

PHYLOGEOGRAPHY AND GENETIC STRUCTURE IN THE *CNEMIDOPHORUS LONGICAUDA* COMPLEX (SQUAMATA, TEIIDAE)

MARTHA M. YOKE^{1,3}, MARIANA MORANDO², LUCIANO J. AVILA², AND JACK W. SITES, JR.¹

¹Department of Integrative Biology and M.L. Bean Life Science Museum, 401 WIDB, Brigham Young University, Provo, Utah, 84602, USA

²CENPAT-CONICET, Boulevard Almirante Brown s/n, U9120ACV, Puerto Madryn, Chubut, Argentina

ABSTRACT: *Cnemidophorus longicauda* is a poorly-studied species of whiptail lizard distributed in the Monte Desert region of Argentina. Some variation in dorsal color pattern has been observed between northern and southern populations along the latitudinal range of the species. Recently, a new species of *Cnemidophorus* (*C. tergoaevigatus*) previously considered as *C. longicauda* has been described, although no comparative morphological study has been conducted. In this study we use the mitochondrial ND4 gene region to examine the phylogeography and genetic structure of this species complex. Phylogenetic reconstruction, Nested Clade Phylogeographic Analysis, and AMOVA reveal deep genetic divergence between northern and southern populations that are geographically separated by the Famatina–Sañogasta Mountains. Because most of the localities of *C. tergoaevigatus* are from the general region of the “northern group” recovered in this study, there is general concordance between geographic patterns of mtDNA and morphological (color pattern) variation, and thus support for the recognition of this species. We discuss this genetic break and the phylogenetic/phylogeographic history of the *Cnemidophorus* in the context of other lizard species with similar distributions, and the implications for understanding the evolutionary history of the temperate South American biota.

Key words: Argentina; *Cnemidophorus*; MtDNA; Nested Clade Phylogeographic Analysis; Phylogeography

PHYLOGEOGRAPHY, a relatively recently developed discipline of population genetics, has made wide use of the mitochondrial DNA locus to make inferences concerning the evolutionary histories of populations within species, and among recently diverged species (Avice, 2000). Most early phylogeographic studies, however, were based on a single gene locus (mtDNA) and simple visual associations of haplotype topologies and geography, followed by qualitative assessments of genealogical histories. More recently, approaches such as Nested Clade Analysis (Templeton et al., 1995; now referred as Nested Clade Phylogeographic Analysis [NCPA], Templeton, 2004) have proven useful in phylogeographic studies which, when accompanied by precise geographic localities, permit statistical tests of the strength of associations between haplotype genealogies and their distributions. Significant associations can then be used as the basis for inferring the most likely historical and/or ongoing demographic processes responsible for any given pattern. However, for any number of reasons — incomplete lineage

sorting, hybridization, selective sweeps, poor taxonomic resolution of species — inferences based on the mtDNA locus alone can be misleading (Funk and Omland, 2003), and the NCPA has been criticized for its inability to differentiate among alternative explanations statistically for a given phylogeographic pattern (Knowles and Maddison, 2002). Despite these challenges, the mtDNA locus is expected to track shallow population/species splits with greater fidelity than nuclear gene loci under many biologically plausible scenarios (Hickerson and Cunningham, 2005; Moore, 1995), and the use of multiple methods to test for genetic structure and to infer demographic history can serve to cross-validate mtDNA-based NCPA results (Morando et al., 2004; Pfenninger and Posada, 2002), or to highlight limitations of particular inferences (Masta et al., 2003).

We have argued elsewhere (Avila et al., 2006; Morando et al., 2003) that phylogeographic studies of poorly-known groups are characterized by imprecise knowledge of species boundaries and geographic distributions (due to limited sampling from poorly-known regions), and normally few external

³ CORRESPONDENCE: e-mail, martha.yoke@gmail.com

data (i.e., independent information from bioclimatic, geological, or paleoecological data; Hugall et al., 2002; Kozak et al., 2006) are available to formulate a priori hypotheses of patterns of divergence. In these circumstances, the NCPA approach is ideal for generating hypotheses about the evolutionary histories of recently derived species and distinct populations. Further, recognition of the limitations of the single-locus approach does not permit immediate adoption of multi-locus coalescent methods (Edwards and Beerli, 2000; Hey and Nielsen, 2004) for estimating historical demographic parameters. This approach (advocated by Knowles, 2004) still requires a reasonably good knowledge of species boundaries and distributions, intra-specific population structure, and the availability of unlinked, non-recombinant nuclear gene regions for implementation (Jennings and Edwards, 2005).

We emphasize two further points. First, hypotheses generated from mtDNA phylogeographic studies based on NCPA can be further tested in a number of ways, including the use of independent genetic markers (Templeton, 2004), and/or the use of multiple statistical methods that make different assumptions about the data or evolutionary histories (Hickerson and Cunningham, 2005). In the absence of paleoecological or bioclimatic information, one can also compare the phylogeographic structures of different co-distributed species (or species groups) within the region of interest (Avice, 2000; Zink, 2002) to see whether similar historical or demographic inferences can be drawn across the same region in unrelated groups. Second, methods are under development to address co-phylogeny issues in both spatial (Lapointe and Rissler, 2005) and temporal contexts (Hickerson et al., 2006), and if these newer approaches are validated, then stronger inferences can be made about the influence of historical events and ecological processes in structuring regional biotas.

Cnemidophorus longicauda is the southernmost distributed member of its genus and is restricted almost exclusively to the Monte Desert biogeographic region of southern and western Argentina. This species has a latitudinal distribution of more than 2000 km.,

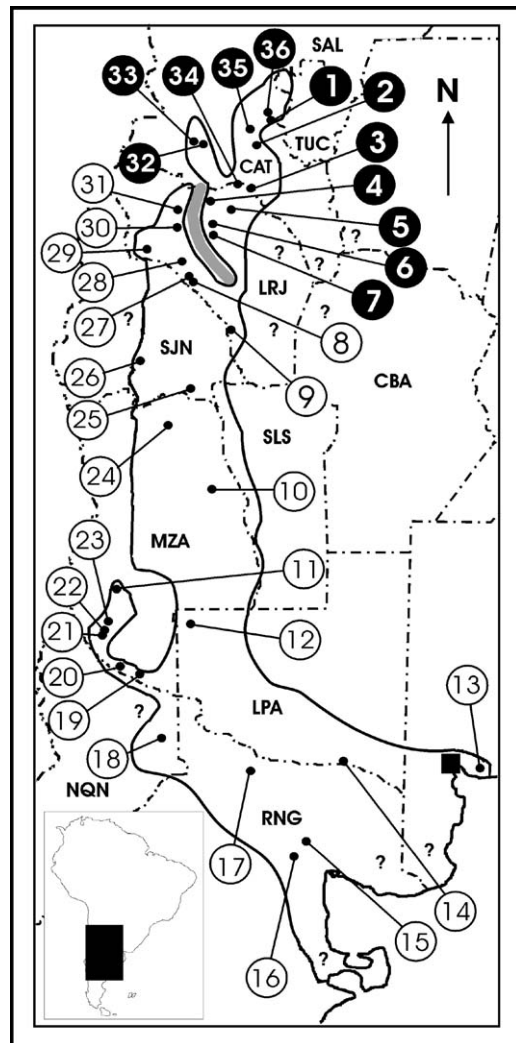


FIG. 1.—Geographical distribution of the *Cnemidophorus longicauda* complex and sampling localities; white circles = Southern clade, black circles = Northern clade. The gray area indicates the location of the Famatina-Sañogasta Mountains, precise localities and sample sizes are summarized in Appendix 1, and question marks identify regions of the Monte Desert in which members of this complex may occur but have not been documented. Three letter codes represent province names: SAL = Salta, TUC = Tucumán, CAT = Catamarca, LRJ = La Rioja, SJN = San Juan, SLS = San Luis, CBA = Córdoba, MZA = Mendoza, LPA = La Pampa, NQN = Neuquén, RNG = Río Negro. Black square = outgroup.

extending from the Calchaquies valleys in the Salta Province in the north to the Atlantic shores of Golfo Nuevo in Chubut Province in the south (Fig. 1). Along this latitudinal

distribution of almost 17°, there is no clear evidence of morphological differentiation between populations, but some variation in dorsal color pattern can be observed between northernmost and southernmost populations, with an intergradation area in the central part of the range (western La Rioja and San Juan Provinces). In the course of this study, a new species of *Cnemidophorus* was described from four localities in La Rioja province (*C. tergoaevigatus*; Cabrera, 2004). This description was made on the basis of only a few individuals, most of which were collected at the beginning of the last century without clear reference to precise localities; the holotype and paratypes total three individuals from four possible localities, two of which represent two mountain ranges; but without precise localities for any individual. Typically southern populations have a conspicuously striped dorsum, whereas the striping is less evident or absent in northern populations. Intermediates of these two patterns are sometimes found in the above-mentioned transitional areas, but no morphological study has been conducted beyond Cabrera's (2004) qualitative assessment of dorsal striping pattern. For convenience, we will in this study refer to both taxa as the *Cnemidophorus longicauda* complex.

Phylogeographic studies in southern South America are scarce and have been made on rodents (Albright, 2004; Palma et al., 2005) and for several species complexes of the lizard genus *Liolaemus* (Avila et al., 2006; Morando et al., 2003, 2004). These authors have suggested that the phylogeographic patterns derived from these studies, which included many inferences of past fragmentation, recent speciation, range expansion, and secondary contact and introgression, could in part be explained by Pleistocene climatic changes. Similar events have been implicated in the evolutionary histories of some vertebrate species in the northern hemisphere (Berggren et al., 2005; Good et al., 2003; Kozak et al., 2006; Zamudio and Savage, 2003), but the southern hemisphere has been studied in much less detail. Evidence from phylogeographic analyses of other groups in the region would help to validate the supposition that the Pleistocene climate changes have significantly

impacted the evolutionary history of the Monte–Patagonian biota.

The population genetic structure of the *C. longicauda* complex has not been studied, and here we use the mtDNA locus to generate hypotheses about the phylogeographic history of this group, as we have done in previous studies of the lizard genus *Liolaemus* (Avila et al., 2006; Morando et al., 2003, 2004). We are aware that uncritical use of the NCPA for a single gene tree may lead to over-interpretation (Knowles, 2004 [Table 1]), but in this case, the absence of climatic, geological, or paleoecological data precludes the *a priori* formulation and statistical testing of multiple alternative hypotheses advocated by Knowles and Maddison (2002). In this study we use conventional phylogenetic inference, in combination with the NCPA of mtDNA sequences, to estimate the genetic substructuring within the *C. longicauda* complex, and to hypothesize historical or demographic processes that may have contributed to its current structure. We cross-validate the NCPA inferences using other methods appropriate for mtDNA haplotypes (Morando et al., 2004; Pfenninger and Posada, 2002), qualitatively compare inferences made for *C. longicauda* in this study with those made for another co-distributed complex of *Liolaemus* (Avila et al., 2006; Morando et al., 2004), and use the most strongly-supported inferences to formulate hypotheses which can be tested with independent genetic markers and utilized in future multi-species studies of the evolution of the Monte–Patagonian biota.

MATERIALS AND METHODS

Taxon Sampling and Laboratory Procedures

Sampling consisted of 73 individuals from 36 localities (Table 1, Fig. 1), including one individual of *C. lacertoides* used as an out-group. Voucher specimens are deposited in the field collection of Luciano J. Avila and Mariana Morando (specified by "LJAMM" and housed in the Centro Nacional Patagónico [CENPAT]), and the M.L. Bean Life Science Museum at Brigham Young University (BYU).

DNA was extracted from liver tissue preserved in 96% ethanol following the protocol

TABLE 1.—Different haplotypes included in the NCPA networks, with localities from which each haplotype was sampled (locality numbers correspond to those in Fig. 1 and Appendix 1).

| Southern network Haplotype number | Locality number | Northern network Haplotype number | Locality number |
|-----------------------------------|------------------------|-----------------------------------|-----------------|
| 1 | 9 | 25 | 35 |
| 2 | 31 | 26 | 2 |
| 3 | 30 | 27 | 1, 2, 35, 36 |
| 4 | 9 | 28 | 2 |
| 5 | 27, 28 | 29 | 34 |
| 6 | 10 | 30 | 3 |
| 7 | 10 | 31 | 4 |
| 8 | 28, 8 | 32 | 5, 4, 34, 6 |
| 9 | 28 | 33 | 34 |
| 10 | 25 | 34 | 2 |
| 11 | 12 | 35 | 32 |
| 12 | 24 | 36 | 33 |
| 13 | 24 | 37 | 33 |
| 14 | 29 | | |
| 15 | 26 | | |
| 16 | 22 | | |
| 17 | 19 | | |
| 18 | 15, 23, 11, 20, 18, 17 | | |
| 19 | 14 | | |
| 20 | 16 | | |
| 21 | 17 | | |
| 22 | 21 | | |
| 23 | 8 | | |
| 24 | 25 | | |

of Fetzner (1999). Quality and quantity of extractions were estimated by electrophoresis on a 1% agarose gel and dilutions were made as needed. Polymerase Chain Reaction (PCR) was then used to amplify a 589 base pair fragment of the mtDNA ND4 gene region. The PCR cocktail and primers used (ND4-F and ND4-R) are those described by Morando et al. (2003); PCR amplicons were checked by electrophoresis on a 1% agarose gel and purified using Milipore cleaning plates. Sequencing was done on an ABI PRISM 3730XL automated DNA sequencer (PE Applied Biosystems) at the BYU DNA Sequencing Center. The resulting sequences were then edited and aligned in Sequencher 3.1.1 (Gene Codes Corp., Inc., Ann Arbor, MI) and translated into amino acids for confirmation of alignment. No indels were present and there were no missing data.

Phylogenetic Analyses

To eliminate redundant haplotypes, we used the program Collapse ver. 1.1 (<http://darwin.uvigo.es/software/collapse.html>) to select unique haplotypes for phylogenetic and phylogeographic analyses. Maximum likeli-

hood (ML) and maximum parsimony (MP) tree searches were executed using PAUP* 4.0b5 (Swofford, 2002), and we used the hierarchical likelihood-ratio test (hLRT), as implemented in the program ModelTest (ver. 3.04; Posada and Crandall, 1998) to select the best-fit model and associated parameters for the ML analysis. A heuristic search of 50 replicates with tree-bisection reconnection (TBR) branch swapping was performed to obtain a ML tree. The MP analysis was performed using an exhaustive branch-and-bound search (Hendy and Penny, 1982). Support for the recovered clades was estimated using nonparametric bootstrapping (Felsenstein, 1985). Bootstrap analysis for both ML and MP analyses consisted of 100 pseudoreplicates with random stepwise addition and TBR branch swapping. Bootstrap values $\geq 70\%$ are considered strong support for a clade (Hillis and Bull, 1993).

Nested Clade Phylogeographic Analysis

Population genealogies above and below the species level are different in nature in sexually reproducing species—pathways of descent are frequently strictly cladogenic

(branching) for many loci if reproductive isolation is complete, whereas these pathways are tokogenic (networked) among individuals sampled from populations interconnected by gene flow. Traditional tree-building algorithms are based on assumptions of branching relationships only, and are therefore violated at the population level (Templeton, 2001). Network methods complement tree-reconstruction methods, and in combination they provide a simultaneous assessment of haplotype relationships at shallow (within species) and deeper (between species) levels of divergence for a molecular marker with sufficient variation to register both histories (Posada and Crandall, 2001).

The NCPA was performed by first constructing statistical parsimony networks of haplotypes using the algorithm of Templeton et al. (1992), as implemented in the program TCS ver. 1.13 (Clement et al., 2000). The algorithm links haplotypes with the smaller number of differences defined by a 95% confidence criterion, and identifies the most probably ancestral haplotype as predicted from coalescent theory (Castelloe and Templeton, 1994). Haplotypes are then organized into a system of nested clades where higher nesting levels correspond to longer evolutionary time, as described by Templeton et al. (1995). Geographic association among haplotypes was first assessed using the nested contingency test described by Templeton and Sing (1993) by permuting clades within a nesting category against geographic locations (which are treated as the categorical variables) using the program GeoDis (ver. 2.0; Posada et al., 2000). GeoDis generates two types of distance values; clade distances (D_C) which measure how geographically widespread individuals from a given clade are, and nested clade distances (D_N) which measure the spread of individuals from a given clade relative to their most closely related clades (those within the same "nesting level"; Templeton et al. 1995).

The null hypothesis of no geographic association within nested clades was tested using the program by comparing observed values of D_C and D_N between tip clades (those with one mutational connection), interior clades (two or more connections), and

interior-tip clade comparison (I-T) within a nested group of clades. The null distribution of these comparisons was derived from 10,000 Monte Carlo permutations of clades against sampling sites. Significant distance values ($P \leq 0.05$) indicate nonrandom haplotype-geographic associations in the clade in which they are found, and when significant associations occur, a biological inference can be made using the most recent version of Templeton's (2004) inference key.

Tests of Neutrality and Molecular Diversity

To evaluate population equilibrium independently of NCPA, Fu's (1997) F_s -test and Tajima's (1989) D -test were implemented using Arlequin ver. 2.0 (Schneider et al., 2000). These tests differ in their statistical power (Ford, 2002; Wayne and Simonsen, 1998), but both assume that populations are in mutation-drift and migration-drift equilibrium. Significant values for either of these tests would suggest either that the populations deviate from: (1) mutation-drift equilibrium (the sequences are not evolving under neutral expectations); or (2) migration-drift equilibrium (a population may have experienced a recent expansion). Using this same software, nucleotide diversity (π ; the probability that two nucleotides from randomly chosen homologous sequences will be different; Nei, 1987), gene diversity (the probability that two randomly chosen haplotypes are different in the sample; Nei 1987), and population structure, were estimated with an AMOVA (Excoffier et al., 1992), and a mismatch distribution analysis (with pairwise distances) was implemented as another test for population expansion. The mismatch distribution is tested with the statistic of "raggedness" (Harpending et al., 1993), which estimates the smoothness of the mismatch distribution, and has been found to distinguish between stationary and expanded populations (Harpending, 1994). A raggedness index of 0.05 or smaller is regarded as significant enough to reject the hypothesis of no range expansion (Harpending, 1994). We also estimated the average pairwise sequence divergence between the two main groups obtained (see Results below) using the Kimura two parameter model and taking into account the intragroup variation (using Arlequin ver. 2.0).

RESULTS
*Phylogenetic Relationships of
 mtDNA Haplotypes*

Thirty-seven unique ingroup haplotypes were resolved (Table 1), and the MP search recovered six equally parsimonious trees (tree length = 146 steps); a strict consensus tree was generated for comparison with ML results. The HKY+G model of evolution was selected by the hLRTs in ModelTest with base frequencies of A = 0.3300, C = 0.3127, G = 0.1178, and T = 0.2396, a ti/tv ratio of 9.1885, and a gamma distribution shape parameter of 0.1295. Using these parameters, the ML analysis yielded one tree ($\ln L = -1467.4605$) that was nearly identical to the strict consensus MP tree. Consequently, only the ML tree is presented here (Fig. 2). Both trees recovered two strongly supported haploclades (bootstrap values for MP and ML = 100) corresponding to reciprocal monophyly for populations north and south of the Famatina Sañogasta Mountains. Within each of these groups, however, there was little phylogenetic structure (see Fig. 2).

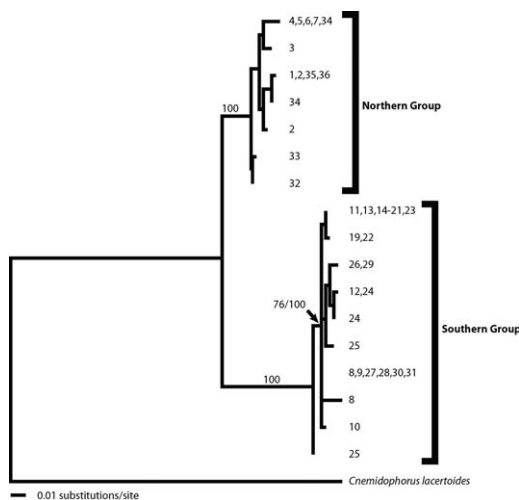


FIG. 2.—Single tree recovered from maximum likelihood search ($\ln L = -1467.4605$); terminal numbers correspond to the localities in Figure 1, and numbers on the branches represent MP and ML bootstrap values, respectively (one value only is given if ML = MP bootstrap proportions).

*Parsimony Network and Nested Clade
 Phylogeographic Analysis*

The TCS algorithm constructed two separate haplotype networks at the 95% confidence level, which correspond to the samples north and south of the Famatina–Sañogasta Mountains. The haplotype network for the southern populations was not fully resolved in that one mutational pathway was equally parsimonious for two alternative connections, thus creating a “loop” in this network (i.e., homoplasy; see dotted line in Fig. 3). Predictions from coalescent theory (Crandall and Templeton, 1993) offer three ‘rules’ for resolving these loops, including: (1) high frequency haplotypes in the population are expected to be found on the interior of a cladogram, and lower frequency haplotypes should be found at the tips of the cladogram; (2) haplotypes with more mutational connections are likely to be older than those with few or no mutational connections to other haplotypes, and older vs. younger haplotypes are expected to be on the interior vs the tips of a cladogram, respectively; and (3) geographic proximity – given equally parsimonious connections between haplotypes from near vs. distant locations, those in geographic proximity are more likely to be related. We resolved the loop in the southern network using the geographic proximity criterion because none of the possible solutions would have created tip haplotypes (making either of the other two criteria difficult to apply). The connection between haplotypes 8 and 18 was selected as the least plausible connection due to the fact that the localities of haplotypes 16–22 are all geographically much closer to each other than any of them are to the localities of haplotype 8 (Fig. 1). This loop was therefore broken, and the solid line joining haplotype 8 to a missing haplotype (solid circle in Fig. 3) was inferred as a more parsimonious connection for implementation of the nesting design.

The nested hierarchical design of these networks is shown in Figs. 3 and 4 (southern and northern groups, respectively). The numbers in these networks correspond to haplotypes, and the localities at which each of these haplotypes is found are shown in Table 1. Some significant distance values were found in all clade levels within the network of the

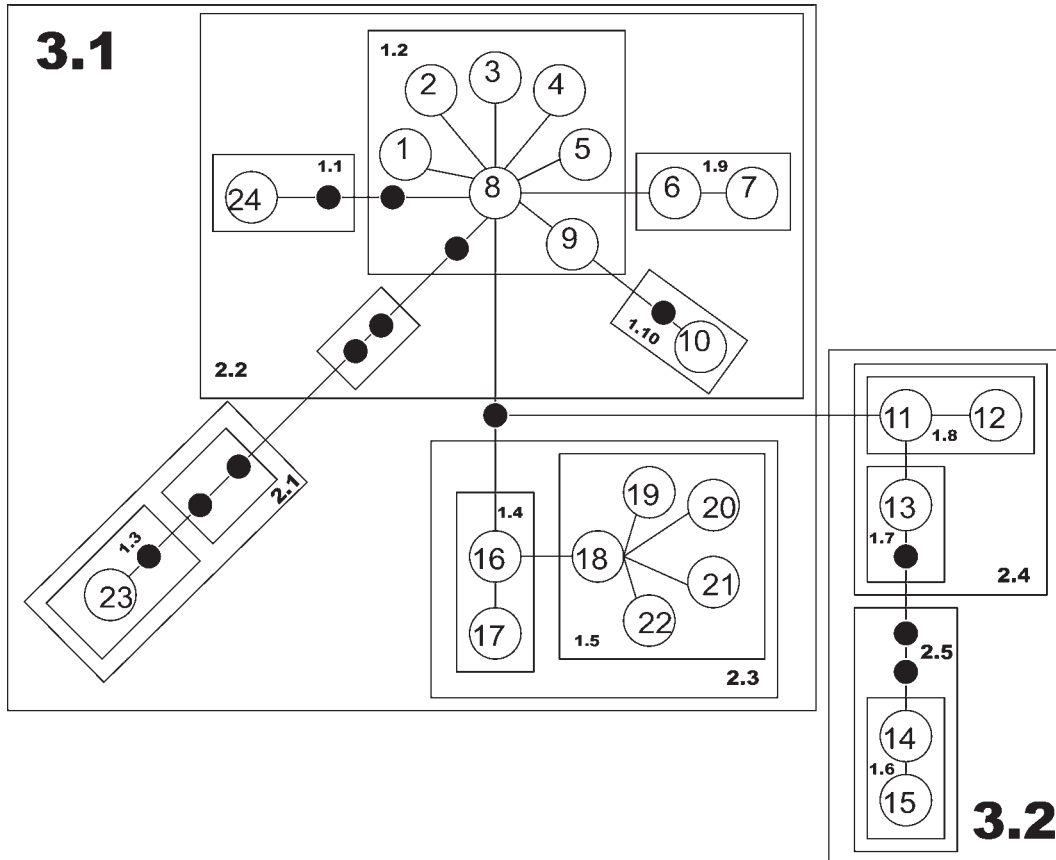


FIG. 3.—Unrooted haplotype network for the southern group with nesting design used for NCPA; the entire network is the estimated 95% probability of haplotype relationships based on the statistical parsimony algorithm implemented by TCS (Clement et al. 2000). Thin lines represent parsimonious connections between haplotypes corresponding to single mutational steps, and unsampled intermediate haplotypes are represented by solid circles. The dotted line indicates an alternative parsimonious connection (see text for the criterion used to break the loop). Haplotype numbers correspond to those shown in Table 1.

southern group (all of the NCPA results are summarized in Table 2). At the lowest nesting level (1-step clades), Templeton's (2004) inference key suggests that a history of restricted gene flow with dispersal by long distance colonization is most plausible for the genetic structure of clade 1-2, and that restricted gene flow with isolation by distance is most plausible for clade 1-8. In the next most inclusive level of 2-step clades, nested clade 2-2 had a significant distance value and a history of range expansion-contiguous range expansion is inferred for the genetic structure of haplotypes grouped into this clade. At the 3-step clade level, clade 3-1 is inferred to have the same cause, while the genetic structure of clade 3-2 is best explained

by a history of fragmentation/isolation by distance. At the total cladogram level, Templeton's key yielded an inference of range expansion-contiguous range expansion for the entire southern network.

Within the northern clade, which contained only three nesting levels, there were no significant distance values at the lowest level. At the second level, clade 2-1 resulted in a fragmentation/isolation by distance inference, and the total cladogram had the same outcome.

Neutrality and Molecular Diversity

The results of the neutrality and diversity tests are shown in Table 3. Overall both Fu's

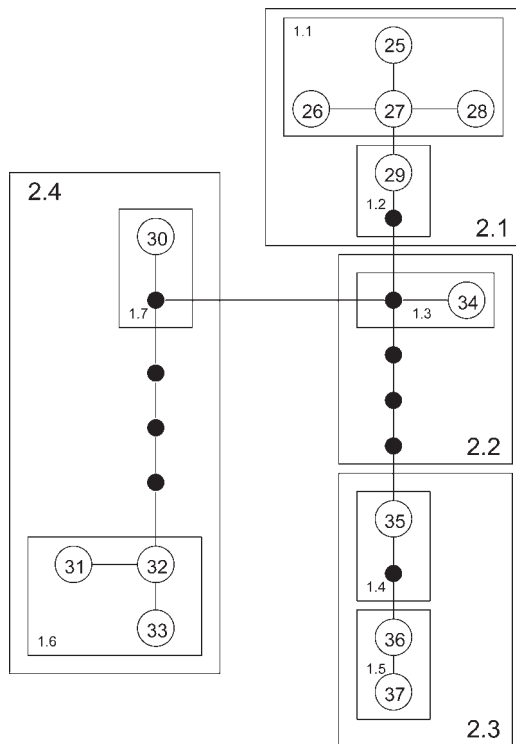


FIG. 4.—Unrooted haplotype network for the northern group with nesting design used for NCPA; symbols are as in Fig. 3, and haplotype numbers correspond to those shown in Table 1.

(1997) *F_s* and Tajima's (1989) *D* were significant (*F_s* was highly significant), indicating either non-neutral evolution or recent population expansions for the southern group, with the exception of clade 3-2, which gave a nonsignificant result. The total clade resulted in an *F_s* value of -12.71 with $P < 0.001$, and a Tajima's *D* of -1.78 with

$P(D \text{ random} < D \text{ obs.}) = 0.027$ (Table 3). Conversely, the northern group did not yield significant total clade values for either analysis, although individually clade 2-1 did result in significant values for both analyses. Fu's *F_s* = -2.06 for the total clade, with $P = 0.191$, and Tajima's *D* = 0.54 with $P(D \text{ random} < D \text{ obs.}) = -0.298$. Nucleotide diversity also differed between these groups, with $0.93 \pm 0.52\%$ for the northern and $0.59 \pm 0.34\%$ for the southern groups, respectively.

The mismatch analysis yielded a unimodal curve (Fig. 5) for the southern group, with a $P(\text{SSDobs.})$ of 0.41 , a raggedness index of 0.03 , and a $P(\text{Ragobs.})$ of 0.39 . This result is consistent with the inference of a recent range expansion obtained from the NCPA analysis (Table 2). For the northern group, however, a bimodal curve (Fig. 5) resulted as well as a $P(\text{SSDobs.})$ of 0.01 , a raggedness index of 0.07 , and a $P(\text{Ragobs.})$ of 0.11 . Thus, for the northern group, the hypothesis of stable demographic history cannot be rejected in favor of a recent expansion. The AMOVA analysis revealed that 84.04% of the total variation was due to between-group (northern and southern) subdivision; 11.05% of the variation was distributed among populations within the two groups, and 4.91% was due to within-sample variation (Table 4). The average sequence variation between the northern and southern groups is 14.12% , and after considering the intragroup variation the corrected estimate is 13.28% .

DISCUSSION

As indicated by the results of our analyses, the *C. longicauda* complex contains a high

TABLE 2.—Summary of NCPA results for clades with one or more significant distance values; inferences were made following the steps in Templeton's (2004) most recent inference key.

| Clade | Chain of inference |
|-----------------------|--|
| Northern group | |
| Clade 2-1 | 1-2-3-4-9-10: Fragmentation/Isolation by distance |
| Total Cladogram | 1-2-3-4-9: PF-10: Fragmentation/Isolation by distance |
| Southern group | |
| Clade 1-2 | 1-2-3-5-6-7: Restricted gene flow/Dispersal with long distance dispersal |
| Clade 1-8 | 1-2-11-17-4: Restricted gene flow/Isolation by distance |
| Clade 2-2 | 1-2-11: Range expansion-12: Contiguous range expansion |
| Clade 3-1 | 1-2-11: Range expansion-12: Contiguous range expansion |
| Clade 3-2 | 1-2-3-4-9-10: Fragmentation/Isolation by distance |
| Total Cladogram | 1-2-11: Range expansion-12: Contiguous range expansion |

TABLE 3.—Results of gene and nucleotide diversity estimates and neutrality tests (n = number of individuals), ** indicates $P < 0.001$, * indicates $P < 0.02$, NS = $P > 0.05$.

| | n | Gene diversity | Nucleotide diversity (π)/% | Tajima's D | Fu's Fs |
|----------------|-----|------------------|----------------------------------|------------|-----------|
| Northern Clade | 25 | 0.87 \pm 0.05 | 0.93 \pm 0.52 | 0.54(NS) | -2.06(NS) |
| Clade 2-1 | 12 | 0.58 \pm 0.16 | 0.11 \pm 0.11 | -1.75* | -2.98** |
| Clade 2-2 | 1 | | | | |
| Clade 2-3 | 4 | 0.83 \pm 0.22 | 0.25 \pm 0.23 | -0.75(NS) | -0.29(NS) |
| Clade 2-4 | 8 | 0.64 \pm 0.18 | 0.30 \pm 0.002 | -1.67(NS) | -0.11(NS) |
| Southern Clade | 46 | 0.912 \pm 0.03 | 0.59 \pm 0.34 | -1.78* | -12.71** |
| Clade 3-1 | 38 | 0.88 \pm 0.04 | 0.42 \pm 0.26 | -2.11* | -11.58** |
| Clade 3-2 | 8 | 0.86 \pm 0.11 | 0.55 \pm 0.36 | 0.97(NS) | -0.12(NS) |

level of genetic substructuring. Namely, the populations in the northern part of the range are widely separated genetically from the more southern populations in the ND4 gene tree (Fig. 2). Geographically, the Famatina-Saňogasta Mountains (elevation up to 6000 m; shaded region in Fig. 1) mark the separation of the two groups and have likely been the barrier to gene flow. The recently described

C. tergoaevigatus is distributed approximately in our localities 3, 4, 5, from the northern group, and between 28 and 30 (locality 3 in the original description) that are recovered in the southern group. The split between the northern and southern clades was strongly supported in both ML and MP analyses, and this leads us to hypothesize that the two groups may represent different species. The northern group comprises almost all of the localities (except locality 3, which lies between our localities 28 and 30, south of the Famatina-Saňogasta mountains) of the recently described *C. tergoaevigatus*. Thus we can hypothesize that our northern group corresponds to this new species and that its distribution extends further north, an extension that will require testing with independent data.

Although there is no extensive morphological study of these species, individuals from the southernmost area of the southern group show a striped dorsal coloration pattern, while those from the northern group are typically not striped. However, individuals south of the Famatina-Saňogasta mountains (southern group) in southern La Rioja and San Juan provinces present both dorsal patterns and this was the main character used for the diagnosis of *C. tergoaevigatus*. All the other morphological characters used by Cabrera (2004) to distinguish between *C. longicauda* and *C. tergoaevigatus* are uninformative with larger sample sizes (L. J. Avila, unpublished data). The main problem with the individuals used as the holotype and paratypes of *C. tergoaevigatus* arise from the fact that all are from a pool of several localities, and in fact,

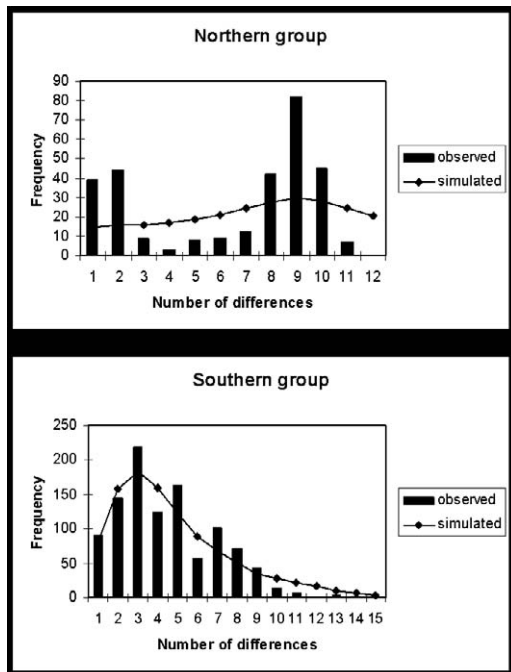


FIG. 5.—Mismatch distributions of pairwise number of mutational differences between individuals from the northern and southern groups. Black bars represent the observed data, and the bold curve is the model fitted to the data.

TABLE 4.—Results of Analysis of Molecular Variance (AMOVA), partitioned at three hierarchical levels.

| Source of variation | df | % variation | Φ statistic |
|---|----|-------------|--------------------|
| Between Northern and Southern groups | 1 | 84.04 | $\Phi_{ct} = 0.84$ |
| Among populations within Northern and Southern groups | 4 | 11.05 | $\Phi_{sc} = 0.69$ |
| Within populations | 65 | 4.91 | $\Phi_{st} = 0.95$ |

three of the lizards used represent four possible localities. These individuals were collected by the mammalogy expedition of J. Yepes and A. R. Zotta in 1933–4, and most probably they collected lizards and mixed all individuals from several localities. The Chilcito and Villa Union localities are small cities but Cerro Velasco and Cerro Famatina are actually large mountain ranges; other lizards (MACN 6827/32) from the same localities were not considered as paratypes but included as “Other referred material”. It is highly probable that no single individual of this sample came from locality three of Cabrera’s (2004) description, and that all individuals from this area are members of our southern clade. All the other specimens examined by this author are included in the geographic range of our northern clade.

The deep phylogenetic break between the northern and southern groups of *C. longicauda* is reflected in the 13–14% average sequence divergence between them. Although there is no calibration to relate this divergence to an absolute time, we can use the 1.3–2% per million year mtDNA sequence variation in reptiles (Macey et al., 1999; Zamudio and Greene, 1997) in a pairwise comparison to set this split in a crude time frame. This approximation dates the separation of the two clades at about 6.6–10.8 million years ago (Mya), in the middle Miocene. Between 9–10 Mya (Pascual et al., 1996), a marine transgression, the Paranaense Sea, isolated the Patagonian area from the northern part of Argentina in a SE-NW direction. A cladistic biogeographic analysis of 14 areas of endemism in southern South America (Flores and Roig-Juñent, 2001) using six insect genera from different families and one plant genus, found evidence for a vicariant event that split a northern area, including the northern Monte, from the central and southern Monte and Patagonian areas. These authors hypothesized that this vicariant event was driven by

transgression of the Paranaense Sea. This epicontinental sea could also have been the barrier that separated the northern and southern populations of the *Cnemidophorus longicauda* complex. As this sea retreated due to changing climates and orographic uplift, relictual populations in the north and south started expansions towards the newly formed great plains in Argentina between 11–3 Mya (Pascual and Bondesio, 1982).

The results obtained for within the northern group may be explained by the geography of the area. This area, the northern Monte Desert or Boreal Desert, is a unique biogeographic region, and recently separated from the Central and Southern Monte Deserts (Burkart et al., 1999). It is composed of several mountain chains presently connected by narrow, sandy valleys creating a terrestrial archipelago of high and low elevation xeric habitats and tectonic islands, thus shaping an ideal region for isolation and allopatric differentiation (Mares et al., 2000; Ojeda et al., 2001). Even very recently a new monotypic rodent genus was described from this area (Mares et al., 2000), and on the basis of diversity of four coleopteran families, the region has been proposed as the highest conservation priority within the Monte Desert (Roig-Juñent et al., 2001). This complex landscape is likely associated with the comparatively greater population structure of the northern group. Both NCPA inferences (fragmentation/isolation by distance) and the bimodal mismatch distribution are consistent with a recent history of demographic stability of these populations. Furthermore, although the sample size of the northern group is approximately half that of the southern group, it contains nearly twice the nucleotide diversity (Table 3). It is interesting to note that basal haplotypes of the northern clade are from the northwestern-most localities (Figs. 1, 2; localities. 32, 33), in the Rio Abaucan Valley. This valley is completely surrounded

by mountains exceeding 3000 m, except for a narrow pass in the northern Famatina Mountains; moreover, the endemic lizard *Liolaemus abaucan* was recently described for this area (Etheridge, 1993). We hypothesize that an ancestral population of *Cnemidophorus* was initially isolated in this region, and subsequently dispersed to extend the group distribution to the south and to the north. Towards the south, the populations encountered the Famatina-Saňogasta Mountains that most probably are a present day barrier to unrestrained dispersal.

All of the analyses of the southern group suggest that a history of relatively recent range expansion may best explain its pattern of genetic variation. In addition to the range expansion inferences made from NCPA, the *F_s*-test (Fu, 1997) gave significant results for this group. Since this test is particularly sensitive to range expansion, it adds confidence to the NCPA inferences, as do the results of the mismatch distribution analysis, which failed to reject the inference of range expansion. The basal haplotype from the southern clade is from locality 25 (Figs. 1, 2), which is geographically situated between the distributional areas of clades 2-2 and 2-3 (Fig. 3) for which contiguous range expansion was inferred. The distributional area of clade 2-3 includes almost all the localities south of locality 25 (except locality 12), a range that is entirely concordant with the distribution of *Liolaemus darwini* (*sensu stricto*) for which contiguous range expansion was also inferred (Morando et al., 2004). Within the southern group a separate fragmented or isolated by distance group is clade 3-2 (Fig. 1, localities 12, 24, 26, 29) with a North-South linear distribution west of locality 25. This basal area of the southern clade is in the southern San Juan Province where a great variety of dorsal patterns (striped, nonstriped, and all intermediate types) were observed (L. J. Avila, unpublished data). This observation supports the idea of an ancestral area from which populations expanded to the north (the region of mixed dorsal pattern morphs) up to the Famatina Mountains, and also towards the south (the smooth dorsal pattern morph).

Although the NCPA may not detect all range expansion events (Templeton, 1998), it

has also been shown to be conservative, and not likely to give false positives (Templeton, 2002). In addition, range expansion inferences have been made for other lizard species in this same region, and occupy adjacent ranges south and west of the *C. longicauda* complex (Morando et al., 2003). These studies collectively provide another cross-validation of the demographic inferences drawn here for recent expansion of southern *C. longicauda* populations, on the basis of shared demographic histories of a co-distributed regional fauna (Avice, 1992). The NCPA has proven useful for examining recent divergences and postulating processes that may have shaped the phylogeographic structure of a species and contributed to speciation, but it is now apparent that a combination of approaches is needed to confidently infer evolutionary processes and demographic histories of recently diverged species or haploclades. Follow-up studies will include other taxa and independent nuclear markers, and address these issues more critically within the framework of regional geological and Pleistocene climatic histories, and using newer statistical methods (Hickerson et al., 2006; Lapointe and Rissler, 2005) to test for similar responses to shared historical events.

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APPENDIX I

Sampling localities (numbering corresponds to Fig. 1), sample sizes, and museum voucher numbers for all lizards used in this study.

| Locality number | Location | Number of Individuals | Geographic coordinates |
|-----------------|---|----------------------------|---------------------------------|
| 1 | Ruta Provincial 47, 20 km S Punta de Balasto, Catamarca | 3 (LJAMM 4271/2/3) | 27°07'30.4" S, 66°13'03.4" W |
| 2 | Ruta Provincial 46, 11 km E Belén, Catamarca | 4 (LJAMM 4259/60/61/62) | 27°42'11.1" S, 66°56'07.7" W |
| 3 | 1 km S Bañado de los Pantanos, La Rioja | 1 (LJAMM 4181) | 28°21'54.5" S, 66°50'02.4" W |
| 4 | Ruta Nacional 40, Km 657, 9 km E Pituil, La Rioja | 2 (LJAMM 4153/4) | 28°32'09.1" S, 67°22'21.2" W |
| 5 | Anillaco, La Rioja | 1 (LJAMM 1917) | 28°49'00.0" S, 66°57'00.0" W |
| 6 | Ruinas de Capayán, La Rioja | 1 (LJAMM 5752) | 29°03'00.9" S, 67°26'39.3" W |
| 7 | 10 km E Anguinan, camino a Sierra del Velasco, La Rioja | 1 (LJAMM 4151) | 29°13'02.1" S, 67°21'02.6" W |
| 8 | Ruta Provincial 510, Km 88, 2 km E Baldecitos, La Rioja | 2 (LJAMM 4075/6) | 30°12'21.3" S, 67°40'26.4" W |
| 9 | Ruta Nacional 141, 7.6 km W Mascasin, La Rioja | 4 (LJAMM 5737/8/9/48) | 31°25'25.1" S, 67°02'50.9" W |
| 10 | Ruta Nacional 146, 10.6 km W La Horqueta, Mendoza | 2 (LJAMM 5731/2) | 34°04'46.4" S, 66°41'48.0" W |
| 11 | El Nihuil, Mendoza | 2 (LJAMM FN 409/10) | 35°03'43.2" S, 68°39'20.7" W |
| 12 | Ruta Provincial 10, 5.1 km E La Humada, La Pampa | 1 (LJAMM 4025) | 36°20'51.3" S, 67°57'04.2" W |
| 13 | Monte Hermoso, Buenos Aires | 1 (LJAMM 4463) | 38°59'13.7" S, 61°21'13.2" W |
| 14 | Ruta Provincial 34, 1 km W Ruta Provincial 11, orillas Río Colorado, La Pampa | 1 (LJAMM FN4) | 38°48'54.4" S, 64°56'24.9" W |
| 15 | Ruta Provincial 4, 84 km S Ruta Nacional 250, Río Negro | 1 (LJAMM FN23) | 40°06'20.2" S, 66°00'26.5" W |
| 16 | Ruta Provincial 4, Laguna del Indio Muerto, Río Negro | 1 (LJAMM FN29) | 40°24'08.5" S, 66°02'44.9" W |
| 17 | Villa Regina, Río Negro | 2 (LJAMM 4492, 5863) | 39°05'45.6" S, 67°03'05.2" W |
| 18 | Ruta Provincial 7, 28,7 km NW Añelo, Neuquén | 1 (LJAMM 5704) | 38°11'04.9" S, 69°01'22.5" W |
| 19 | 100 km W Gobernador Ayala, Mendoza | 1 (LJAMM 5097) | 37°15'46.0" S, 68°54'34.0" W |
| 20 | Ruta Provincial 180, 21 km S cruce El Clavado, Mendoza | 1 (LJAMM 5142) | 37°00'04.0" S, 69°05'18.0" W |
| 21 | Ruta Nacional 40, El Zampal, Mendoza | 1 (LJAMM 5715) | 36°30'45.6" S, 69°39'54.7" W |
| 22 | Ruta Nacional 40, 1.5 km N El Zampal, Mendoza | 1 (LJAMM 4068) | 36°29'44.7" S, 69°40'08.6" W |
| 23 | Ruta Nacional 40, Los Frisos, 5 km N El Zampal, Mendoza | 2 (LJAMM 4054/5) | 36°28'53.8" S, 69°38'52.5" W |
| 24 | Ruta Nacional 142, 18.2 km N Costa de Araujo, Mendoza | 3 (LJAMM 4068/9/70) | 32°36'57.2" S, 68°20'01.4" W |
| 25 | Ruta Provincial 20, 2.8 km W Encón, San Juan | 4 (LJAMM 4071/2/3/4) | 32°11'39.1" S, 67°49'12.2" W |
| 26 | Matagusanos, San Juan | 2 (LJAMM 2344/5) | 31°14'30.0" S, 68°38'59.0" W |
| 27 | Ruta Provincial 26, Km 139, La Rioja | 1 (LJAMM 1969) | 29°50'42.0" S, 67°57'57.0" W |

APPENDIX I
Continued.

| Locality number | Location | Number of Individuals | Geographic coordinates |
|--|--|-------------------------|---------------------------------|
| 28 | Ruta Nacional 76, Km 158, 18 km S Pagancillo, La Rioja | 4 (LJAMM 4061/2/3/4) | 29°41'45.1" S, 68°01'28.6" W |
| 29 | Ruta Nacional 40, 2 km S Guandacol, La Rioja | 3 (LJAMM 2305/6/7) | 29°34'59" S, 68°31'40" W |
| 30 | Ruta Nacional 76, Km 198, 9 km S Villa Unión, La Rioja | 1 (LJAMM 4066) | 29°23'00.7" S, 68°13'09.9" W |
| 31 | Ruta Nacional 76, 2 km N Villa Castelli, La Rioja | 1 (LJAMM 4065) | 29°00'20.2" S, 68°12'26.7" W |
| 32 | 10 km N Medanitos, camino a Tatón, Catamarca | 1 (LJAMM 2346) | 27°28'13" S, 67°35'13" W |
| 33 | Ruta Provincial 34, 16 km S Palo Blanco, Catamarca | 3 (LJAMM 2341/2/3) | 27°26'50" S, 67°40'49" W |
| 34 | Ruta Nacional 60, 2.1 km W Alpasinche, La Rioja | 3 (LJAMM 4263/4/5) | 28°17'49.0" S, 67°04'02.8" W |
| 35 | Ruta Provincial 43, Puerta de Corral Quemado, Catamarca | 2 (LJAMM 4279/80) | 27°14'44.0" S, 66°54'01.9" W |
| 36 | Ruta Nacional 40, 6 km W Punta de Balasto, Catamarca | 3 (LJAMM 4274/5/6) | 26°59'45.8" S, 66°11'40.1" W |
| Outgroup <i>Cnemidophorus</i> <i>lacertoides</i> | Bahía Blanca, Buenos Aires | (LJAMM 4477) | 38°04'04.6" S, 61°58'46.6" W |