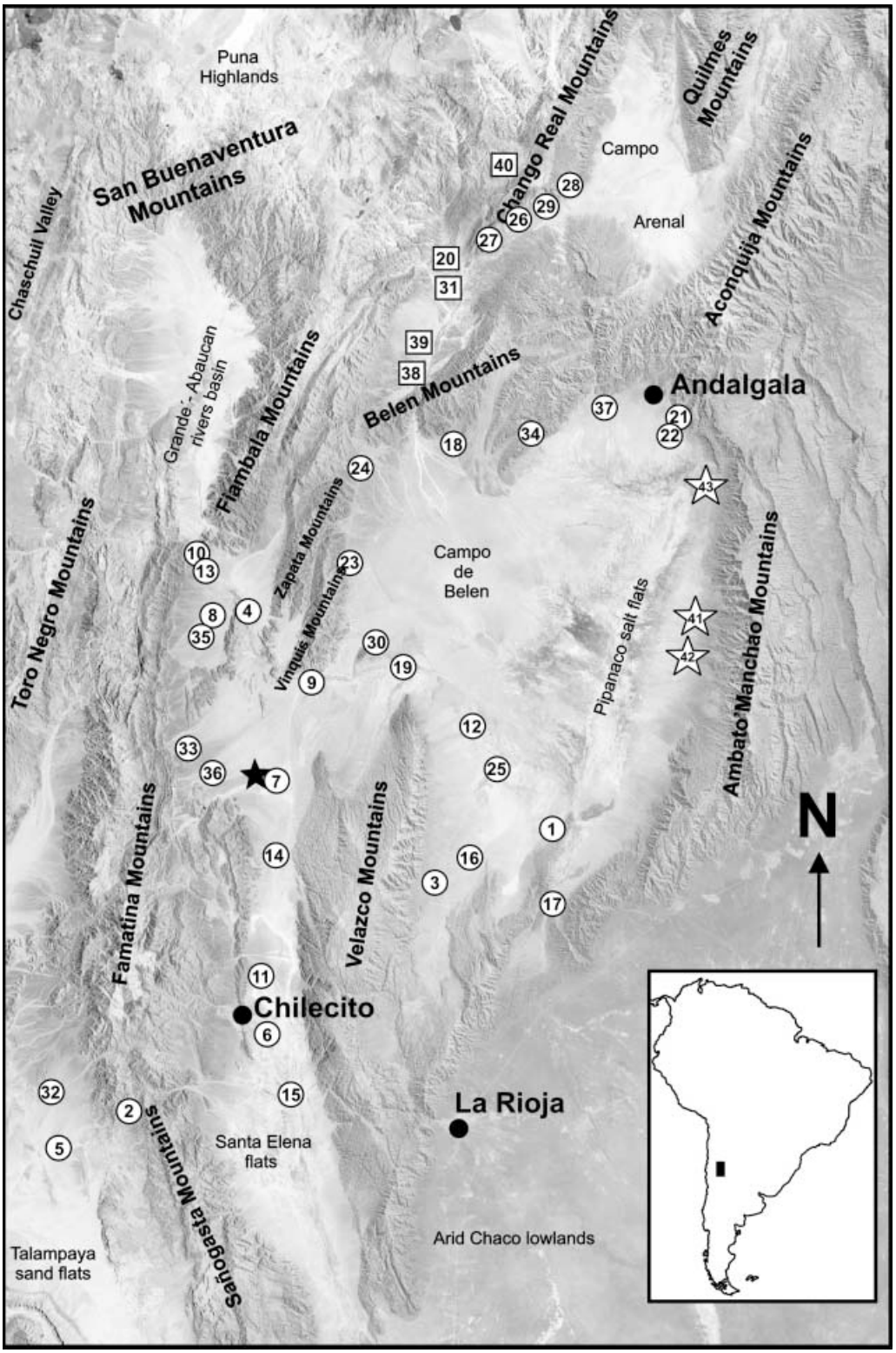

Fig. 1 Geographic distribution of the taxa included in this study. Samples localities for *Liolaemus. koslowskyi* (*sensu* stricto) are in circles. Squares and stars mark sampled localities for *L. koslowskyi* N and *L. koslowskyi* E, respectively, black star is the type locality of *L. koslowskyi*. Locality numbers correspond to those in Table 1 and Appendices I and II.

Table 1 summarizes the number of individuals sequenced per locality and distributional information for all taxa used in this study (Table 1, Fig. 1), and voucher specimens are deposited in the LJAMM herpetological collection (now housed in the Centro Nacional Patagónico (CENPAT), Puerto Madryn, Argentina), Fundación Miguel Lillo (FML, San Miguel de Tucumán, Argentina), Museo de Ciencias Naturales La Plata (MLP.S, La Plata, Argentina) and M.L. Bean Life Science Museum (BYU, Provo, UT). Museum voucher numbers are listed by taxon and locality in Appendix I, and museum acronyms follow Leviton *et al*. (1985).

Laboratory procedures

Total genomic DNA was extracted from liver/muscle tissues preserved in 96% ethanol, following the protocol developed by Fetzner (1999). Three micro litres of extraction product were electrophoresed on 1% agarose gel to estimate the quality and amount of genomic DNA, and sample dilutions were performed, where necessary prior to polymerase chain reaction (PCR) amplification. A 830-bp fragment of cyt-b gene region was amplified via PCR following Morando *et al*. (2003), using the light strand primers GluDGL (Palumbi 1996) and cyt-b1 (Kocher *et al*. 1989), and the heavy strand primer cyt-b3 (Palumbi 1996; cyt-b amplification was not possible for *L. quilmes*). Cyt-b2 (Palumbi 1996) and F1 (Whiting *et al*. 2003) were used as internal sequencing primers. For a subset of 18 *L. koslowskyi* individuals plus all the nonfocal taxa and outgroups (except for *L. quilmes* 7, see below) we amplified a fragment of 732 bp of the 12S mitochondrial gene using primers as described in Morando *et al*. (2003). Double-stranded PCR amplified products were checked by electrophoresis on a 1% agarose gel, purified using a MultiScreen PCR (mu) 96 (Millipore Corp., Billerica, MA) and directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction (Applied Biosystems, Foster City, CA). Excess of Dye Terminator was removed with MultiScreen HV (Millipore Corp.), and sequences were fractionated by polyacrylamide gel electrophoresis on an ABI3730xl DNA Analyser DNA sequencer (PE Applied Biosystems, Foster City, CA) at the DNA Sequencing Center at BYU. Sequences were deposited in GenBank under accession numbers EU795736 to EU795772 and EU822955 to EU823098.

Sequence alignments

Sequences were edited and aligned using the program SEQUENCHER 3.1.1 (Gene Codes Corp. Inc., Ann Arbor, MI), and translated into amino acids for confirmation of alignment. In the cytochrome region no indels were present. The 12S fragment did not present many ambiguous positions and no structural models were needed for this alignment; a few small gaps (4–7 bp) were inserted to maximize nucleotide identity in conserved blocks.

Phylogenetic analyses

We estimated a distance tree for the complete cyt-b data set of 164 individuals (144 ingroup (focal species) terminals, 18 nonfocal terminals and two outgroup taxa) using parameters estimated with a model of molecular evolution selected with MODELTEST (v3.06; Posada & Crandall 1998). In this tree, the three main ingroup clades were concordant with the three networks obtained with TCS (see below). Considering this, we used a subset of 18 singleton haplotypes representing the three *koslowskyi* networks for subsequent tree based phylogenetic methods. The cyt-b and 12S data sets were combined to estimate model parameters with MODELTEST (v3.06; Posada & Crandall 1998), and the best fit model of evolution (38 taxa –18 haplotypes from the focal +20 nonfocal terminals, with 1562 bp) with the Akaike criterion was GTR + I + Γ (Yang 1994; Gu *et al*. 1995). With parameters calculated under this model we ran 10 maximum likelihood (ML) replicates and 10 000 maximum parsimony (MP) pseudoreplicates for nonparametric bootstrap analyses (Felsenstein 1985). Distance, MP and ML criteria were implemented using PAUP* 4b5 (Swofford 2002). A Bayesian analyses was performed with this reduced data set using MRBAYES 2.0 (Huelsenbeck & Ronquist 2001) independently two times for 2×10^6 generations and sample frequency = 500. We determined when stationarity was reached (in order to discard the 'burn-in' samples) by plotting the log-likelihood scores of sample points against generation time; when the values reached a stable equilibrium, before 20 000 generations in all cases, stationarity was assumed. The equilibrium samples (3961 trees) were used to generate a 50% majority rule consensus tree. The percentage of samples that recover any particular clade on this tree represents that clade's posterior probability; these are the *P*-values, and we consider $P = 95\%$ as evidence of significant support for a clade (Huelsenbeck & Ronquist 2001). Recent simulation analyses indicate that although Bayesian support values are usually higher than those from nonparametric bootstrap, they appear to provide a much closer estimate of phylogenetic accuracy (Wilcox *et al*. 2002).

Nested clade phylogeographical analyses

The complete set (no missing data) of 144 sequences (830 bp) was used for these analyses. Nested clade phylogeographical analyses (NCPA; Templeton *et al*. 1995; Templeton 1998) were used to infer the population history of *L. koslowskyi*. The program TCS v1.13 (Clement *et al*. 2000), was used to construct haplotype networks and nesting categories were assigned following Templeton and Sing (1993) and Templeton *et al*. (1995) . The networks were then used for NCPA, which was implemented with the GEODIS program (v2.0, Posada *et al*. 2000). All the statistical analyses were performed using 10 000 Monte Carlo replications. Statistically significant results were interpreted following the inference key of

Templeton (November 2005, <http://darwin.uvigo.es/software/geodis.html>).

We are fully aware that the NCPA method has generated controversy (Panchan & Beaumont 2007; Garrick *et al*. 2008; Petit 2008a,b; Templeton 2008), but we use these analyses cautiously and in the spirit of generating more refined hypotheses for poorly known groups (Avila *et al*. 2006).

Neutrality tests and molecular diversity analysis

To test the validity of the assumption that the base variation is evolving under approximately neutral expectations, we used the McDonald–Kreitman (1991) test (hereafter M–K) as implemented in the program DNASP v3.99.4 (Rozas & Rozas 1999), on the 810 bp of the cyt-b gene fragment. We also used the same program to estimate the average number of nucleotide substitutions per site between selected clades. To assess population equilibrium independent of the NCPA inferences, we implemented Tajima (1989) *D*-test and Fu (1997) *F*'s test. For the main clades identified in the phylogenetic analyses and NCPAs (see below), we calculated the uncorrected average pairwise genetic distances using PAUP* 4b5 (Swofford 2002). We also estimated gene diversity (Nei 1987; p. 180) and nucleotide diversity (π, the mean of pairwise sequence differences, Nei 1987; p. 257) for these same clades. The parameter θ can be estimated using π or the number of segregating sites (S). If evolution is neutral then both estimates give the same value of θ, and this can be compared via the Tajima's test (Tajima 1989) to assess whether impacts of selection or population change can be detected (see below). As a final assessment of population demographic histories, we performed mismatch analyses for *L. kosloswskyi* and other identified clades and their fit to Poisson distributions was assessed by Monte Carlo simulations of 1000 random samples. The sum of squared deviations (SSD) and raggedness indexes (Rgg) (Harpending 1994) between observed and expected mismatch distributions were used as a test statistic with *P*-values representing the probability of obtaining a simulated SSD larger or equal to the one observed. This index takes larger values (Rgg > 0.05) for multimodal distributions expected in stationary populations, relative to unimodal and smoother distributions typical of expanding populations (Rgg < 0.05). We calculated the probability of observing by chance a higher value of the Rgg than the observed one P (Rag_{obs}), under the hypothesis of population expansion. As informed by the phylogenetic and NCPA results, we performed three AMOVAs (Excoffier 2001) to estimate: (i) the genetic structure between the *L. koslowskyi* complex, *L. koslowskyi* N (north), *L. koslowskyi* E (east) (see below); (ii) the genetic structure among the two main clades within the *L. koslowskyi* complex (clades 5-1 and 5-2); and (iii) the genetic structure between the clades included in 5-1 and 5-2 (group 1: 4-1, 4-2, 4-3; group 2: 4-4, 4-5).

The nucleotide diversity, population structure, mismatch analyses, and Tajima and Fu tests were performed with the program ARLEQUIN v2.00 (Schneider *et al*. 2000). This combination of methods permits us to independently evaluate specific NCPA inferences tied to population growth — including dispersal or range expansions — by statistical tests based on completely different assumptions.

Results

Phylogenetic analyses

The cyt-b distance tree recovered three main clades within the *L. koslowskyi* samples; one including sequences from 115 individuals, which contains the type locality of the species and we refer to as *L. koslowskyi*. The second clade included sequences from 18 individuals (*L. koslowskyi* N), and the third clade included 11 sequences (*L. koslowskyi* E; result not shown). These three clades are congruent with the three networks obtained with the Nested Phylogenetic Clade Analyses (see below), and because haplotypes within these clades are very similar, we selected 18 singleton haplotypes representing these networks (*L. koslowskyi* haplotypes: 7, 18, 22, 25, 27, 28, 33, 36, 47, 48, 50, 63, 64, 67; *L. koslowskyi* N: 4, 12; *L. koslowskyi* E: 3, 9; Appendices I and II) for further tree-based phylogenetic analyses. Figure 2 (the ML topology) represents our best estimate of the phylogenetic relationships of the *darwinii* group, based on the combined data set with cyt-b and 12S sequences. We present this tree as we obtained similar results with the MP and Bayesian analyses. The ML tree recovers two main clades with high support (85% parsimony bootstrap (PB), > 0.95 posterior probability (PP)), one includes *L. darwinii*, *L. olongasta*, *L. laurenti* and *L. chacoensis* (clade 1), and the other including all other species except *L. uspallatensis* (clade 2). Different authors have proposed relationships in the *darwinii* group including a variable number of taxa, and using morphological and/or molecular data (Etheridge 2000; Schulte *et al*. 2000; Morando 2004; Cruz *et al*. 2005; Avila *et al*. 2006; Abdala 2007), and all of these are congruent in recovery of clades 1 and 2 except Etheridge (2000).

Within these two clades the level of congruence is variable between authors, but all recovered *L. albiceps*, *L. irregularis* and *L. lavillai* as closely related (clade 3 in Fig. 2). Schulte *et al*. (2000), Morando *et al*. (2004), Cruz *et al*. (2005) and Avila *et al*. (2006) recovered *L. quilmes* as the sister taxon of *L. koslowskyi*, but this topology is not resolved in this study. For this reason we included several taxa under the name *L. quilmes* from different areas and all these taxa + *L. espinozai* were recovered as a monophyletic group with high support (clade 4; 99% PB, > 0.95 PP). The long branches in this clade suggest the possibility of several candidate species under this name. The relationships between *L. koslowskyi*, the *L. quilmes* clade, *L. abaucan* and the (*L. albiceps* + *L. irregularis* + *L. lavillai*)

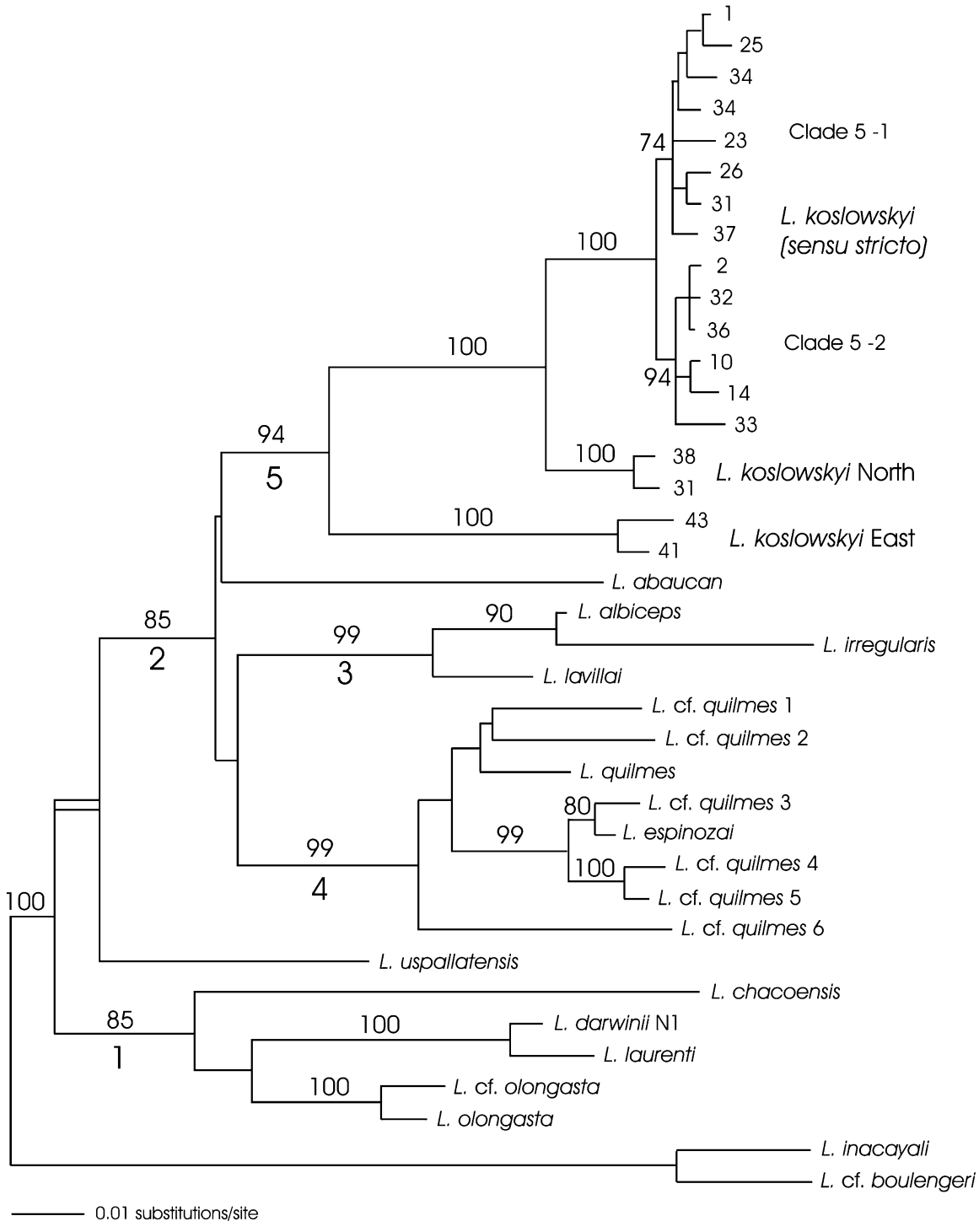

Fig. 2 Single ML tree (–Ln l = 743 471 446); numbers above branches are MP bootstrap values > 70%, thick branches represent PP values > 0.95, and the open branch > 0.85; single numbers below selected branches are clades described in the text.

clade do not have strong support with any of the phylogenetic methods we implemented.

Nested clade phylogeographical analyses

Application of the Templeton *et al*. (1992) algorithm (as implemented in TCS) for the cyt-b haplotypes in *L. koslowskyi* (Fig. 3, Appendix II) showed that sequences differing by up to 10 substitutions have at least a 0.95 probability of being parsimoniously connected. By this criterion we obtained three networks corresponding to the three clades recovered with the distance tree. The biggest network includes 115 sequences including haplotypes from the type locality, thus we referred to this network as *L. koslowskyi* (Fig. 3A). The second network, *L. koslowskyi* N (Fig. 3B) includes 18 individuals (representing 12 haplotypes (Appendix II)) from five localities (20, 31, 38, 39, 40; Fig. 1); and the third network, *L. koslowskyi* E (Fig. 3C) includes 11 individuals representing 9 haplotypes (Appendix II) from three localities (41, 42, 43; Fig. 1). Two individuals from localities included in *L. koslowskyi* N (one each from localities 20 and 31) had haplotypes shared with the *L. koslowskyi* clade network (clades 1-23). Small sample sizes of these last two networks precluded NCPA analyses, so we only carried out these analyses on the *L. koslowskyi* clade and summarize inferences in Table 2.

For low level clades with significant D_c or D_n values (clades 1-4, 1-26, 1-55, 2-13) either the geographical sampling was incomplete or the genetic resolution was not sufficient to discriminate between alternative processes. For clade 3-2, which has a wide distribution, it was possible to infer restricted gene flow with isolation by distance (Table 2); for clade 3-4, which includes haplotypes from the northern distribution of clade 3-2 as well as haplotypes from an isolated area in northern-most part of the *koslowskyi* distribution (locations 26 and 29), it was possible to infer either isolation-by-distance or past fragmentation. At the fourth level, for clade 4-1, it was not possible to discriminate between range expansion, colonization and restricted dispersal or gene flow, but considering the neutrality test and mismatch analyses (Table 3) the most plausible explanation for the observed genetic signature in this clade would be range expansion. For clades 4-3 and 4-4 we inferred contiguous range expansion in agreement with results from other analyses (Table 3). For clade 4-5, it was possible to hypothesize restricted gene flow with isolation by distance, but a significant Fu test (Table 3) implies a range expansion. This clade includes two individuals sharing a haplotype from locality 19, which is located within the distribution of clade 4-1, and is the only locality from which very different haplotypes are included in different higher level clades. This pattern may be evidence of ongoing or recent hybridization (Avila *et al*. 2006). There were two clades at the highest nesting level, an eastern clade (5-1) with the widest and northernmost distribution, and a western clade (5-2) with the southernmost distribution. For clade 5-1, contiguous range expansion explained the observed pattern, in agreement with other results (Table 3); and for clade 5-2 we inferred restricted gene flow with isolation-by-distance, but other analyses are consistent with a range expansion explanation.

Neutrality tests and molecular diversity analysis

Results of the M–K test are consistent with neutral evolution in almost all comparisons within the ingroup and for the nonfocal group as a whole compared with the outgroup (*L. koslowsky*/*L. koslowsky* N: Fisher's exact test $P = 0.476$ NS; *G*-value = 0.946, $P = 0.33$ with Williams and Yates correction = NS; *L. koslwosky*/*L. koslwosky* E: Fisher's exact test $P = 0.050$ NS; *G*-value = 5.029, $P = 0.0249$* with Williams correction = 4.835, $P = 0.027$* and Yates correction = 3.458, $P = 0.062$ NS; *L. koslowsky* N/*L. koslwosky* E: Fisher's $P = 0.26$ NS; *G*-value = 1.5, $P = 0.22$ NS; with Williams' correction = 4.208, P = NS; *G*-value with Yates' correction = 3.03, $P = 0.081$ NS; *L. koslwoskyi* complex/*L. abaucan*: Fisher's exact test $P = 0.322$ NS; *G*-test = 2.11, $P = 0.14$ NS with Williams and Yates corrections = NS; *L. koslwoskyi* complex/*L. quilmes* = Fisher's exact test $P = 0.082$ NS; *L. koslwoskyi* complex/*L. uspallatensis*: Fisher's exact test $P = 0.2120$ NS; *G*-value = 2.552, $P = 0.110$, Williams correction $P = 0.119$ and Yates correction $P = 0.233$ NS).

The average number of nucleotide substitutions per site (D_{xy}) between all *L. koslowskyi* samples and *L. abaucan* was 0.094; between the *L. koslwoskyi* complex and *L. koslwoskyi* N was 0.042, between the *L. koslwoskyi* complex and *L. koslwoskyi* E was 0.082; and between *L. koslwoskyi* N and *L. koslwoskyi* E was 0.078.

Estimations of nucleotide and gene diversity and θ, results of Tajima and Fu tests, and the Rgg for all samples of the *L. koslowskyi* complex, as well as for the main clades within it, are summarized in Table 3. In almost all clades the Tajima and Fu tests and the mismatch analyses were consistent with range expansion expectations.

Results of the AMOVA analyses are summarized in Table 4, and showed that most of the genetic variation was strongly partitioned between the *L. koslowskyi* clade and *L. koslowskyi* N and *L. koslowskyi* E (77.4%). Within *L. koslowskyi*, the variance was approximately equally distributed among groups (46.44%) and among populations within groups (53.56%) for both higher level nesting clades (5-1 and 5-2). Variance was also approximately equally partitioned among groups (30.37%), among populations within groups (37.18%), and within populations (32.46%) for the fourth level clades (third AMOVA).

Discussion

The mtDNA phylogeny for lizards of the *L. darwinii* group including 43 populations of *L. koslowskyi* from throughout its

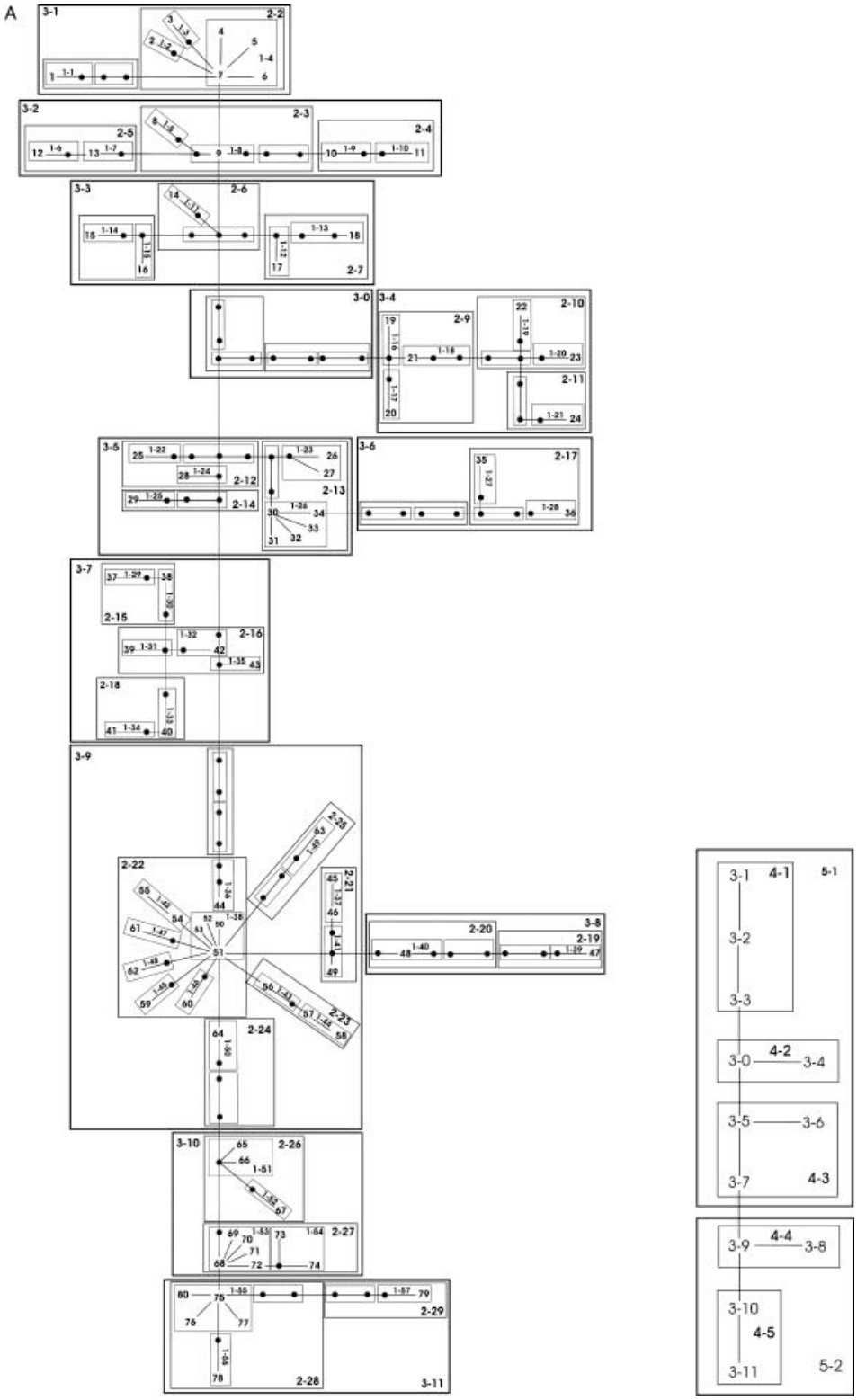

Fig. 3 A–C. Networks of *Liolaemus koslowskyi* with associated nested design. Designations of different haplotypes within these, as well as their frequencies and geographical locations, are summarized in Appendix II. —A. *L, koslowskyi (sensu stricto)*. —B. *L. koslowskyi* N. —C. *L. koslowskyi* E.

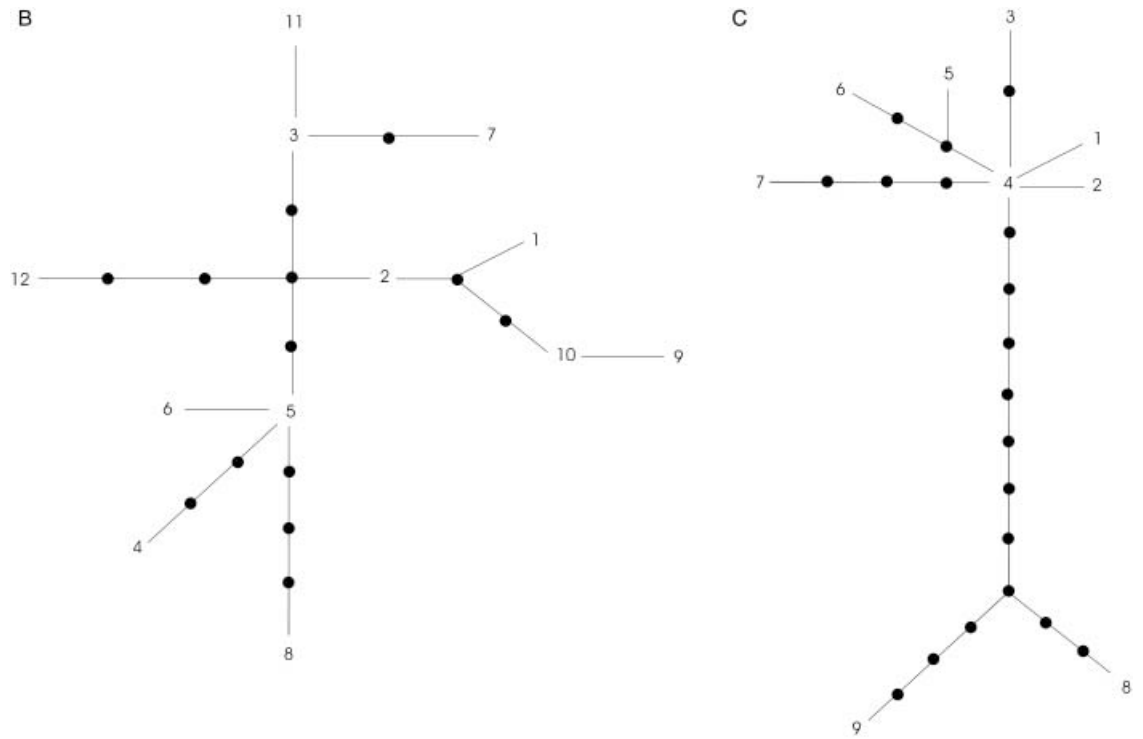

Fig. 3 *Continued*.

Table 2 Clades identified by resolved haplotype network/nesting design (Fig. 3) for which statistically significant clade distances (Dc) and/or nested clade distances (Dn) were obtained. The NCPA inferences are: IbD, isolation by distance; RE, range expansion; CRE, continuous range expansion; GF, gene flow; C & RD, colonization and restricted dispersal; RGFwIbD, restricted gene flow with isolation by distance; F, fragmentation; IGS, incomplete geographical sampling. Underlined acronyms identify most plausible interpretations confirmed from other analyses; * indicate conflict with inferences from other analyses.

Clade	χ^2	*P*	Inference chain
Liolaemus koslowskyi			
Clade 1-4	26.0	0.06	1-2-3-5-15-16-18: F/RE/IbD
Clade 1-26	12.1	0.49	1-2-3-5-6: RE/C & RD/GF
Clade 1-55	17.0	0.04	1-2-3-5-15-16-18: NO: F/RE/IbD
Clade 2-13	14.0	0.03	1-19-20: IGS
Clade 3-2	16.0	0.29	1-19-20-2-11-17-4-: RGFwIbD
Clade 3-4	11.2	0.18	1-2-11-17-4-9–10: F/IbD
Clade 3-5	34.0	0.02	1-19-20: IGS
Clade 4-1	28.5	0.21	1-2-3-5-6: RE/C & RD/GF
Clade 4-3	42.6	0.00	1-2-11: RE –12: CRE
Clade 4-4	18.2	0.07	1-2-11: RE –12: CRE
Clade 4-5	12.3	0.11	1-2-3-4: RGFwIbD*
Clade 5-1	79.2	0.00	1-2-11: RE –12: CRE
Clade 5-2	42.7	0.00	1-2-3-4: RGFwIbD*
Total cladogram	110.1	0.00	1-2: interior/tip status cannot be determined

range, recovered these populations in a well-supported clade (clade 5, Fig. 2), with *L. koslwoskyi* E as the sister terminal to all others. *Liolaemus quilmes*, *L. espinozai* and six candidate species closely related to *L. quilmes* were recovered in a well-supported monophyletic group (clade 4), that together with the well-supported clade (*L. lavillai* + (*L. irregularis* + *L. albiceps*)) constitute the sister group of (*L. koslowskyi* + *L. abaucan*) clade with moderate support (clade 2). Although *L. calchaqui*, *L. crepuscularis* and *L. ornatus* were not included in this work, these results are congruent with those of Abdala (2007), which included these three species in a phylogeny based on 128 morphological characters and 1776 mitochondrial base pairs.

Within *L. koslowskyi* clade 5 we recovered three well-supported haploclades in agreement with the three networks recovered with the TCS algorithm (Fig. 3). Also, the AMOVA results show that most of the genetic variation (77%) is partitioned among these three groups. Most of the haplotypes, including those of the type locality of *L. koslowskyi* were recovered with high support in a monophyletic group we referred to as *L. koslowskyi* (*sensu stricto*). The basal group within the *L. koslowskyi* clade, *L. koslowskyi* E, includes all the individuals (11; Appendices I and II) from localities 41, 42 and 43 (Fig. 1). These localities are located east of the salt flats of 'Salar de Pipanaco' and these haplotypes are approximately 7% divergent from those of *L. koslowskyi* (*sensu stricto*),

Table 3 Estimates of gene and nucleotide diversity (π) and two different estimates of the parameter (θ_π and θ_S) for the different clades identified in the phylogenetic analyses. Tajima's *D*-statistic and Fu's *F*'s test are given with associated level of significance, SSD'*P* = sum-of-square deviations probability for mismatch analyses, and Rgg is the raggedness index with its associated probability. Numbers under the *D*, *F* and SSD'*P* estimates are probability values (NS, not significant).

	N	Gene diversity	Nucleotide diversity (in percentage)	θ_π	θ_S	Tajima's *D*	Fu's *F*	SSD'*P*	Rgg
Liolaemus koslowskyi	115	0.990 (0.003)	1.508 (0.7591)	12.520 (6.30)	22.189 (5.56)	–1.423 0.042	-3×10^{38} 0.008	0.0013	0.0026 NS
L. k. North	18	0.948 (0.033)	0.593 (0.339)	4.921 (2.811)	6.396 (2.546)	–0.906 NS	–3.380 0.051	0.0058	0.020 NS
L. k. East	11	0.963 (0.510)	0.837 (0.481)	6.945 (3.992)	9.218 (3.958)	–1.137 NS	–1.809 NS	0.0205	0.0205 NS
L. k. clade 51	64	0.980 (0.007)	1.233 (0.632)	10.239 (5.251)	17.131 (4.828)	–1.378 0.049	–11.305 0.005	0.0017	0.0055 NS
L. k. clade 5-2	51	0.982 (0.008)	0.827 (0.439)	6.863 (3.644)	12.669 (3.812)	–1.589 0.035	–11.771 0.003	0.0109	0.0101 NS
L. k. clade 4-1	26	0.957 (0.027)	0.666 (0.369)	5.529 (3.059)	9.172 (3.234)	–1.489 0.043	–7.443 0.005	0.0031	0.011 NS
L. k. clade 4-2	9	0.833 (0.126)	0.582 (0.356)	4.833 (2.952)	5.887 (2.780)	–0.868 NS	–0.077 NS	0.0782	0.249 NS (0.08)
L. k. clade 4-3	29	0.951 (0.024)	0.963 (0.513)	7.995 (4.257)	10.185 (3.486)	–0.796 NS	–4.757 0.047	0.0094	0.018 NS
L. k. clade 4-4	25	0.977 (0.022)	0.471 (0.272)	3.913 (2.262)	9.269 (3.300)	–2.183 0.001	–13.337 0.0000	0.0016	0.021 NS
L. k. clade 4-5	26	0.954 (0.022)	0.419 (0.246)	3.477 (2.041)	6.551 (2.410)	–1.717 0.019	–8.174 0.0010	*1	

*1 = Least square procedure to fit model mismatch distribution and observed distribution did not converge after 1800 steps.

Table 4 AMOVA for (1) *L. koslowskyi*, *L. koslowskyi* north, *L. koslowskyi* east; (2) *L. koslowskyi* clades 5-1 and 5-2; (3) *L. koslowskyi* clades 4-1, 4-2, 4-3 (included in clade 5-1) and 4-4, 4-5 (included in clade 5-2). All statistically significant.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
(1) Among populations	2	953.195	19.197	77.40
Within populations	141	790.221	5.604	22.60
Fst = 0.77403				
(2) Among populations	1	219.561	3.791	46.44
Within populations	113	494.100	4.373	53.56
Fst = 0.46437				
(3) Among groups	1	219.561	2.473	30.37
Among pops. within groups	3	203.299	3.028	37.18
Within populations	110	290.801	2.644	32.46

Fsc = 0.534; Fst = 0.675; Fct = 0.304.

which implies a divergence time of approximately 5 mya (following the argument developed by Morando *et al.* (2007)). There is evidence that this isolated valley is rich in relatively young taxa, as new mammalian genera have been recently described for this area (*Andalgalomys olrogi*, Williams & Mares 1978; *Pipanacoctomys aureus*, Mares *et al.* 2000), and new species of the rodent genera *Ctenomys* and *Eligmodontia* endemic to this valley are in the process of being described (Mares *et al.* 2000). Although detailed studies, including morphological data, are necessary to assess the status of the *L. koslowskyi* E populations, these results suggest that it likely represents an undescribed species.

The third clade, *L. koslowskyi* N, includes almost all the haplotypes from localities 20, 31, 38, 39 and 40 (Fig. 1), except two tip haplotypes (haplotypes 26 and 27, clade 1-2, Fig. 3B; Appendix II) from localities 20 and 31 that are recovered within the '*L. koslowskyi*' network. These haplotypes are related to those from localities 26–29, geographically very close and located in a narrow valley that connects them. It is plausible that, as this narrow valley was formed, these populations were interconnected by some level of gene flow. The level of genetic divergence between *L. koslowskyi* N and *L. koslowskyi* (corrected average pairwise distance [PiXY – (PiX + PiY)/2]: 3.20%) implies a rough divergence time of 2.5 mya, but our results are insufficient to assess if *L. koslowskyi* N constitutes a separate lineage.

The *L. koslwoskyi* network has the highest nucleotide diversity of the three clades, and includes two subsets at the most inclusive nesting level (Fig. 3A, clades 5-1 and 5-2). Clade 5-1 is the north-eastern-most area of *L. koslowskyi* and clade 5-2 includes the south-western-most localities (Fig. 4). A valley between the Velasco and Vinquis mountains

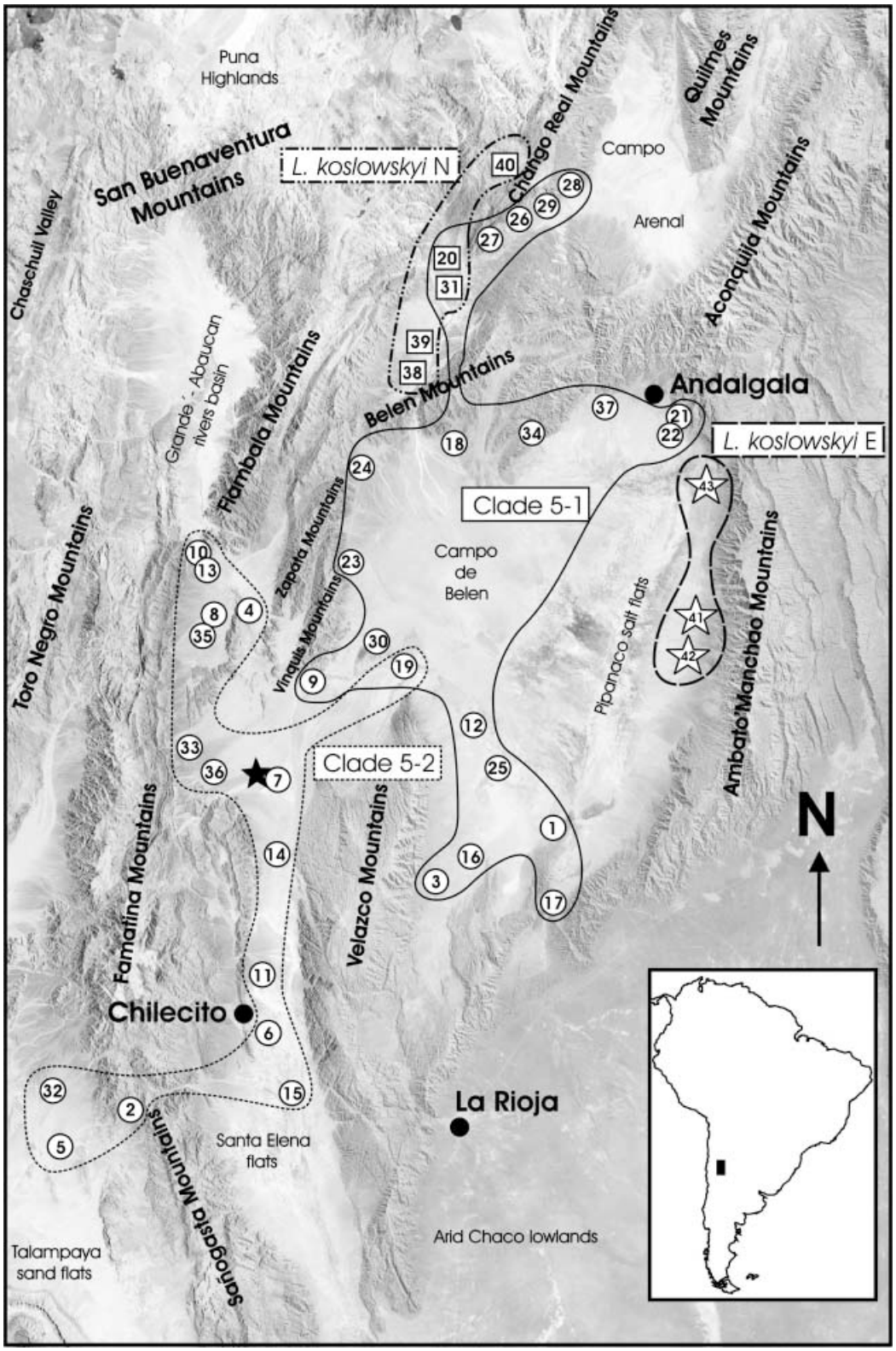

Fig. 4 Geographic distribution of the higher clades of *Liolaemus koslowskyi* (*sensu stricto*) *and L. koslowskyi* E (stars) and *L. koslowskyi* N (squares). Black star: type locality of *L. koslowskyi*. Locality numbers correspond to those in Table 1 and Appendices I and II.

geographically connects these two clades, and in the northern part of this valley (Fig. 4, locality 19), we found two groups of divergent haplotypes in sympatry. Almost all of these haplotypes are tip haplotypes, suggesting that gene flow between clades 5-1 and 5-2 is occurring through this valley. For clade 5-1 as well as for two of the fourth level clades we inferred continuous range expansions, in agreement with the hypotheses that when climatic conditions are adequate, these lizards disperse through these valleys to maintain some level of gene flow. This NCPA inference is consistent with a highly significant mismatch distribution (Table 3) consistent with a recent demographic expansion at this same clade level. At lower nesting levels few inferences were possible, but results from NCPA, neutrality tests, and mismatch analyses suggest that restricted gene flow as well as expansions in some areas contribute to the genetic structure we observe in *L. koslowskyi*.

Liolaemus koslowskyi is genetically deeply structured, and during the history of these populations it is possible that lizards colonized surrounding areas. This is a geographically complex area and probably these colonizations were associated with the relatively recent orogeny of the region, as well as Pleistocene climatic oscillations. In general, the series of Andean and pre-Andean mountain chains that rim the isolated valleys are fairly young, with most of the uplift having occurred since the Miocene. Indeed, significant uplift took place in the Pliocene or even as recently as the Pleistocene (Pascual & Ortiz Jaureguizar 1990). One of these colonization events could have promoted eastern expansion and later isolation of populations east of the Pipanaco salt flats (*L. koslovskyi* E). A similar event could have promoted the divergence of the *L. koslowskyi* N populations through some small N–S connections in the Belen Mountains, interconnecting the Campo de Belen and Campo Arenal, and later the opening of the narrow valley between the Belén and Chango Real Mountains created a passage through which gene flow may now be occurring (Fig. 4).

The highly structured *L. koslowskyi* clade suggests that it has a relatively ancient history in a land-locked, topographically rich archipelago of habitat and tectonic islands that are themselves relictual mountains and valleys. Lizard species that may have colonized the region before the period of uplift would be expected to have had their geographical ranges broken into numerous populations and diversify over time. It is highly probable that several expansion–contraction cycles have fostered the dispersal into valleys and subsequently restricted gene flow to permit divergence of some populations. Our results suggest that this may what has promoted speciation of *L. koslowskyi* E, and a high level of divergence of *L. koslowskyi* N. This hypothesis specifies a temporal sequence of events that can now be tested with appropriate nuclear markers and coalescent methods (Rosenblum *et al.* 2007).

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Appendix I

Specimens of reference with museum voucher numbers listed by locality. Outgroups and individuals of *L. koslowskyi* with * were used in phylogeny reconstruction.

L. koslowskyi: Loc. 1: LJAMM (1911)*. Loc. 2: LJAMM (1912)*. Loc. 3: LJAMM (1913). Loc. 4: LJAMM (1957, 58). Loc. 5: LJAMM 2291. Loc. 6: LJAMM 4152. Loc. 7: LJAMM 4157–4159. Loc. 8: LJAMM 4169. Loc. 9: LJAMM 4170–71. Loc. 10: LJAMM 4174*. Loc. 11: LJAMM 4175-76-77-78. Loc. 12: LJAMM 4179–80. Loc. 13: LJAMM 4197, 4752–53. Loc. 14: LJAMM 4206*–07, 5827-28-29. Loc. 15: LJAMM 4217–18, 4755-56-57-58–59. Loc. 16: MLP.S 2509–10, BYU 48166. Loc. 17: LJAMM 4247–48. Loc. 18: LJAMM 4257–58. Loc. 19: BYU 48167-68-69, MLP.S 2512–13. Loc. 20: LJAMM 4281-82-83-84-85-86. Loc. 21: LJAMM 4301. Loc. 22: LJAMM 4304–05, BYU 48174, MLP.S 2525. Loc. 23: LJAMM 4307*-08–09. Loc. 24: BYU 48183–84, MLP.S 2520–21. Loc. 25: LJAMM 4317*-18. Loc. 26: LJAMM 4347, BYU 48178-79-80*, MLP.S 2524. Loc. 27: LJAMM 4369, MLP.S 2522–23, BYU 48175-76-77. Loc. 28: LJAMM 4383. Loc. 29: BYU 48181–82, MLP.S 2518–19. Loc. 30: LJAMM 5011–12. Loc. 31: LJAMM 5015–16*-20*. Loc. 32: LJAMM 4760-61-62-63*-64-65-66. Loc. 33: LJAMM 4819*. Loc. 34: LJAMM 4827-28-29-30*-31*-32. Loc. 35: LJAMM 4833-34-35-36-37-38. Loc. 36: LJAMM 4844-45-46-47–48*-49–50. Loc. 37: LJAMM 4851-52-53-54–55*-56-57-58. Loc. 38: LJAMM 4287*-88, MLP.S 2516–17, BYU 48172–73. Loc. 39: LJAMM 5013–14. Loc. 40: LJAMM 5008-09-10. Loc. 41: LJAMM 4822*-23-24-25-26. Loc. 42: LJAMM 4297–98. Loc. 43: MLP.S 2514–15, BYU 48170–71*.

Nonfocal taxa:

L. abaucan: LJAMM 2360; *L. albiceps*: LJAMM 2648; *L.* cf. *quilmes* 1: MLP.S 2526; *L.* cf. *quilmes* 2: LJAMM 4417; *L.* cf. *quilmes* 3: BYU 48227; *L.* cf. *quilmes* 4: MLP.S 2527; *L.* cf. *quilmes* 5: BYU 48233; *L.* cf. *quilmes* 6: LJAMM 4422; *L.* cf. *olongasta*: LJAMM 2292; *L. chacoensis*: MLP.S 2508; *L. darwinii* N1: LJAMM 2275; *L. espinozai*: MLP.S 2530; *L. irregularis*: LJAMM 2630; *L. laurenti*: LJAMM 4161; *L. lavillai*: MLP.S 2531; *L. olongasta*: LJAMM 2377; *L. quilmes*: LJAMM 4375; *L. uspallatensis*: BYU 48121.

Outgroups:

L. cf. *boulengeri*: LJAMM 2165; *L. inacayali*: FN165.

Appendix II

Haplotype number, locality and specimens of reference used for nested clade analyses.

Haplotype number	Locality number	Number of individuals	Haplotype number	Locality number	Number of individuals
L. koslowskyi (115)			52	32	1 (LJAMM 4762)
1	17	1 (LJAMM 4248)	53	36	1 (LJAMM 4848)
2	16	1 (MLP.S 2510)	54	15	2 (LJAMM 4218, 4757)
3	37	1 (LJAMM 4856)	55	32	1 (LJAMM 4764)
4	25	1 (LJAMM 4318)	56	11	1 (LJAMM 4177)
5	30	1 (LJAMM 5012)	57	15	1 (LJAMM 4756)
6	24	2 (BYU 48184, MLP.S 2520)	58	15	1 (LJAMM 4755)
7	1	1 (LJAMM 1911)	59	11	1 (LJAMM 4175)
	16	1 (MLP.S 2509)	60	32	1 (LJAMM 4760)
	17	1 (LJAMM 4247)	61	5	1 (LJAMM 2291)
	23	2 (LJAMM 4308, 4309)	62	32	1 (LJAMM 4766)
8	34	1 (LJAMM 4827)	63	15	1 (LJAMM 4758)
9	37	1 (LJAMM 4851)	64	32	1 (LJAMM 4763)
10	9	1 (LJAMM 4171)	65	35	1 (LJAMM 4835)
	30	1 (LJAMM 5011)	66	19	2 (BYU 48167, 48168)
11	24	2 (BYU 48183, MLP.S 2521)	67	33	1 (LJAMM 4819)
12	3	1 (LJAMM 1913)	68	7	1 (LJAMM 4159)
13	19	1 (MLP.S 2512)		14	1 (LJAMM 5829)
14	16	1 (BYU 48166)		35	1 (LJAMM 4837)
15	12	1 (LJAMM 4180)	69	35	1 (LJAMM 4833)
16	12	1 (LJAMM 4179)	70	4	1 (LJAMM 1958)
17	19	2 (BYU 48169, MLP.S 2513)		14	1 (LJAMM 5827)
18	25	1 (LJAMM 4317)	71	14	1 (LJAMM 4207)
19	34	1 (LJAMM 4828)	72	13	1 (LJAMM 4197)
20	34	1 (LJAMM 4829)		36	1 (LJAMM 4845)
21	26	3 (BYU 48179, MLP.S 2524, LJAMM 4347)	73	13	1 (LJAMM 4753)
	29	1 (BYU 48181)	74	13	1 (LJAMM 4752)
22	23	1 (LJAMM 4307)	75	8	1 (LJAMM 4169)
23	18	1 (LJAMM 4257)		35	2 (LJAMM 4836, 4838)
24	18	1 (LJAMM 4258)	76	4	1 (LJAMM 1957)
25	34	1 (LJAMM 4831)	77	36	1 (LJAMM 4844)
26	20	1 (LJAMM 4286)	78	7	1 (LJAMM 4157)
27	31	1 (LJAMM 5016)	79	35	1 (LJAMM 4834)
28	37	1 (LJAMM 4855)	80	36	4 (LJAMM 4846, 4847, 4849, 4850)
29	22	1 (MLP.S 2525)	*L. koslowskyi* N (sample size = 18)		
30	26	1 (BYU 48178)	1	20	1 (LJAMM 4282)
	28	1 (LJAMM 4383)	2	20	1 (LJAMM 4283)
	29	2 (BYU 48182, MLP.S 2519)	3	20	1 (LJAMM 4285)
31	27	1 (BYU 48175)		38	1 (LJAMM 4290)
32	27	1 (BYU 48176)		39	1 (LJAMM 5013)
33	26	1 (BYU 48180)	4	38	1 (LJAMM 4287)
	27	4 (LJAMM 4369, MLP.S 2522, 2523, BYU 48177)	5	20	2 (LJAMM 4281, 4284)
34	29	1 (MLP.S 2518)		38	1 (LJAMM 4288)
35	9	1 (LJAMM 4170)	6	38	1 (LJAMM 4289)
36	34	2 (LJAMM 4830, 4832)	7	38	1 (LJAMM 4291)
37	37	1 (LJAMM 4852)	8	38	1 (LJAMM 4292)
38	22	1 (LJAMM 4304)	9	40	2 (LJAMM 5008, 5010)
39	22	1 (LJAMM 4305)	10	40	1 (LJAMM 5009)
40	37	1 (LJAMM 4854)	11	39	1 (LJAMM 5014)
41	22	1 (BYU 48174)	12	31	2 (LJAMM 5015, 5020)
42	21	1 (LJAMM 4301)	*L. koslowskyi* E (sample size = 11)		
43	37	3 (LJAMM 4853, 4857, 4858)	1	41	1 (LJAMM 4826)
44	32	1 (LJAMM 4761)	2	41	2 (LJAMM 4823, 4825)
45	6	1 (LJAMM 4152)	3	43	1 (BYU 48171)
46	11	1 (LJAMM 4176)	4	43	1 (LJAMM 4294)
47	14	1 (LJAMM 4206)	5	43	1 (LJAMM 4295)
48	10	1 (LJAMM 4174)	6	43	1 (LJAMM 4293)
49	15	1 (LJAMM 4759)	7	42–41	2 (LJAMM 4298, 4824)
50	2	1 (LJAMM 1912)	8	42	1 (LJAMM 4297)
51	11	1 (LJAMM 4178)	9	41	1 (LJAMM 4822)
	15	1 (LJAMM 4217)			
	32	1 (LJAMM 4765)			
	14	1 (LJAMM 5828)			