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Congeneric phylogeography: hypothesizing species limits and evolutionary processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata: Liolaemini)

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In poorly known groups for which data are insufficient to develop biologically plausible model-based approaches to phylogeographical analyses, a 'first hypotheses' protocol is suggested as offering the best way to generate hypotheses for subsequent model-based tests. Preliminary hypotheses are formulated about species boundaries and population processes in three species complexes of the *Liolaemus boulengeri* group in the context of mtDNA 'congeneric phylogeography'. The temperate South American *Liolaemus* provides a model with ancient and recent allopatric divergence across ecologically and geologically complex landscapes, incipient speciation, secondary contact, and discordance between molecular and morphological patterns of variation. Moderately dense sampling of widely distributed 'inertial' species in the Patagonian Steppe has revealed hidden genetic and probably species diversity, and also hinted at demographic and historical processes that may have shaped the histories of these taxa. Five of the seven focal species of the present study were paraphyletic for mtDNA genealogies, suggesting that they represent complexes of species, and nested clade phylogeographical analysis (NCPA) analyses suggest that different historical and demographic processes have shaped the observed patterns. Introgression and incomplete lineage sorting are hypothesized as being the cause of some of the observed paraphyly. Provisional delimitations of species are proposed and NCPA is used to generate hypotheses of population history, all of which are subject to further testing. Multi-faceted studies, involving phylogenetic assessments of independent molecular markers and morphological variation across codistributed taxa with estimates of niche breadths in a landscape context, will likely yield the most promising returns for cross-validation of hypotheses of population and speciation histories.

ADDITIONAL KEYWORDS: Argentina – evolution – mitochondrial DNA – paraphyly – Patagonia – phylogeny – South America – speciation.

INTRODUCTION

Phylogeographical studies of single species or closely-related taxa focus on how evolutionary processes operate in natural populations (Avise, 2000), but the abundance of these studies in the literature belies the difficulties inherent in recovering complex demographic histories (Knowles, 2004). In animals, most studies have relied on the mtDNA locus to make inferences about population histories and demographic processes (Avise, 2004) and, although neither tree-based nor summary statistical methods are fully adequate (Hey & Machado, 2003), the limitations of single-gene trees are widely appreciated (Funk & Omland, 2003; Templeton, 2004).

One commonly used method of analysing mtDNA sequences for these kinds of studies is the nested clade analysis (NCA, Templeton, Routman & Phillips, 1995), which provides a statistical test of geographical population structure, and an inference key to suggest the most plausible biological causes for significant structure. A recent extension of the nested clade phylogeographical analysis (NCPA) now provides a formal statistical framework for cross-validation of single

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locus inferences via the use of multiple unlinked gene regions (Templeton, 2001, 2003). The original NCA has been criticized because it did not estimate any degree of confidence for a particular causal inference, nor did it distinguish statistically among alternative interpretations (Knowles & Maddison, 2002). Limited simulations suggest that the NCA inference key may frequently be misled (Knowles & Maddison, 2002; but see also Templeton, 2004) and, although this is not the only population structure estimator susceptible to such error (Abdo, Crandall & Joyce, 2004), the widespread use of the NCA for single gene trees does offer the possibility for over-interpretation if appropriate caution is not applied (Knowles, 2004).

Further progress will obviously depend on the incorporation of unlinked nuclear markers into phylogeographical analyses (Rosenberg & Nordborg, 2002; Hey & Machado, 2003; Templeton, 2004), but a larger issue is the fundamental shift away from the traditional null hypothesis testing approach in ecology and evolutionary biology toward model selection, in which several competing hypotheses are evaluated simultaneously (Johnson & Omland, 2004). This is precisely the argument for the emergence of 'statistical phylogeography' (Knowles & Maddison, 2002). However, implementation of this paradigm will require that it successfully confront two major issues: (1) the stochasticity of coalescent histories of unlinked gene regions (Hudson & Turelli, 2003) and (2) the potentially complex and varied histories of species and populations (including migration, admixture, divergence in isolation or with gene flow, population bottlenecks and expansions; Knowles, 2004). Conducting such a study requires that an investigator: (1) collect data for multiple gene genealogies (Wakeley & Hey, 1998; Templeton, 2003); (2) specify a sufficient number of alternative historical hypotheses to approximate biologically reality, but not to offer so many alternatives that spurious findings become likely (Johnson & Omland, 2004); (3) make decisions about a model's complexity (complex models may incorporate more biologically meaningful parameters, but at the expense of requiring more data to distinguish among alternative hypotheses; Knowles, 2004); and (4) integrate external data, such as palaeoecological or bioclimatic information (for an example, see Hugall *et al*., 2002).

Although this is the ideal approach, several factors currently limit the widespread use of such a methodology in phylogeographical studies. First, independent nuclear genetic markers remain unavailable for many nonmodel organisms, and the upfront time and cost for their development may be nontrivial (Avise, 2004; Morin *et al*., 2004). Second, statistical phylogeographical models that both accurately represent the histories of populations or species (which may need to incorporate a wide array of potential processes), and in a manner in which alternative hypotheses can be distinguished statistically with available data, are not yet developed (Knowles, 2004: 4). Finally, in many poorly studied groups of organisms from poorly sampled regions, there may be little or no usable external data from which to develop plausible a priori hypotheses necessary for the statistical phylogeographical approach. In these cases, the NCPA 'first hypothesis' approach would seem a good place to begin, and the mtDNA locus often will have to be used at least as the 'first pass' marker to assess general patterns of population variation, and to formulate more specific hypotheses that can be further tested with other classes of markers. On the positive side, this locus should track recent population splits with higher fidelity than a single nuclear marker under many biologically plausible scenarios, and mtDNA phylogeographical analyses have become increasingly sophisticated as the limitations of the single-gene approach are better understood (Funk & Omland, 2003; Ballard & Whitlock, 2004; Hickerson & Cunningham, 2005). For example, the assumption of neutral evolution is now routinely tested, and cross-validation of NCPA inferences is possible by a number of independent criteria (Pfenninger & Posada, 2002; Masta, Laurent & Routman, 2003; Carstens *et al*., 2004; Morando *et al*., 2004).

Accurate species identifications are usually taken as a given in phylogeographical studies, and coalescent-based statistical phylogeographical methods assume accuracy of species trees (Edwards & Beerli, 2000) but, in poorly known groups, early species descriptions were often based on a limited number of characters, insufficient geographical sampling, and methods of analysis that would be judged inadequate by present-day standards. These groups present additional challenges to phylogeographical studies because they are characterized by the presence of 'inertial species' (Good, 1994); nominal taxa in which the 'species limits are set solely by historical precedence'. Such 'species' are propagated in the literature on the basis of recurrent citation of original names, whereas geographical variants are frequently given new names and misresolution of species boundaries continues. These species often have very large geographical ranges that are typically artefacts of inadequate taxonomy (homonymy; Gaston, 2003), and phylogeographical studies of such taxa can be severely compromised by the underrepresented biodiversity. In relatively recently evolved species in which allopatric isolation has been the chief cause of speciation, niche conservatism and morphological crypsis may also be widespread (Wiens, 2004a), further challenging empirical species delimitation and phylogeographical inference. Well-supported species delimitation in such groups will require many of the

same protocols, as will statistical phylogeography. Clear a priori criteria for testing species boundaries, appropriate geographical sampling, and multiple independent characters and analyses are required for resolution of these and related evolutionary questions (Sites & Marshall, 2003, 2004).

We have recently initiated studies to address some issues of species delimitation, phylogenetic relationships, and phylogeography in the species-rich South American lizard genus *Liolaemus*. This large genus contains at least 170 recognized species; it is characterized by a rapid rate of discovery of new species (Espinoza, Lobo & Cruz, 2000; Nuñez, Navarro & Veloso, 2000; Etheridge, 2001; Abdala, 2002, 2003; Martínez Oliver & Lobo, 2002; Avila, 2003; Avila, Pérez & Morando, 2003; Espinoza & Lobo, 2003; Etheridge & Christie, 2003; Pincheira-Donoso & Nuñez, 2003; Verrastro *et al*., 2003; Avila *et al*., 2004), and many recognized species have large distributions that extend over 1500 km along north–south axes through topographically diverse Andean or Patagonian landscapes. These landscapes are remote and poorly sampled for most nominal species, and the tectonic and climate history of the region has almost certainly fostered both extensive recent speciation and distributional shifts (e.g. in response to glacial cycles; Markgraf & McGlone, 2004). Therefore, most of the issues of unavailable nuclear gene markers, insufficient knowledge for development of biologically relevant a priori phylogeographical hypotheses, and inertial species, are likely to be manifested in *Liolaemus*. It is precisely in these cases that the NCPA is of greatest value, and two mtDNA phylogeographical studies on different groups have been completed to highlight this point (Morando *et al*., 2003, 2004).

In the first study, Morando *et al*. (2003) delimited 'candidate' species using recently proposed criteria by Wiens & Penkrot (2002) and Templeton (2001), and suggested that, for widely distributed poorly known taxa, the number of species of *Liolaemus* could possibly be two-fold that which is currently recognized. The second study hypothesized that introgression and incomplete lineage sorting probably contribute to the two observed patterns of mtDNA paraphyly in the *Liolaemus darwinii* species complex (Morando *et al*., 2004). Both studies presented phylogeographical hypotheses inferred from the mtDNA only, but in the context of recognized limitations of the single-locus approach (Wakeley & Hey, 1998; Irwin, 2002; Shaw, 2002; Templeton, 2003). In the present study, this earlier work is extended in the context of mtDNA 'congeneric phylogeography' (Funk & Omland, 2003) to formulate preliminary hypotheses about species boundaries and population processes in three species complexes of the *Liolaemus boulengeri* group.

THE FOCAL *LIOLAEMUS* SPECIES GROUPS

The southern temperate South America herpetofauna is dominated in species richness by lizards of the clade Liolaemini, which includes the genera *Liolaemus* (> 170 species), *Phymaturus* (> 16 species), and *Ctenoblepharys* (one species). The [(*Liolaemus* + *Phymaturus*) *Ctenoblepharys*] topology of this clade is well-supported by molecular and morphological data (Schulte, Valladares & Larson, 2003).

The present study focuses on the *boulengeri* group (= *boulengeri* series of Schulte *et al*., 2000), and emphasizes most of the species not included in the *wiegmannii* and *darwinii* groups [i.e. *Liolaemus boulengeri* Koslowsky, 1898; *Liolaemus canqueli* Cei, 1975; *Liolaemus cuyanus* Cei & Scolaro, 1980; *Liolaemus donosobarrosi* (Cei, 1874); *Liolaemus fitzingerii* (Dumeril & Bibron, 1837); *Liolaemus inacayali* Abdala, 2003; *Liolaemus melanops* Burmeister, 1888; *Liolaemus martorii* Abdala, 2003; *Liolaemus morenoi* Etheridge & Christie, 2003; *Liolaemus rothi* Koslowsky, 1898; *Liolaemus xanthoviridis* Cei & Scolaro, 1980; and several undescribed species now confused with *L. boulengeri*, *L cuyanus*, *L. rothi*, and *L. melanops*. Nomenclatural and taxonomic details are provided in Appendix 1]. Details on the taxonomic background of this complex are provided elsewhere (Cei, 1973a, b, 1975a, b, 1980, 1986, 1990, 1993, 1998; Cei & Scolaro, 1977a, b, 1980, 1983; Scolaro & Cei, 1977; Cei *et al.*, 1980; Scolaro, Cei & Arias-de-Reyna, 1985; Etheridge, 1993, 1995; Etheridge & Christie, 2003) as well as in Appendix 1.

MATERIAL AND METHODS

TAXON SAMPLING AND OUTGROUP CHOICE

Mitochondrial DNA sequence data were collected from a total of 293 lizards, of which 283 samples from 100 localities represented the majority of the named species of the *L. boulengeri* group. Populations of species originally recognized under the names *L. boulengeri* (here the *boulengeri* complex), *L. rothi* (the *rothi* complex), *L. cuyanus* (the *cuyanus complex*), *L. fitzingerii*, and *L. xanthoviridis* (the *fitzingerii* complex), *L. canqueli* and *L. melanops* (the *melanops* complex), were the 'focal species' (Wiens & Penkrot, 2002) of the present study. Samples were also included for *L. donosobarrosi* Cei 1974 (the *donosobarrosi* group) and *L. morenoi* Etheridge & Christie (2003) (the *melanops* complex); these groups are likely closely related to the above-listed groups, but among-group phylogenetic relationships are uncertain. Members of the *darwinii* group, *L. chacoensis* Shreve, 1948, the *wiegmannii* group, and *L. pseudoanomalus* Cei, 1981 were included as nonfocal species within the *boulengeri* group; whereas ten additional species were used as outgroups [nine representing *Liolaemus* species from

other groups (Etheridge, 1995; Schulte *et al*., 2000) and *Phymaturus indistinctus* (considered the sister genus of *Liolaemus*; Etheridge, 1995; Schulte *et al*., 2000)]. *Phymaturus indistinctus* Cei & Castro, 1973 was used as the universal outgroup, thus allowing the position of the nonfocal species of the *boulengeri* group and outgroup *Liolaemus* species to remain unconstrained in all phylogenetic reconstructions, with respect to the focal taxa.

The number of individuals sequenced per gene region (arranged by species complex) and distributional information for all individuals used in the present study are provided in Appendix 2. Divergence profiles were established for three mtDNA gene regions following Morando *et al*. (2003) and, as a result, more individuals were included for the cytochrome *b* region, followed by the ND4 and 12S fragments, in decreasing order of divergence. A subset of this total was also sequenced for two nuclear gene regions (see below) to better resolve deeper phylogenetic relationships within the *boulengeri* group. Voucher specimens are deposited in the LJAMM herpetological collection (Centro Nacional Patagónico CENPAT-CONICET, Puerto Madryn, Argentina); Fundación Miguel Lillo (FML; Tucumán, Argentina); M.L. Bean Life Science Museum, Brigham Young University (BYU); Museo de La Plata (MLP.S; La Plata, Argentina); and San Diego State University (SDSU). Museum numbers of all voucher specimens are listed by locality in Appendix 3, and museum acronyms are used in accordance with Leviton *et al*. (1985).

Laboratory procedures

Protocols for DNA extraction, mtDNA primer descriptions, polymerase chain reaction (PCR), and sequencing procedures follow Morando *et al*. (2003) for the mtDNA cytochrome *b*, ND4, and 12S gene regions. After preliminary phylogenetic reconstructions identified the most inclusive well-supported clades, one individual of each group/complex was used to sequence two nuclear genes regions (c-mos and gapdh). Primers G73 and G78 (Saint *et al*., 1998) were used for c-mos under PCR conditions: 93 °C for 3 min (94 °C for 1 min; 52 °C for 1 min; 72 °C for 1 min) × 40–75 °C for 5 min to obtain a fragment of 509 bp. Amplification of 303 bp of the gapdh gene region used primers GAPDH-H and GAPDH-L (Friesen *et al*. 1997) under PCR conditions: 96 °C for 3 min (94 °C for 30 s; 54–56 °C for 30 s; 72 °C for 45 s) × 40–72 °C for 7 min. Most sequences were edited using the program Sequencher 3.1.1 (Gene Codes Corp. Inc.), and the protein-coding regions cytochrome *b*, ND4, and c-mos were translated into amino acids for confirmation of alignment. Alignment of the 12S region was performed with CLUSTAL X (Thompson *et al*., 1997), using the default settings for gap and mismatch penalties, with subsequent manual adjustments. Ten positions could not be aligned unambiguously and were deleted. Missing data were coded as '?'. Coding regions (cytochrome *b* and the first part of ND4) did not present stop codons or indels, and average base frequencies show strong bias against guanine on the light strand (cytochrome *b*: A = 0.28, C = 0.27, G = 0.14, T = 0.29; ND4: A = 0.33, C = 0.27, G = 0.12, T = 0.27; 12S: A = 0.20, C = 0.25, G = 0.19; T = 0.35). These features are characteristic of the mitochondrial genome but not nuclear-integrated copies of mtDNA genes (Macey *et al*., 1997).

Phylogenetic analyses

Single gene regions

Only nonredundant cytochrome *b* haplotypes (selected with the program Collapse version 1.1; http://bioag.byu.edu/zoology/crandall_lab/programs.htm), were used for a Bayesian analysis (156 haplotypes, 464 bp for all individuals without missing data, the fastest evolving region of the three sequenced), to test for exclusivity of haplotypes at each locality. The Bayesian analyses were run twice using MrBayes 2.0 (Huelsenbeck & Ronquist, 2001) based on the model of evolution GTR + I + Γ (Yang, 1994; Gu, Fu & Li, 1995). A priori, the specific parameter values were uniform and were estimated as part of the analysis. To more thoroughly explore the parameter space, Metropolis-Coupled Markov Chain Monte Carlo simulations were also run with four incrementally heated chains, using the default values. From a random starting tree, 1.5×10^6 generations were run, and the Markov chains were sampled at intervals of 100 generations to obtain 15 000 sample points. Stationarity was estimated (to discard the 'burn-in' samples) by plotting the log-likelihood scores of sample points against generation time; stationarity was assumed when the values reached a stable equilibrium (between 170 000 and 450 000 generations). The equilibrium samples (the 13 300 and 10 500 trees retained after burn-in) were used to generate a 50% majority rule consensus tree. Two additional separate analyses were conducted on the ND4 and 12S regions to examine phylogenetic congruence between these and the cytochrome *b* haplotype tree (Leaché & Reeder, 2002). All were performed only under a Bayesian framework (see below) to detect potential areas of incongruence. Again, the GTR + I + Γ model (Yang, 1994; Gu *et al*., 1995) was used; the ND4 and 12S sequences reached stationarity before 100 000 and 250 000 generations, respectively.

Combined gene regions

Results of the cytochrome *b* exclusivity analysis were used to select a subgroup of 130 terminals for which

combined analyses for the three mitochondrial gene regions were performed. This combined data set (2255 bp) was used to estimate phylogenetic relationships under maximum likelihood (ML), maximum parsimony (MP), and Bayesian methods.

For MP analysis, all characters were equally weighted, and a heuristic search was conducted with 100 replicates of random addition with Tree Bisection and Reconnection (TBR) branch-swapping, and gaps coded as missing data, using PAUP*, version 4.0b4b (Swofford, 2001). A nonparametric bootstrap analysis (Felsenstein, 1985) with 10 000 replicates (hsearch nreps = 5), was performed to obtain the MP bootstrap proportions in the BYU supercomputer facility.

For ML analysis, the combined data set was analysed under the general time reversible model with proportion of invariable sites with a discrete gamma distribution (GTR + I + Γ, Yang, 1994), which was selected as the best fit model of nucleotide substitution using Modeltest, version 3.04 (Posada & Crandall, 1998). A heuristic search with five replicates using the TBR branch-swapping algorithm was performed to obtain the ML tree. A nonparametric bootstrap analysis with 100 replicates (maxtrees = 1000, addseq = random, nreps = 1, timelimit = 5) was performed to obtain the bootstrap proportions for the ML tree (all ML analyses were also performed in the BYU supercomputer facility).

Using Mr Bayes 2.0 (Huelsenbeck & Ronquist, 2001) with the same model, specific parameter values were estimated as part of the analysis for 2×10^6 generations, with four incrementally-heated chains, and sampled at intervals of 100 generations to include 20 000 data points. Stationarity was reach before 40 000 generations and, after discarding these first 400 trees (burn-in), the 50% majority rule tree was obtained from the remaining 9600 data points. To avoid local entrapment, two independent analyses were ran and compared for convergence to similar log-likelihood mean values (Huelsenbeck & Bollback, 2001; Leaché & Reeder, 2002). The posterior probabilities were also compared for individual clades obtained from the separate analyses for congruence to ensure convergence of the two analyses.

Because the deepest splits in the mtDNA trees were not resolved with strong support, the analyses was further extended on a reduced data set of single individuals representing each strongly supported group (bootstrap > 95%, Bayesian posterior probability 0.9–1.0, except for the *donosobarrosi* group which has 83–86% ML and MP bootstrap values), by including the nuclear gene regions (c-mos, gapdh). Separate Bayesian analyses were performed on the nuclear genes for 2×10^6 generations using the HKY + Γ model of evolution (Hasegawa, Kishino & Yano, 1985); stationarity was reach before 4000 generations and no incongruences were found. The combined data set of 3287 bp was used for phylogenetic analyses under Bayesian, MP, and ML criteria. All characters were equally weighted for MP, and searches were conducted via the branch-and-bound algorithm (gaps coded as a fifth character), and bootstrap values calculated using 10 000 pseudoreplicates. The ML analysis of the combined data was again based on the GTR + I + Γ model, with a heuristic search of 50 replicates, TBR branch-swapping, and 100 replicates to obtain bootstrap values. The Bayesian analysis also used the GTR + I + Γ model in two independent runs of 1.5×10^6 generations, and sampling every 500 generations.

Statistical parsimony and nested clade phylogeographical analyses

Geographic sampling was deemed adequate in three groups of focal species to use nontree based methods for population inferences. Statistical parsimony was used to construct haplotype networks for cytochrome *b* sequences (584, 550, and 557 bp for the *fitzingerii*, *melanops*, and *donosobarrosi* groups, respectively) with the program TCS, version 1.06 (Clement, Posada & Crandall, 2000; http://bioag.byu.edu/zoology/crandall_lab/programs.htm), and nesting categories were assigned following Templeton *et al*. (1995) and Templeton & Sing (1993). The networks were then used for the NCPA, as implemented with the GeoDis program, version 2.0 (Posada, Crandall & Templeton, 2000; http://bioag.byu.edu/zoology/crandall_lab/programs.htm). All statistical analyses were performed using 10 000 Monte Carlo replications and ambiguous connections (loops) in the networks were resolved using approaches from coalescent theory (Crandall & Templeton, 1993; Crandall, Templeton & Sing, 1994). Statistically significant associations (haplotypes with geography) were interpreted following the revised inference key of Templeton (2004; http://bioag.byu.edu/zoology/crandall_lab/programs.htm).

To detect secondary contact between lineages for which previous fragmentation was inferred, Templeton (2001) proposed an extension of the NCPA. To implement this extension, first the average population clade distance (APCD) is calculated with GeoDis; this distance measures the average clade distance from the geographical centre for the involved haplotypes or clades found in each population and each nesting level. Second, the statistical significance of these measures is evaluated with 10 000 random permutations of clades against sampling locality. In panmictic populations, all haplotypes and clades should have the same geographical centre, and this distance is expected to be the same for all populations. Under isolation by distance, the lower clade levels are expected to have small positive average population clade dis-

tances that approach zero with increasingly inclusive clade levels. However, if haplotypes from previously fragmented clades are now united in a single population, the average population clade distance is expected to remain high or even increase with clade level, until a maximum is reached at the clade level where the fragmentation was inferred (Templeton, 2001; Pfenninger & Posada, 2002).

Congeneric phylogeography, hypothesis testing, and cross-validation of NCPA inferences

Animal mtDNA phylogenetic and phylogeographical studies reveal that the nonmonophyly (paraphyly/polyphyly) of mtDNA gene trees is often well supported, taxonomically widespread, and usually more common than previously appreciated (Funk & Omland, 2003). Patterns of nonmonophyly may reflect aspects of allele history that provides important insights into species biology, and Funk & Omland (2003) listed four biological causes that could produce such patterns.

First, inadequate phylogenetic information may result of weak signal in the data (i.e. they provide too few synapomorphies to recover a robust tree, and/or misleading homoplasies in a few sites can confound the few variable sites). At shallow levels of divergence, an attempt was made in the present study to obtain sufficiently dense population sampling to implement NCPA and other methods and, at deeper levels of divergence, nuclear genes were included to improve resolution of, as well as support for, older phylogenetic relationships.

Second, inaccurate species limits; when the taxonomic circumscription of the nominal species fails to correspond with the patterns of gene flow, the misidentification of inter- or intraspecific variation can lead to over- or under-resolution of true species boundaries, which will seriously compromise all other evolutionary inferences (Sites & Marshall, 2003, 2004). Examples of both were discovered in an earlier study of the *L. elongatus–kriegi* complex (Morando *et al*., 2003), and the same approach is used here to delimit 'candidate' species.

Third, interspecific hybridization is common in animals and often leaves mtDNA alleles of one species introgressed into the gene pool of another. This phenomenon was recently hypothesized for the Liolaemus darwinii complex (Morando *et al*., 2004) and, here, morphological observations were used to contrast with molecular results, as well as a recent extension of the NCPA to detect secondary contact (Templeton, 2001, 2004).

Fourth, incomplete lineage sorting, is expected but more difficult to demonstrate conclusively as a source of nonmonophyly of mtDNA alleles for several reasons (Funk & Omland, 2003). Nevertheless, a combination of methods can provide stronger support for an inference drawn from any single method, and narrow the range of plausible hypotheses about mechanisms and processes of divergence (Funk & Omland, 2003; Hickerson & Cunningham, 2005).

For each of the three complexes studied in detail here, this approach was adopted and an attempt made to reject or reduce the number of plausible alternative hypotheses to the extent that is statistically or qualitatively feasible. Because the NCPA has been criticized for limitations in a number of contexts (Knowles & Maddison, 2002; but see Templeton, 2004), inferences tied to population growth (including dispersal or range expansions) were cross-validated by statistical tests based on completely different assumptions (Masta *et al*., 2003; Morando *et al*., 2004). First, the neutrality tests of McDonald & Kreitman (1991), *D*-test (Tajima, 1989), and Fu (1997) were implemented, and then inferences about the demographic histories were further tested by mismatch analyses (with pairwise distances) and the 'raggedness' index (Harpending, 1994). Population structure was estimated by performing analyses of molecular variance (AMOVA; Excoffier, 2001), and calculating the corrected average pairwise genetic distances (taking into account the intrapopulation mean divergences of the two groups being compared) using the Tamura & Nei (1994) model of evolution, for the three complexes recovered in the phylogenetic analyses and for which NCPAs were implemented. Gene diversity (Nei, 1987: 180) and nucleotide diversity (π, the mean of pairwise sequence differences, Nei, 1987: 257) were also estimated for these complexes. The nucleotide diversity, population structure, and neutrality test analyses were performed with the software ARLEQUIN, version 2.001 (Schneider, Roessli & Excoffier, 2000), and the M-K-test was implemented in the program DNASP (Rozas & Rozas, 1999). These tests are presented and interpreted in the context of the caveats described by Morando *et al*. (2004).

RESULTS

Phylogenetic analysis

Sequences were deposited in GenBank under accession numbers AY173871, AY173791, AY173800, AY173721, AY367852, AY367875, AY367821, AY367823, AY367826/7, AY367854, AY367855, AY367878, AY367880, AY367882, AY367883, AY367792, AY367794, AY367796, AY367797, AY367849, AY367851, AY389288, AY389247, and DQ237286–237846. Two independent Bayesian runs performed for all individuals with the cytochrome *b*

partition yielded similar results, but haplotypes at several localities were not exclusive; some haplotypes were interdigitated with haplotypes from localities or other groups (data not shown). Thus, more than one individual was included per locality in these cases for the combined analyses.

Separate analyses of the three mtDNA gene partitions recovered some topological differences among terminals for the three complexes studied in detail with population genetic methods. The major discrepancies between the gene partitions were the position of *L. pseudoanomalus*, and the relationships of the *boulengeri* complex and *darwinii* group. These last groups are weakly supported by cytochrome *b* and ND4 [posterior probabilities (*PP*) = 0.73, 0.66] as sister taxa, but not by the 12S region. One other conflict was apparent; the cytochrome *b* tree recovered the *donosobarrosi* group as the sister taxon of the *fitzingerii* complex (*PP* = 0.78), whereas ND4 and 12S recovered the *melanops* complex as the sister taxon of *fitzingerii* (*PP* = 0.57, 0.97). Because all of these conflicts were weakly supported, we combined all three regions for all subsequent mtDNA analyses. The M-K-test was nonsignificant for the whole data set, and thus neutrality could not be rejected. Figure 1 presents the ML mtDNA tree, and shows the following major patterns. First, many groups previously recognized by earlier workers (i.e. the *montanus* section of Schulte *et al*., 2000) are recovered as well supported clades (most are identified by brackets in Fig. 1); but relationships among most of these are not well resolved. Exceptions include the basal position of the *chiliensis* group, and the successively nested positions of the *lineomaculatus* and *montanus* sections (Fig. 1).

Several species are recovered in a strongly supported clade that is recognize here as the *boulengeri* complex, including *L. boulengeri*, the recently described *L. inacayali* (Abdala, 2003), at least two undescribed species previously confused with *L. boulengeri* (*Liolaemus* sp. nov. 1 and 3; Fig. 1), and some populations that correspond to the name *L. rothi*. The mtDNA sequences recover a strongly supported *darwinii* group, and within group relationships are similar to those obtained by Schulte *et al*. (2000). At least two well-supported species complexes are included in this group, a *quilmes* complex that includes *Liolaemus quilmes* and several likely undescribed species, and a *darwinii* complex (Morando *et al*., 2004). The well-supported *rothi* complex includes *L. rothi* and an undescribed species (*Liolaemus* sp. nov. 4) from the slopes of Somuncura Plateau in central Patagonia. Another strongly supported clade is the (*fitzingerii* group + *donosobarrosi* group); the three terminals in this clade are strongly supported and represented by relatively dense population sampling, and thus these clades were selected for the more detailed congeneric phylogeographical studies described below. *Liolaemus pseudoanomalus* is modestly supported as the sister taxon of the *wiegmannii* group, and relationships within the *wiegmannii* group are similar to those hypothesized by Schulte *et al*. (2000). *Liolaemus wiegmannii* also appears to be a complex of several undescribed species and the name *wiegmannii* complex is used to designate this clade (Fig. 1).

To address the issue of inadequate phylogenetic resolution in weakly-resolved regions of Figure 1, ML analysis were performed on a reduced subset of taxa by including one representative of each of the clades provisionally named in Figure 1. The nuclear gene regions were added to this matrix and a slightly more resolved phylogeny was recovered (Fig. 2), but most of the among-clade relationships between the main groups remain unresolved. However, support for the deeper relationships of the [(*fitzingerii* + *melanops*) + *donosobarrosi*] clade are either unchanged or greatly improved at all measures of support (Fig. 2). The weak resolution of phylogenetic relationships at other nodes in Figure 2 does not significantly impact inferences made about species limits and population processes within the three focal groups.

Congeneric phylogeography: the fitzingerii group

Our mtDNA hypothesis recovers a strongly supported (*fitzingerii* complex + *melanops* complex) clade (Fig. 1), and this relationship is corroborated by the nuclear sequences (Fig. 2). Figure 3 presents the ML analysis of the *melanops* complex, and recovered two strongly supported groups that are designated as north and south clades. Statistical parsimony analysis links all haplotypes differing by a maximum of ten nucleotides and, with this criterion, two separate networks (corresponding to north and south clades in Fig. 3) were recovered that differed by 15 nucleotides (Fig. 4). The geographical relationships of the nested networks to haplotype distributions is shown in Figure 5. The south clade includes individuals from the type locality of *L. melanops* (Fig. 5, locality 20) and, within this clade, haplotypes corresponding to *L. canqueli* (localities 13–16) are recovered as a strongly supported as a monophyletic group (Fig. 3) and included in clade 2.1 in the NCPA (Fig. 4). Inferences from the NCPA (Table 1) suggest a general pattern of range expansion//continuous range expansion at the highest nesting level for the south clade. It was found that five individuals from locality 11 had haplotypes from the south clade, and three had haplotypes corresponding to the north clade, suggesting that individuals from these two clades are in sympatry in this locality (Fig. 5).

The north clade includes a recently described species (*Liolaemus morenoi*; Etheridge & Christie, 2003)

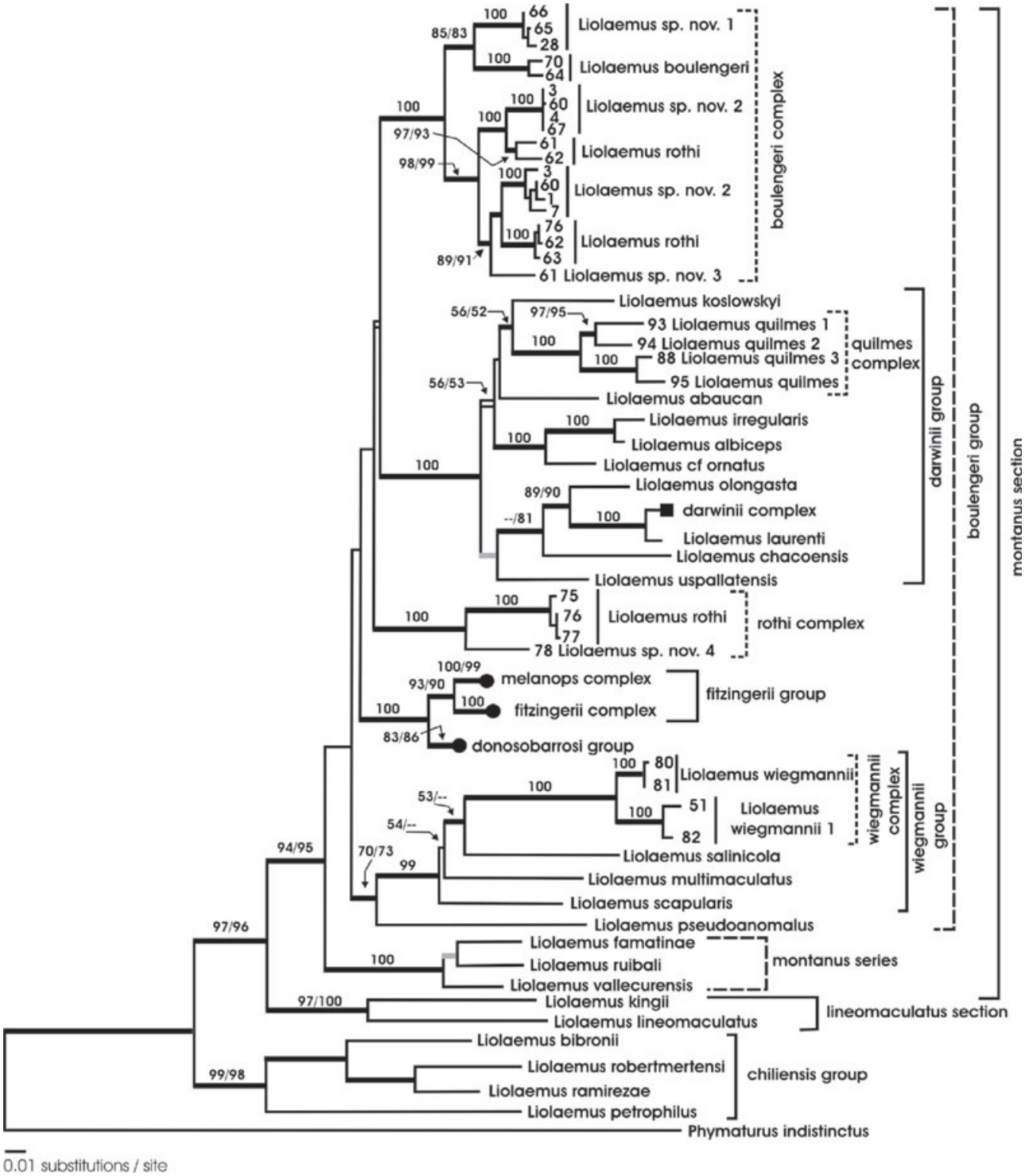

Figure 1. Maximum likelihood tree (–lnL = 27415.08895) for combined mtDNA gene regions of the main complexes and groups of the *Liolaemus boulengeri* series (the most inclusive dotted bracket) and nonfocal taxa used in this study. Numbers above selected branches represent likelihood and parsimony bootstrap values, respectively, and the thick black, grey, and white branches have Bayesian posterior probabilities = 0.9–1.0, 0.8–0.9, and 0.5–0.7, respectively. Numbers at some terminals correspond to localities listed in Appendix 2; solid circles indicate complexes/group that are shown in detail in subsequent figures, and the solid square identifies the *darwinii* complex studied in detail by Morando *et al*. (2004).

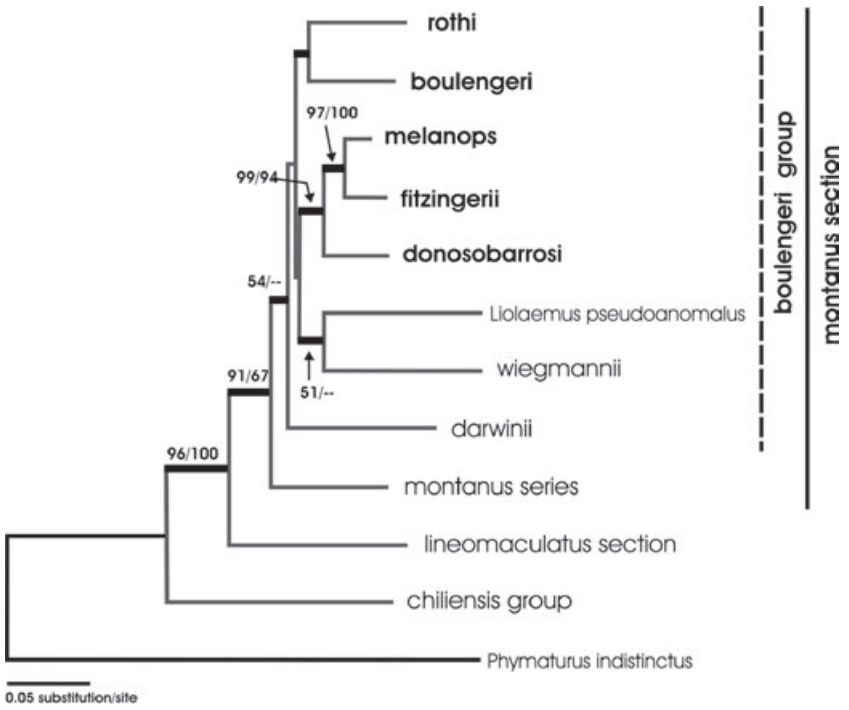

Figure 2. Maximum likelihood tree (–lnL = 13460.0925) for five gene regions (three mtDNA + two nuclear) for a subset of taxa representing all moderately to strongly supported groups recovered in the mtDNA tree (Fig. 1). Numbers above branches and thick black branches and solid circles and square are interpreted as indicated in Figure 1.

recovered as basal with ML (Fig. 3), but two individuals from locality 12 are recovered as basal with strong support in the Bayesian tree ($PP = 0.99$; not shown). Statistical parsimony recovers the *L. morenoi* haplotype 13 steps from haplotype 8 in clade 3-1 (Fig. 4), along with another recently described species (*L. martorii*; Abdala, 2003; but see Appendix 1) also included in this clade (Fig. 3, localities 9 and 10). No inference was possible for the entire north clade but, for less inclusive clades, patterns of range expansion (clade 2-1, RE-CRE), and restricted gene flow with isolation-by-distance (clade 3-1, RGF-ID) were inferred (Table 1).

Cross-validation for range expansion was evident in a significant value for Fu's test (*L. melanops* north and *melanops* complex, Table 2), and also in the mismatch distribution ($P_{SSD} = 0.77$) and a low value in the raggedness index ($Rag_I = 0.008$; $P = 0.83$). For the south clade, although none of the neutrality tests gave significant results, and the $P_{SSD} = 0.07$ is only marginally nonsignificant, the $Rag_I = 0.048$ ($P = 0.18$) is low, in general agreement with the NCPA range expansion inference. Results from the AMOVA show that the majority of genetic variance is distributed between the north and south clades (Table 3), for which the average corrected pairwise genetic distance is 2.72%.

Figure 6 presents the ML analysis of the *fitzingerii* complex: all the *L. fitzingerii* and *L. xanthoviridis* haplotypes are recovered as a strongly supported monophyletic group, and statistical parsimony links all haplotypes into a single network ($P < 0.05 = 10$ nucleotides; Fig. 7). Nested clades are plotted geographically in Figure 8. The type locality of *L. xanthoviridis* is between our localities 38 and 40 but, because haplotypes from these localities appear in different clades of the complex, none of these clades can be unambiguously named as *L. xanthoviridis*. However, individuals from locality 38 are more similar to the original description of *L. xanthoviridis* (Cei & Scolaro, 1980) but this species is very difficult to recognize because differences with *L. fitzingerii* are solely based on coloration and a poorly defined scale character.

The phylogenetic results obtained by all methods (MP, ML, and Bayesian) recovered very similar topologies. The most basal haplotype represents locality 38 (the northernmost locality for the distribution of this complex), and all other haplotypes are recovered in one of two large clades. The most strongly supported is clade 3–5 (Fig. 6), representing the north-east part of the distribution (Fig. 8, inset), and the second weakly supported clade includes three groups. The basal (clade 3-2) is strongly supported and is confined to the north-eastern part of the range of the complex (Fig. 8). This group includes individuals with a colour pattern corresponding to *L. xanthoviridis*. Clade 3-3 is strongly supported and is also confined to the

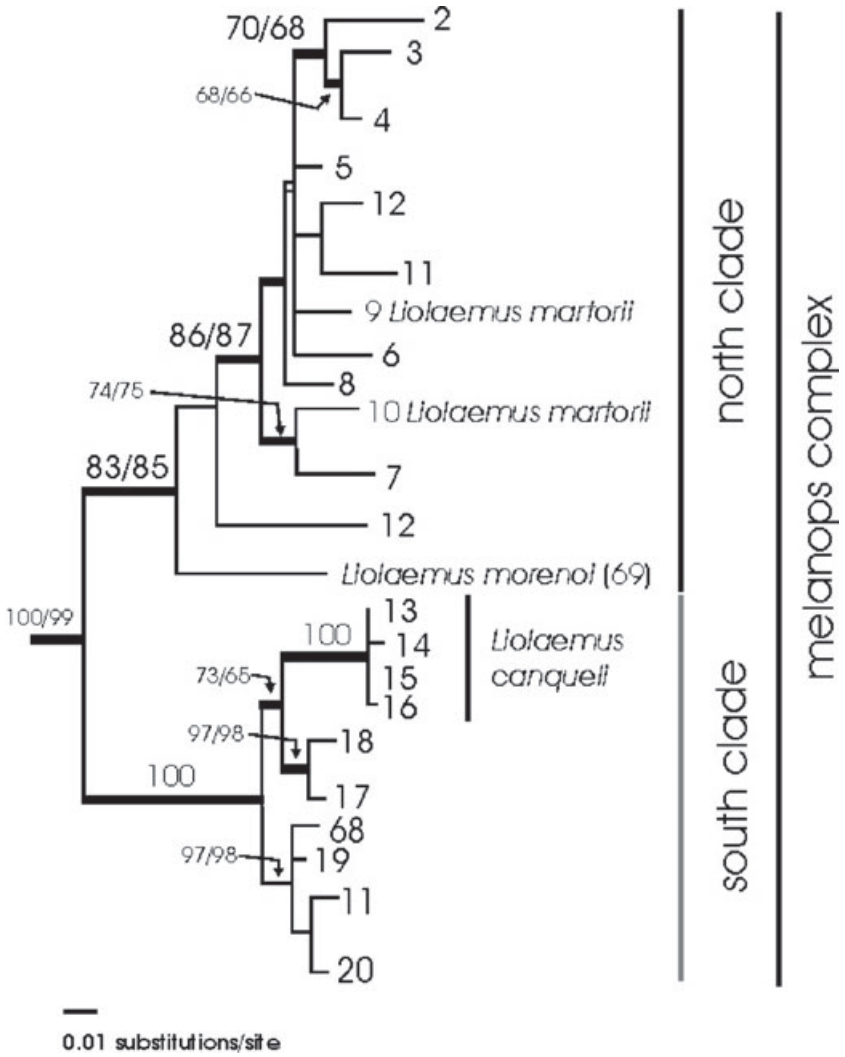

Figure 3. Maximum likelihood mtDNA tree for the *melanops* complex. Numbers above branches and thick black and white branches are interpreted as indicated in Figure 1. Terminal numbers correspond to localities in Appendix 2.

north-eastern part of the distribution (Fig. 8), and a well-defined clade 4-1 includes all haplotypes from a large area representing the southern part of the distribution (Fig. 8).

The statistical parsimony network recovered ambiguous connections (the three loops marked with an arrow in Fig. 7), which were resolved following the geographical criteria from coalescent theory (Crandall & Templeton, 1993). The NCPA led to an inference of allopatric fragmentation (AF, Table 1) for clade 2-1, but without power to reject the null hypotheses of random association. This caveat in the cytochrome *b* based inference led us to apply NCPA to the ND4 region and, for this clade, a long-distance colonization, possibly coupled with subsequent fragmentation or past fragmentation with range expansion, was inferred (Table 1). The same inference was obtained at the third level (clade 3-1) with both genes, and for clade 4-1. The recommendation following this inference in the new key (Templeton, 2004), is to perform a supplementary test for secondary contact (originally described by Templeton, 2001).

The extensive overlap of clades in the north-eastern range (Fig. 8, inset) suggests a history of colonization of the southern areas from the north-east area. The gene diversity is lower in the south, and the nucleotide diversity in clade 4-1 (south) is one quarter of that in clade 4-2 (north-east; Table 2); this pattern is consistent with expectations of a range expansion to the south, as is the significant Fu's test (Table 2). Results from the mismatch analyses also support a model of range expansion for the north-east and southern clades ($P_{SSD} = 0.12$, and $P_{SSD} = 0.59$), with low (0.027, 0.039) and significant values ($P = 0.27$ and 0.74) for

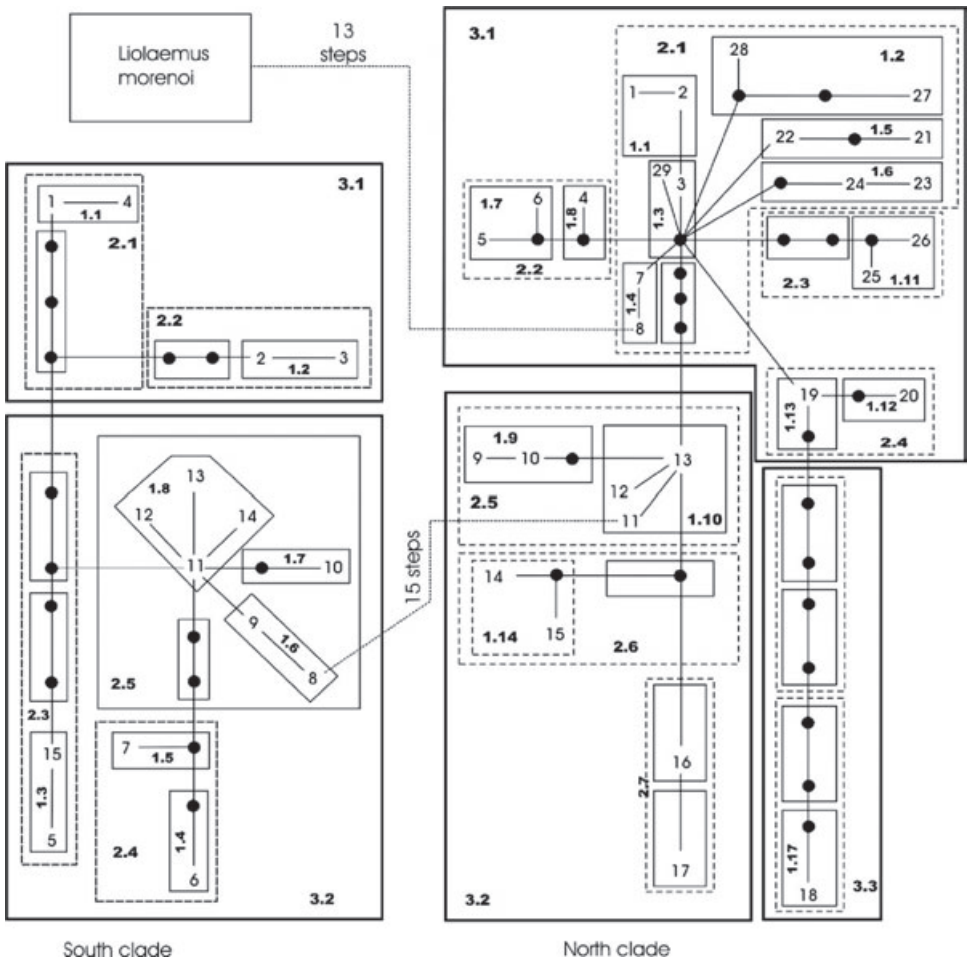

Figure 4. Unrooted cytochrome *b* haplotype networks for south and north clades of the *melanops* complex; haplotypes are designated by numbers (Appendix 3), black dots are intermediate haplotypes not present in the sample, and each line represents a single mutational step connecting two haplotypes. Clade numbers correspond to those shown in Figures 3, 5.

the raggedness index, respectively. AMOVA analyses (Table 3) show that the complex is highly structured at all levels. This deep genetic structure within the *fitzingerii* complex evident from the phylogenetic and NCPA results, together with the pattern observed in clades 2-1 and 4-1 (historical fragmentation between the south and the north-east followed by range expansion to the south and a secondary contact), led us to apply the NCPA test for secondary contact.

Figure 9 shows the results of this test; at the three-step clades, the average clade distance for locality 34 was significantly greater than expected under the assumption of panmixia. At the four-step clades, locality 41 shows a significantly greater distance than expected under panmixia. This locality is the one included in clade 4-1 for which a long-distance colonization event, possibly followed by a fragmentation, was inferred (Table 1). The minimum corrected genetic divergence between the south and the northern clades is 1.73% and, assuming a range of estimates for cytochrome *b* for other reptiles of 0.5–1.4% pairwise substitutions per million years (Giannasi, 1997; Zamudio & Green, 1997; Malhotra & Thorpe, 2000), the separation of these two groups is at least 1.2 Mya and as old as 3.5 Mya, but with caveats (Graur & Martin, 2004).

Congeneric phylogeography: the donosobarrosi group

This group includes three strongly supported clades (Fig. 10; localities 85–86, 98–99), the most basal of which includes at least two likely undescribed species from the Mendoza and La Pampa provinces (Fig. 11) previously referred to as *L. boulengeri* by Cei (1986). A second clade includes *L. donosobarrosi* and two other populations from the Neuquén province that likely represent a different species (Fig. 11). This clade is the sister taxon of the strongly supported *cuyanus* complex (Fig. 10).

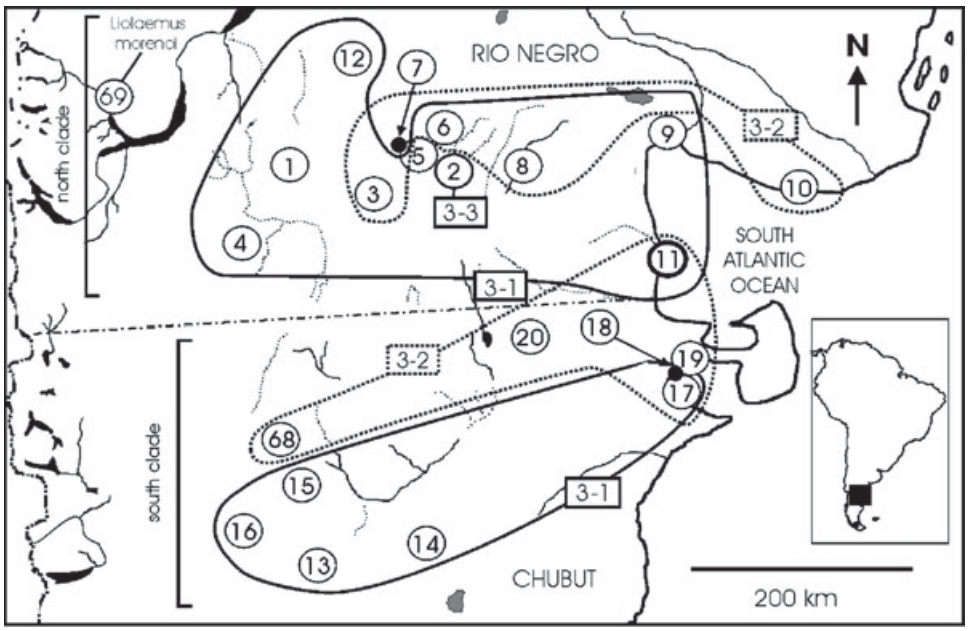

Figure 5. Distribution of haplotypes of the *melanops* complex, with the associated nesting design for some clades relating the haplotypes from these localities (Fig. 4); numbers correspond to localities in Appendix 2.

Table 1. Summary of nested clade phylogeographical analysis for clades showing statistically significant associations between haplotypes and geography

Clade nesting	Permutational χ^2 statistic	*P*	Chain of inference	Inference
melanops complex				
Clade 2-1 north clade	79.73	0.000	1-2-11-YES-12-NO	RE-CRE
Clade 3-1 north clade	47.23	0.013	1-19-20-2-11-17–4-NO	RGF with ID
Clade 3-2 south clade	16.41	0.072	1-2-3-4-NO	RGF with ID
Entire cladogram south	23.99	0.000	1-2-11-YES-12-NO	RE-CRE
fitzingerii complex				
Clade 1-11	3.00	0.336	1-19-20-2-11-17–4-NO	RGF with ID
Clade 2-1 cytochrome *b*	8.00	0.122	1-19-20-2-11-17-4-9-NO	AF
Clade 2-1 ND4	46.00	0.013	1-19-20-2-11-YES-12-YES-13-YES	LDCwPF or PFwRE*
Clade 3-1 (1)	39.00	0.000	–19-20-2-11-YES-13-YES	LDCwPF or PFwRE*
Clade 3-2 cytochrome *b*	10.00	0.020	1-19-20-2-3-5-15-16-18-NO	F or RE or ID
Clade 3-2 ND4	2.40	0.5†	1-19-20-2-11-17-4-9-10-NO	F/ID
Clade 4-1	36.05	0.001	1-2-11-YES-12-YES-13-YES	LDCwPF or PFwRE*
Clade 4-2	38.43	0.000	1-2-3-4-9-10-NO	AF†
Entire cladogram (1)	65–75	0.000	1-2-11-YES-12-NO	RE-CRE
cuyanus complex				
Clade 2-2	2.4	0.496	1-2-11-YES-12-NO	RE-CRE
Clade 3-2	63.84	0.000	1-19-20-11-YES-12-NO	RE-CRE
Entire cladogram	0.00	0.000	1-19-2-3-4-9–10-NO	F/ID

For some clades, the chi-square test was not significant (*fitzingerii* complex, clades 1-11, 2-1, 3-2, and *cuyanus* complex, clade 2-2), but clade and/or nested clade distances were statistically significant (not shown). RGF-ID, restricted gene flow with isolation-by-distance; RE-CRE, range expansion/continuous range expansion; AF, allopatric fragmentation; LDC-PF, long distance colonization with past fragmentation; PFwRE, past fragmentation with range expansion; F, fragmentation..
*LDC possibly coupled with subsequent Fragmentation or PF followed by RE. Secondary contact test and independent evidence for population growth.
†Localities 35 and 36 are mutationally connected to other clades by a larger than average number of steps (clade 1-8 and 2-4); this is additional evidence for an allopatric fragmentation.

Table 2. Sample sizes, estimates of gene and nucleotide diversity (π in percentage), and two different estimates of the paramenter θ (θ_π and θ_S) for different clades identified in the phylogenetic and nested clade phylogeographical analyses

	N	Gene diversity	Nucleotide diversity (π) %	θ_π	θ_S	Tajima's *D*	Fu's *P*
melanops complex	74	0.964 ± 0.012	2.450 ± 1.234	13.47 (6.78)	17.64 (4.83)	–0.79 NS	0.004
Liolaemus melanops north clade	42	0.959 ± 0.021	1.099 ± 0.593	6.045 (3.26)	11.38 (3.59)	–1.65*	0.0001
Liolaemus melanops south clade	32	0.802 ± 0.069	0.957 ± 0.528	5.266 (2.90)	6.952 (2.44)	–0.86 NS	NS
fitzingerii complex	69	0.895 ± 0.030	1.546 ± 0.798	9.030 (4.66)	9.575 (2.81)	–0.18 NS	0.07
Clade 4–1	44	0.759 ± 0.064	0.394 ± 0.244	2.305 (1.43)	3.908 (1.42)	–1.3 NS	0.002
Clade 4–2	25	0.936 ± 0.030	1.470 ± 0.378	8.590 (4.58)	7.945 (2.88)	0.30 NS	NS
Clade 3–2	10	0.777 ± 0.137	0.437 ± 0.289	2.555 (1.69)	4.241 (2.04)	–1.8*	NS
Clade 3–3	7	0.714 ± 0.180	0.146 ± 0.133	0.857 (0.78)	1.224 (0.83)	–1.35 NS	0.02
Clade 3–5	8	0.893 ± 0.085	1.051 ± 0.637	6.142 (3.72)	5.013 (2.49)	1.14 NS	NS
cuyanus complex	31	0.918 ± 0.035	1.895 ± 0.987	10.56 (5.50)	12.01 (3.99)	–0.4 NS	NS
Liolaemus cuyanus S	4	0.500 ± 0.265	1.167 ± 0.834	6.500 (4.65)	7.090 (4.15)	–0.84 NS	NS
Liolaemus cuyanus N	27	0.900 ± 0.045	1.378 ± 0.738	7.675 (4.11)	8.561 (3.03)	–0.38 NS	NS
Clade 3–1	6	0.800 ± 0.172	0.251 ± 0.205	1.400 (1.14)	1.314 (0.91)	0.33 NS	NS
Clade 3–2	21	0.847 ± 0.069	1.178 ± 0.647	6.561 (3.60)	7.504 (2.83)	–0.48 NS	NS

Standard errors for estimates are shown in parentheses. Tajima's *D* statistic with associated level of significance (*$P < 0.05$; NS, nonsignificant) and associated levels of significance for Fu's *F*-test.

Table 3. Analysis of variance among clades of the *melanops*, *fitzingerii*, and *cuyanus* complexes: percentage of the total variance that is explained by the different clade levels, and fixation indices (Φ)

Source of variation	d.f.	% variation	Φ statistic
melanops complex			
Among clades north and south	1	72.35%	
Within populations	72	27.65%	$\Phi_{ST} = 0.72$
fitzingerii complex			
Among clades 4–1 and 4–2	1	67.68%	
Within populations	67	32.32%	$\Phi_{ST} = 0.68$
Among clades 4–1 and subclades within 4–2	1	29.17%	$\Phi_{CT} = 0.29$
Among populations within groups	2	53.15%	$\Phi_{SC} = 0.75$
Within populations	65	17.68%	$\Phi_{ST} = 0.82$
cuyanus complex			
Among clades 3–1 and 3–2	1	51.96%	
Within populations	25	48.05%	$\Phi_{ST} = 0.52$

d.f. = degrees of freedom.

Two clades are recovered within the *cuyanus* complex (Fig. 10), one of which includes three more southern populations (Fig. 11, localities 44, 45, and 51), together with an individual from the northern part of the distribution (locality 53); this clade is refered to here as *L. cuyanus* south. Statistical parsimony analysis connected all haplotypes separated by ten or less nucleotide differences, but haplotypes from the '*L. cuyanus* south' clade were not interconnected with other networks from this complex (Fig. 12). The second clade was weakly supported and included all other haplotypes from the northern part of the distribution (*L. cuyanus* north); the majority of these are included in clade 3-2 (Fig. 12), for which a range expansion (Table 1, RE-CRE) was inferred. A history of fragmentation or isolation-by-distance was inferred for the entire network (Table 1).

DISCUSSION

In the absence of sufficient information to develop a biologically plausible model-based approach, it is sug-

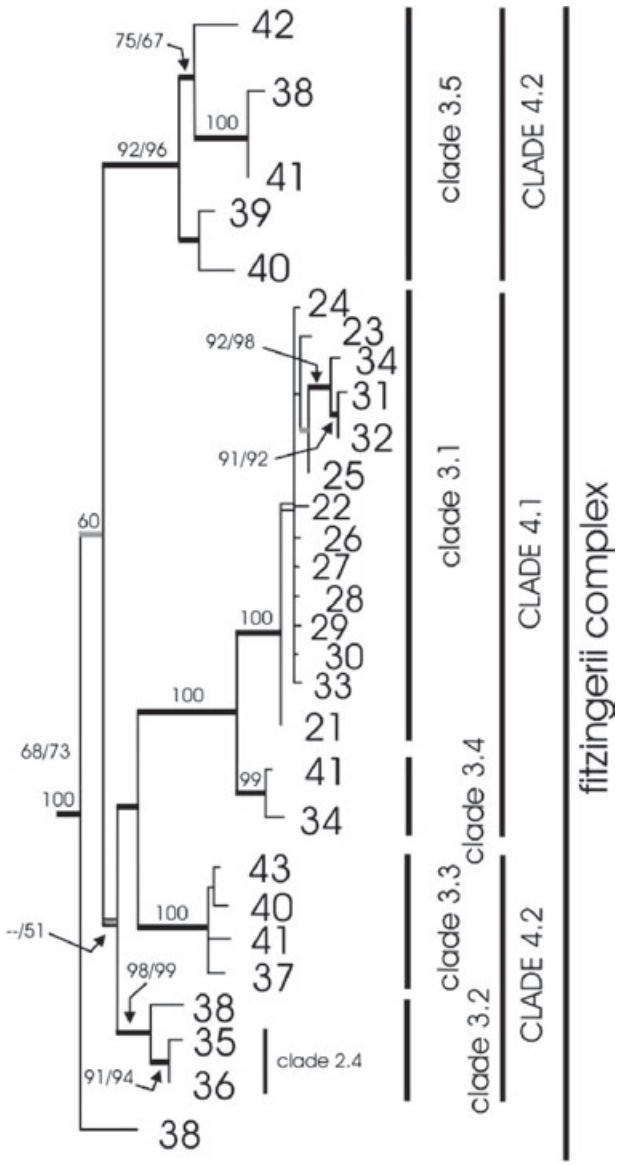

Figure 6. Maximum likelihood tree for the *fitzingerii* complex. Numbers above branches and thick black, grey, and white branches are interpreted as indicated in Figure 1; clade numbers correspond to those shown in Figures 7, 8.

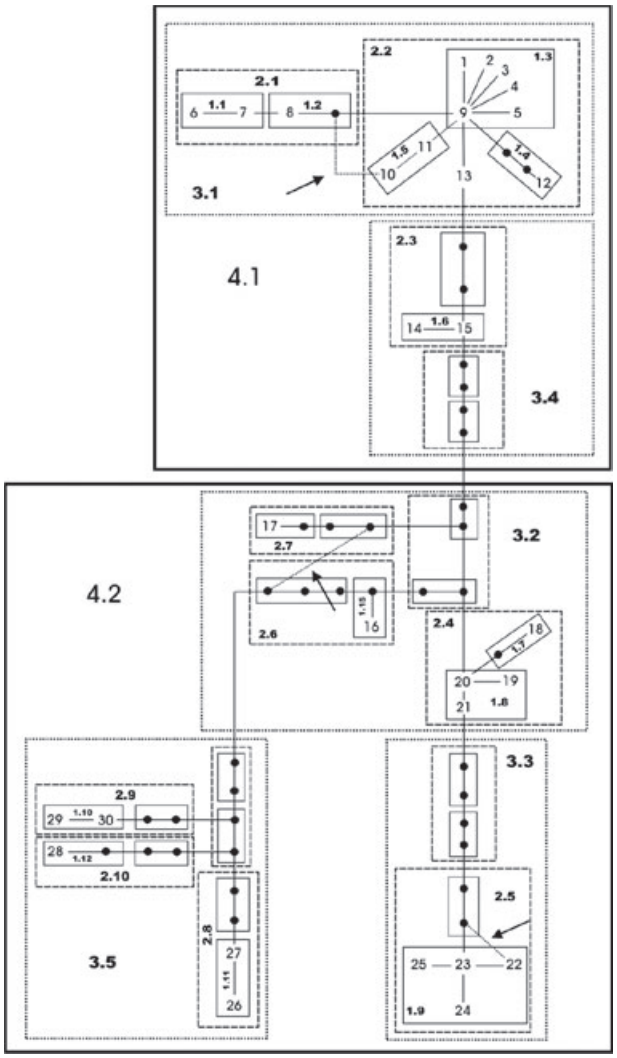

Figure 7. Unrooted cytochrome *b* haplotype networks for the *fitzingerii* complex; haplotypes are designated by numbers (Appendix 3), black dots are interpreted as indicated as in Figure 4, dotted lines and arrows identify ambiguous connections between haplotypes, and clade numbers correspond to those shown in Figures 6, 8.

gested that the 'first hypothesis' protocol offers the best way to generate hypotheses for subsequent model-based tests. Specifically, in widely distributed poorly known groups, this approach is a necessary first step for outlining more explicit hypotheses of species limits, phylogenetic relationships, and phylogeographical patterns, as long as limitations of mtDNA genealogies are recognized (Funk & Omland, 2003; Ballard & Whitlock, 2004). As in earlier papers (Morando *et al*., 2003, 2004), only 'candidate species' and phylogeographical hypotheses are identified here, pending more thorough investigation by multiple lines of evidence and other approaches.

Emerging hypotheses of species boundaries

The inertial species concept of Good (1994) is an appropriate metaphor for the focal species of this study; it is fully expected that a number of candidate species would be discovered in the broadly distributed nominal species (Morando *et al*., 2003). In several of the focal species included here (e.g. *L. boulengeri*, *L. fitzingerii*, *L. melanops*), extreme inter- and intrapopulational variation in morphology, coupled with

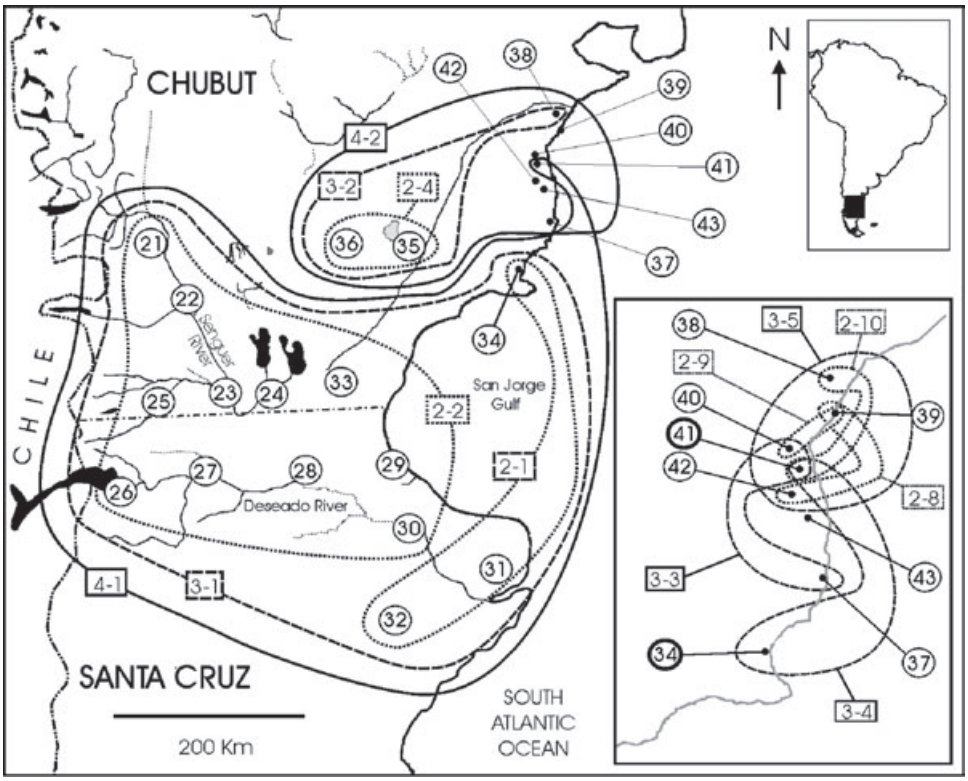

Figure 8. Distribution of haplotypes of the *fitzingerii* complex, with the associated nesting design for some clades relating the haplotypes from these localities (Fig. 7). Inset: detailed area of the north-eastern part of the distribution, with associated nested design. Localities 40 and 41 identify areas of sympatry between *Liolaemus xanthoviridis* and *Liolaemus fitzingerii*. Black dots represent the locality of *L. fitzingerii*, bold circles represent localities for which the secondary contact test was significant (34 and 41), and numbers correspond to the localities in Appendix 2.

sexual and ontogenetic variation, makes delimitation of species very difficult (Etheridge, 1992, 1993; L. J. Avila, unpubl. data).

Liolaemus boulengeri Koslowsky, 1898, was considered as a broadly distributed species characterized by geographical variation in morphology and coloration (Cei, 1986) between the northern populations in Mendoza province and the southernmost populations in the Santa Cruz province. This range spans an approximately linear distance of 1200 km, across an ecologically and topographically complex landscape, which should provide opportunities for allopatric or ecological speciation. The better studied *L. darwinii* complex, also initially considered as a single broadly distributed species with geographical variation in morphology and coloration, was found to be comprised of several species (Etheridge, 1992, 1993, 2001; Lobo & Kretzschmar, 1996; Cei & Scolaro, 1999); thus, it may be expected that a similar pattern could be found in *L. boulengeri*. In the present study, a north–south transect was sampled along the complete geographical range of *L. boulengeri*, and the mtDNA gene genealogy suggests that this 'species' may be a complex of as many as ten species with largely allopatric distributions (Fig. 1).

The hypothesized species diversity in the *L. boulengeri* complex precluded the use of NCPA for these samples; the best supported hypothesis here suggests that these entities do not form a monophyletic group (Fig. 1). For example, some very distinct terminals under the name *L. boulengeri* are recovered within the *L. darwinii* complex, to the exclusion of other *L. boulengeri* terminals (Morando *et al*., 2004; Fig. 2). Populations from localities 28, 66, and 65 (Figs 1, 2) appear to comprise a distinctive species that is the sister group of the true *L. boulengeri* (L. J. Avila, unpubl. data), represented in the sampling here by localities 64 and 70. These taxa are related to another clade that includes *L. inacayali*, and at least two undescribed species, well defined by morphological characteristics (here identified as *Liolaemus* sp. nov. 2 and 3; Fig. 1), and haplotypes from individuals of *L. rothi*.

In the *melanops* complex, *L. canqueli* is recovered as monophyletic with strong support, but this species as well as *L. morenoi* are nested within haplotypes of

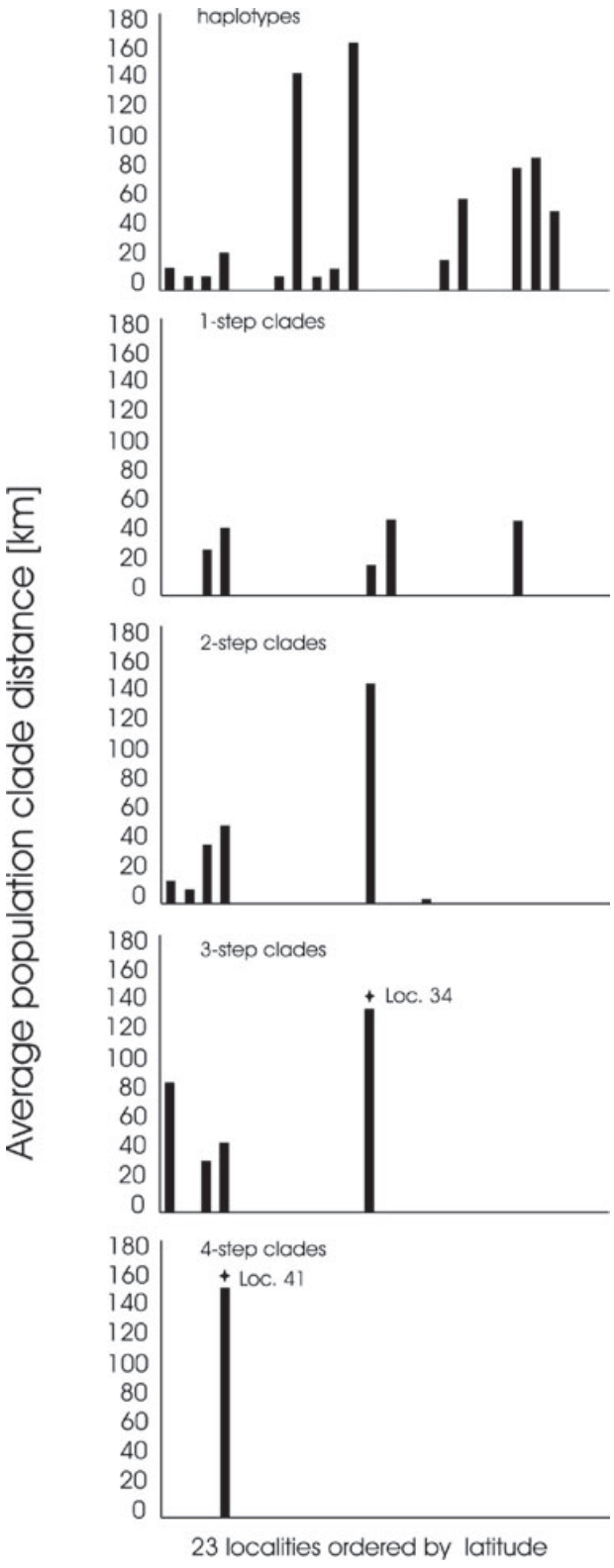

Figure 9. Average population clade distances (km) in the *fitzingerii* complex for all clade levels. Significantly large clade distances at the 1% level are marked by an asterisk followed by the locality number. Loc., locality.

L. melanops, rendering this last species paraphyletic (Fig. 3). Lizards from the north clade of the *melanops* complex occupy habitats ranging from typical austral Monte to ecotonal areas at the edges of the Somuncurá Plateau, to typical Patagonian Steppe in the western extreme of their range. Within this clade, occupying habitats of typical Patagonian Steppe, lizards from localities 1, 3, and 4 (the western range of the north clade; Fig. 3) are very distinctive morphologically, with robust and larger body sizes [snout–vent length (SVL) of up to 90 mm] and more scales around the midbody (65–87) than all other lizards from the northern clade (SVL ≤ 75 mm; midbody scales 59–68), and form a group well supported by the Bayesian analysis. One lizard from locality 2 (Fig. 5) has the same haplotype as lizards from localities 1, 3, and 4, but it is morphologically similar to other individuals from the eastern region of the north clade. Based on this pattern, it is hypothesized that lizards from these localities may represent a different species.

Lizards from localities 9, 10, and 11 (Fig. 5) inhabit coastal dunes and have some chromatic differences and are slightly smaller than lizards from localities with ecotonal characteristics in the central area of the distribution, but lizards from these dunes are not recovered in the same clade in the phylogenetic analyses, nor in the NCPA. In agreement with those observations, lizards from locality 9 and another site near our locality 10 were recently described as a new species: *L. martorii* (Abdala, 2003); but Cei & Scolaro (2003) revalidated the name *Liolaemus goestchi* for these populations, thus invalidating *L. martorii*. This nomenclatural issue aside, it is hypothesized that these costal populations constitute a different species. Different haplotypes from these coastal localities are related to haplotypes from the central area that include the ancestral haplotypes, and may represent yet another undescribed species.

In the south clade, lizards from localities 13–16 (Fig. 5) were recovered as a strongly supported group concordant with the morphologically distinctive species *L. canqueli*. Individuals from locality 19 are morphologically similar to those from localities 17–18, but these groups are recovered in different clades by both tree reconstruction and network methods (Figs 3, 4). There is not enough evidence to suggest species boundaries for these populations, but locality 20 is the type locality for *L. melanops*.

The northernmost of the two clades of the *fitzingerii* complex is confined to a relatively small area (Fig. 8) and is characterized by a set of genetically diverse and highly structured populations that are morphologically variable in colour pattern, whereas the southernmost clade occupies a large area and is characterized by genetically and morphologically homogeneous populations. The type locality of *L. fitzingerii* is from the

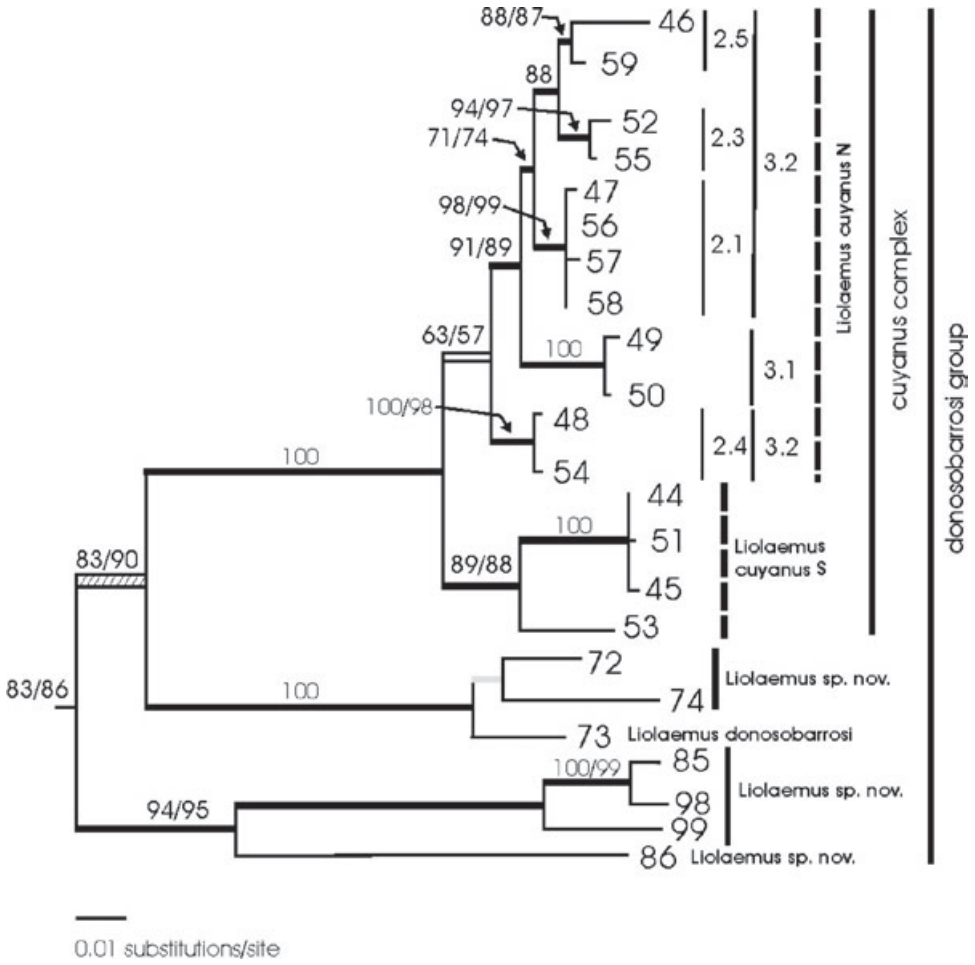

Figure 10. Maximum likelihood tree for the *donosobarrosi* group. Numbers above branches and thick black, grey, and white branches are interpreted as indicated in Figure 1; clade numbers correspond to those shown in Figures 11, 12.

southern area (Fig. 8, near locality 31) and, along the coast in the north-east, it occurs in sympatry with *L. xanthoviridis* (Cei, 1986; this region of sympatry includes localities 40–41 in Fig. 8). Individuals from this area are extremely variable in coloration; the original description of *L. xanthoviridis* was based on coloration; thus the sampled lizards cannot be unambiguously assigned to this species or to *L. fitzingerii*. The southern clade is recovered (with some support) as monophyletic by all three tree reconstruction methods (Fig. 6), but haplotypes from the geographical range of *L. xanthoviridis* are grouped in three separate clades, rendering *L. fitzingerii* paraphyletic. Individuals from localities 35 and 36 (Fig. 8, clade 3-2) have a different colour pattern, and may represent a different species (considered as *L. canqueli* by Cei, 1986), but *L. xanthoviridis* would still be paraphyletic with clade 3-5 and 3-3 overlapping in their distribution. A detailed study of the presumed area of sympatry between *L. fitzingerii* and *L. xanthoviridis* is needed to more accurately assess species limits in this relatively small area.

A strongly supported *cuyanus* complex within the *donosobarrosi* group (Fig. 10) includes two strongly supported clades; a northern group including the type locality of *L. cuyanus*, and a southern group that is geographically separated with the exception of one individual from the north (Fig. 11, locailty 53). Statistical parsimony criteria do not interconnect northern and southern haplotypes, and the individual from locality 53 is separated from both networks (Fig. 12). A geographical gap separates the northern and southern clades of *L. cuyanus* in the same manner as found in the *L. darwinii* complex (Morando *et al*., 2004). This gap could be a sampling artefact but, if real, probably reflects a shared vicariant history between these complexes. Although additional sampling is needed, it is hypothesized that the northern and southern clades of the *cuyanus* complex may represent different species.

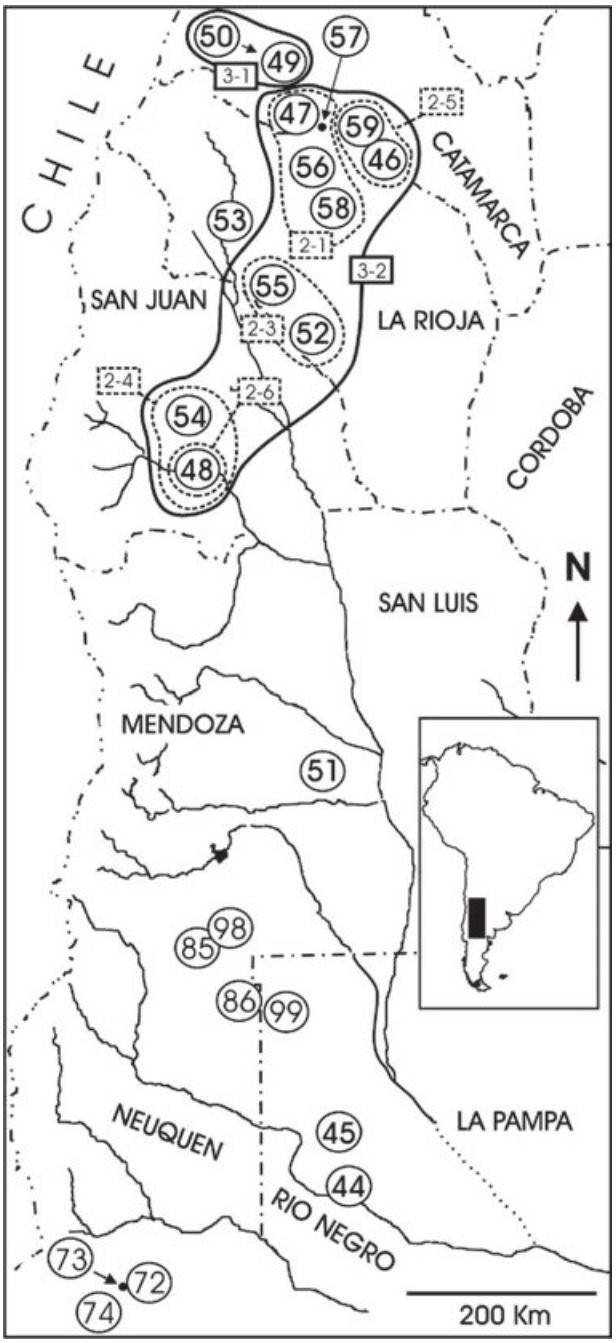

Figure 11. Distribution of haplotypes of the *donosobarrosi* group, with the associated nesting design for some clades relating the haplotypes from these localities (Fig. 12). The black dot represents the locality of *Liolaemus cuyanus*.

EMERGING HYPOTHESES ABOUT HISTORICAL/ DEMOGRAPHIC PROCESSES

The recovery of *L. boulengeri* haplotypes within the *L. darwinii* complex (Morando *et al*., 2004; Fig. 2) renders the former paraphyletic, and frequently cited demographic or evolutionary explanations for this pattern include incomplete lineage sorting and interspecific hybridization (Funk & Omland, 2003). Previous studies on other *Liolaemus* complexes suggest that both are very plausible explanations for some observed patterns (Morando *et al*., 2003, 2004). In the *boulengeri* clade in Figure 1, morphological characters unambiguously diagnose *L. rothi* as a distinct species relative to *L. inacayali*, and *Liolaemus* sp. 3, but *L. rothi* haplotypes are not recovered as monophyletic or as concordant with geography (Fig. 1). The localities from which haplotypes of *L. rothi* are recovered within the *boulengeri* complex (localities 61, 62) are recovered with haplotypes from *L. inacayali* (localities 3, 4, 60, 67) and, elsewhere, localities 62, 63, 76 (*L. rothi*), recovered with haplotypes from *L. inacayali* (localities 1, 3, 7, 60), are from the periphery of the geographical range of the *L. boulengeri* localities. The most plausible explanation for these observations is that mtDNA alleles from one species (*L. inacayali*) introgressed into *L. rothi*, either historically or possibly by ongoing interspecific hybridization. It is suggested that incomplete lineage sorting is not likely in this case because the *L. boulengeri* and *L. rothi* complexes are distantly related and phylogenetic evidence suggests their reciprocal monophyly. Mitochondrial DNA is thought to be more susceptible to introgression across species boundaries (Funk & Omland, 2003), but nuclear markers will be needed to further evaluate these alternatives in the *L. rothi* complex.

In the *melanops* complex, most of the ancestral haplotypes are found in ecotonal environments in localities 5 and 8 (Fig. 5), and the NCPA inference is that ancestral populations expanded from these areas to all other areas included in the sampling. Because many of these peripheral isolates have been described as distinct species (*L. martorii*, *L. morenoi*, *L. canqueli*), divergence in allopatry is inferred to be responsible for diversification in this group.

It is hypothesized that lizards from localities 9, 10, and 11 (*L. martorii*) constitute a distinct species. Although detailed morphological study and more dense sampling are needed, this pattern is consistent with a process of incomplete sorting of mtDNA lineages coupled with accelerated morphological divergence in this species. Morando *et al*. (2004) suggested that this process could explain some patterns of polyphyly observed in the *L. darwinii* group more parsimoniously than hybridization, on the basis of different geographical and phylogenetic corollaries of these processes. Funk & Omland (2003) caution that similar patterns can be generated by more than one process and that these often cannot be unambiguously distinguished with mtDNA alone.

The present distributions of these populations suggest that ongoing introgression or ancient hybridization is less likely to be the cause of the observed

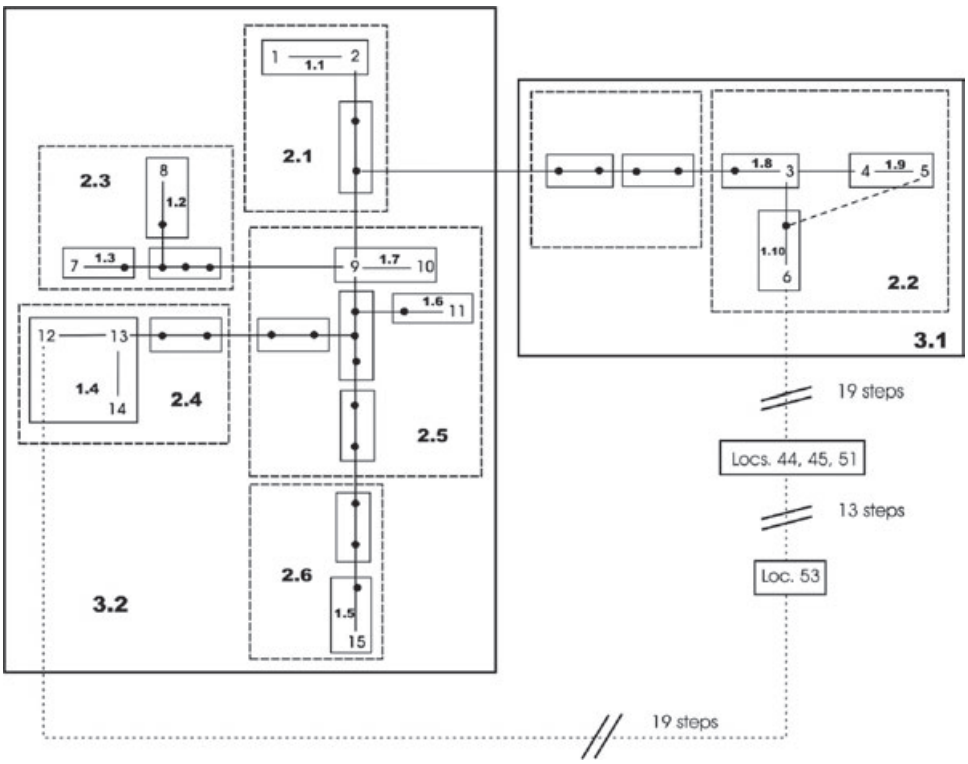

Figure 12. Unrooted cytochrome *b* haplotype network for *donosobarrosi* group. Haplotypes are designated by numbers (Appendix 2). Clade numbers correspond to those shown in Figures 10, 11. Black dots convey the same information as in Figure 4.

patterns. If incomplete lineage sorting is the most plausible explanation for the observed paraphyly in this clade, it can be hypothesized that selective pressures on morphological or secondary sexual traits in these populations has led to morphological differentiation decoupled from differentiation of the mitochondrial genome. Indeed, a close relationship between morphological and molecular rates is unexpected because very little of the genome is directly connected to adaptive change, and most molecular change will either be effectively stochastic (Bromham *et al*., 2002), or driven by other selective pressures (Ballard & Whitlock, 2004). This provides a scenario in which some morphological characters can coalesce more rapidly than some molecular markers under strong selective forces (Moran & Kornfield, 1993; Wikelski & Trillmich, 1997; Abell, 1998; Lebas, 2001; Kwiatkowski & Sullivan, 2002).

In the southern clade, restricted gene flow with isolation-by-distance is the inferred process relating these populations to eastern populations 17 and 18 (Fig. 5). The ancestral haplotype 11 in the network (Fig. 4) is found in localities 11, 17, and 20 (type locality of *L. melanops*; Fig. 5), and a range expansion is inferred from the north-eastern to the south-western part of this clade distribution (Fig. 5). Haplotypes from locality 11 are recovered in the north and south clades, but lizards representing both haplotypes from this locality are morphologically identical, and similar to lizards from the coastal localities in the north clade (Fig. 5, locs. 9, 10). It is hypothesized that this region could be an area of present or past secondary contact, but denser geographical sampling and the use of additional markers is required to corroborate any hypothesis about the processes underlying the pattern in this region.

In the *fitzingerii* complex, a few individuals from the north-eastern localities 34 and 41 share haplotypes with populations from southern localities 31 and 32 (Fig. 8), and several lines of evidence suggest a range expansion occurred from the north-eastern area into the southern Patagonian Steppe. The NCPA leads to the inference for long-distance colonization, possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion for clade 2-1. This same history is also inferred for clade 3-1; both of these clades include haplotypes from southern localities 31 and 32 and north-eastern locality 34, for which the test of Templeton (2001) for secondary contact was significant (Fig. 9). At the most inclusive clade 4-1, which also includes a haplotype from another northern locality (41), the same NCPA inferences were

made, and the secondary contact test was also significant for locality 41 (Fig. 9).

Masta *et al*. (2003) proposed an extension of Templeton's inference key whenever a long distance colonization process is inferred, and their extension to this inference for this clade would lead to the conclusion that there is insufficient evidence to discriminate between long-distance colonization vs. past fragmentation followed by range extension. It is hypothesized that most likely there was a range expansion to the south in small increments, followed by extinction of intermediate populations (given the long distance between these localities). Furthermore, because significant evidence was obtained for secondary contact, it is suggested that marine regressions associated with one or more recent cycles of glaciations extended the coast line eastward from the current shoreline, and this expanded coastal region could have served as a connection between northern and southern areas to permit contact and introgression along the coastal area of the San Jorge Gulf (Fig. 8). Currently, some islands in this gulf are inhabited by populations of *L. fitzingerii*, which is evidence of a more extensive shoreline in the past that was inhabited by these lizards. Climatic changes associated with glacial cycles may also have fostered expansion of lizard populations into the interior of the steppe along the Senguer and Deseado river basins.

In the northern area of the *fitzingerii* complex, there is not enough evidence to associate one of the several paraphyletic clades to the nominal *L. xanthoviridis*, most of which have overlapping distributions. The paraphyly and deep splits observed in this clade (Fig. 6) are likely the result of stochastic lineage sorting and coalescent processes (Irwin, 2002), but two hypotheses could be tested by further studies. First, *L. xanthoviridis* may be comprised of two species, each confined to relatively small area that may have served as refugia during glacial cycles; or *L. xanthoviridis* is a single highly structured species with a history of repeated episodes of expansion/colonization to the south, and *L. fitzingerii* originated as a peripheral isolate during one of these events. These same processes may also explain the origin of the distinct clade 2-4, and the subsequent population expansion that brought *L. fitzingerii* into contact with *L. xanthoviridis*, resulting in the presence of shared ancestral haplotypes in the latter species. The sampling effort in this region was considerable in the present study, but the results presented here show that morphological conservatism in this group has masked an interesting secondary contact zone, and methodological advances in hybrid zone analyses now offer opportunities for dense localized sampling and additional study to make strong inferences about some evolutionary processes (Phillips, Baird & Moritz, 2004).

Within the *L. donosobarrosi* group, the individual from locality 53 in the north, whose haplotype is related to those from the south, is morphologically similar to the other northern individuals, and it is reasonable to hypothesize that these lineages have not been isolated for a sufficient time to attain reciprocal monophyly. If this is correct, then the haplotype from locality 53 is an ancestral southern haplotype retained in the northern populations after isolation. In the *L. darwinii* complex, evidence of incomplete lineage sorting was found in populations approximately codistributed with populations from this complex (Morando *et al*., 2004). Detailed morphological analyses, the inclusion of nuclear genes, and additional sampling in the gap area are required to critically delimit species in both complexes. If more evidence is found for this apparent congruence of processes in phylogenetically distantly related complexes (Fig. 1), then it would imply the existence of a relatively recent barrier to gene flow that affected different clades in similar ways.

Refining and further testing phylogeographical hypotheses

Moderately dense sampling of the widely distributed 'inertial' species in the Patagonian Steppe revealed hidden genetic and probably species diversity, and also hinted at demographic and historical processes that may have shaped the histories of these taxa (Morando *et al*., 2003, 2004). Five of the seven focal species in the present study were paraphyletic for mtDNA genealogies, suggesting that they represent complexes of species (Fig. 13), and NCPA analyses suggest that different historical and demographic processes have shaped the observed patterns. Imperfect taxonomy is one of the main causes of paraphyly in poorly known and undersampled species (Funk & Omland, 2003) and, although, a priori, the initial sampling for most of these species seemed appropriate based on their historical definitions, mtDNA divergence was so deep in some of them (*L. rothi* and *L. boulengeri*) that implementation of the NCPA was precluded. Furthermore, for these two complexes, morphology and distributional data suggest that observed genetic patterns fit the expectations of introgression (another cause of paraphyly), and further character and population sampling should distinguish whether introgression is ongoing or historical.

Another cause of paraphyly is incomplete lineage sorting, which is likely in recent divergence events, as may be the case in the *cuyanus* complex. This pattern may also be due to intense morphological selection on peripheral isolates, which would result in incongruence between molecular and morphological characters; this is the working hypothesis for the observed

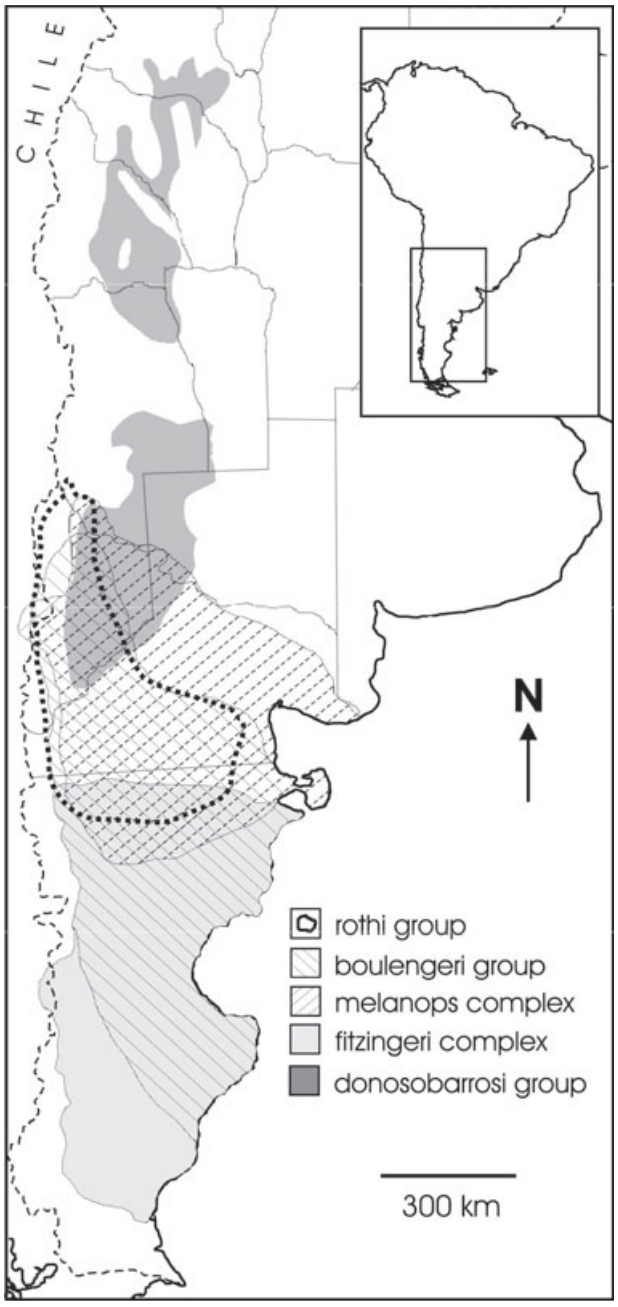

Figure 13. Approximate geographical distribution of focal complexes/group used in this study.

patterns in the *melanops* complex. The population structure and history of the *fitzingerii* group, the most densely sampled in the present study, appears to be so complex that hypotheses about species boundaries and population histories are difficult to assess. *Liolaemus fitzingerii* is a reasonably well-defined species whose boundaries become fuzzy in the area of sympatry with *L. xanthoviridis*, at least in some localities where secondary contact was inferred. *Liolaemus xanthoviridis* includes genetically highly structured and morphologically variable populations that are not recovered as a monophyletic group. This relatively small geographical area could have served as a refugium during the recent glacial cycles in which isolated populations reached certain levels of differentiation before some of them subsequently expanded to the south and differentiated into *L. fitzingerii*. This pattern is consistent with the 'leading edge' hypothesis of population expansion (Hewitt, 2000).

Although the uncertainty of species limits in these complexes compromises the ability to reconstruct robust phylogeographical hypotheses (Agapow *et al*., 2004), the present study has offered both provisional delimitations of species and used NCPA to generate hypotheses of population history, all of which are subject to further testing. In many respects, the temperate South American *Liolaemus* provides a model similar to lizards of the *Sceloporus grammicus* complex (J. C. Marshall, unpubl. data), and salamanders of the genera *Ensatina* (Wake, 1997) and *Batrachoseps* (Jockusch & Wake, 2002) in western North America: ancient and recent allopatric divergence across ecologically and geologically complex landscapes, incipient speciation, secondary contact, and discordance between molecular and morphological patterns of variation. Multi-faceted studies, involving phylogenetic assessments of independent molecular markers and morphological variation across codistributed taxa with estimates of niche breadths in a landscape context (Manel *et al*., 2003; Cicero, 2004; Graham *et al*., 2004; Wiens, 2004b), will likely yield the most promising returns for cross-validation of hypotheses of population and speciation histories.

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APPENDIX 1

BRIEF SUMMARY OF THE TAXONOMIC HISTORY OF THE INGROUP

Species of *Liolaemus* range ecologically from the humid Valdivian forest to the dry Atacama Desert, but the majority are found in the arid and semiarid regions of Argentina and Chile, where up to six species may occur in sympatry in a structurally simple habitat. The majority of *Liolaemus* are terrestrial (or saxicolous), insectivorous (but a substantial number are herbivorous or omnivorous), both oviparous and viviparous parity modes are common, and as in other iguanids, *Liolaemus* exhibit complex social behaviour (Hellmich, 1951; Donoso Barros, 1966; Cei, 1986, 1993; Frost & Etheridge, 1989; Halloy, Etheridge & Burghardt, 1998; Etheridge & Espinoza, 2000).

Etheridge (1995) proposed several major clades within *Liolaemus* based on 'shared, apparently derived' morphological characteristics. A large number of species were placed in the *montanus* group and a subset of these, characterized by a patch of enlarged and spinose scales on the posterior proximal thigh, was named the *boulengeri* group Etheridge (1995). A cladistic analyses of mtDNA sequences (Schulte *et al*., 2000) recovered a monophyletic *boulengeri* group which included two species (*L. pseudoanomalus* and *L. chacoensis*) not having the morphological characters used by Etheridge (1995) to define the *boulengeri* group. The *boulengeri* group of Etheridge (1995) included several clades: the *wiegmannii* group Etheridge (1995) for which there is a strong evidence for monophyly (Etheridge, 2000; Schulte *et al*., 2000), a *darwinii* complex (Etheridge, 1993, 2000, 2001; the *darwinii* group of Morando *et al*., 2004); and the remaining species of the *boulengeri* group of Etheridge (1995) are phylogenetically poorly resolved. In a behavioural study, Halloy *et al*. (1998) used the name *fitzingerii* complex to include *L. boulengeri*, *L. canqueli*, *L. donosobarrosi*, *L. fitzingerii*, *L. melanops*, and *L. xanthoviridis*, and present a maximum parsimony tree based on behavioural characters for 19 species of the *boulengeri* group. After the study by Halloy *et al*. (1998), Etheridge (2000) and Etheridge & Christie (2003) did not use the name *fitzingerii* complex. The name *fitzingerii* 'complex' or 'group' was consistently used by Cei (and collaborators) in a series of works for a set of species that now includes *L. canqueli*, *L. cuyanus*, *L. fitzingerii*, *L. melanops*, and *L. xanthoviridis* (Cei, 1973a, 1975a, 1979, 1986; Cei & Scolaro, 1977a, b, 1980, 1983; Scolaro & Cei, 1977; Scolaro *et al*., 1985). Cei (1986) coined the name *donosobarrosi* group for *L. donosobarrosi*, *rothi* group for *L. rothi*, and included *L. boulengeri* in a *darwinii* group. Several species were recently described (*L. inacayali*, *L. loboi*, *L. mapuche*, *L. martorii* *L. morenoi*, *L. sagei and L. tehuelche*), and hypothesized to be related to *L. boulengeri* (Abdala, 2002, 2003; Etheridge & Christie, 2003). *Liolaemus inacayali*, *L. martorii*, and *L. morenoi* are included in the present analysis and probably some specimens identified here as *L.* cf. *rothi* could be *L. loboi*. Cei & Scolaro (2003) resurrected *L. goestchi* sinonimizing *L. martorii*. Nomenclatural problems with some of these species are not discussed here and the name *L. melanops* is used for all of these populations.

APPENDIX 2

Number of individuals in all ingroup and outgroup taxa (by locality) used in the present study; locality numbers (in parentheses) match those on the distribution maps and in Appendices 3 (which gives museum voucher numbers for all specimens) and 4 (which lists all haplotype numbers identified in the networks).

Province/department	Locality	mtDNA gene regions			Coordinates
		Cytochrome *b*	ND4	12S	
melanops complex					
Río Negro					
25 de Mayo	(1) Ruta Provincial 6, 64 km NE Ingeniero Jacobacci	2	2	1	40°53′S 69°17′W
9 de Julio	(2) Ruta Provincial 66, 2.5 km NW Comicó	6	1	1	41°04′S 67°31′W
25 de Mayo	(3) Ruta Nacional 23, 14 km W Aguada de Guerra	3	3	1	41°09′S 68°30′W
25 de Mayo	(4) Cari Lauquen Lagoon, 7 km N Ingeniero Jacobacci	3	3	1	41°13′S 69°24′W
25 de Mayo	(5) Los Menucos	1	1	1	40°50′S 68°05′W
25 de Mayo	(6) Ruta Nacional 23, 9 km E Sierra Colorada	8	6	3	40°33′S 67°39′W
25 de Mayo	(7) Cantera Las Lajas, 7.5 km W Los Menucos	1	1	1	40°51′S 68°10′W
Valcheta	(8) Ruta Provincial 60, 10 km SW Chipauquil	7	7	3	41°00′S 66°44′W
San Antonio	(9) Piedra Colorada beach, Las Grutas	3	4	1	40°50′S 65°07′W
Adolfo Alsina	(10) Bahía Creek	4	4	1	41°04′S 63°57′W
San Antonio	(11) El Salado beach	8	8	2	41°37′S 65°01′W
El Cuy	(12) El Cuy	3	3	2	39°54′S 65°49′W
Chubut	(13) Ruta Provincial 53, 7–20 km SE Paso de Indios	4	3	2	43°56′S 68°50′W
Paso de Indios	(14) Valle de los Mártires, Ruta Nacional 25, Km 249, 19 km E intersección Ruta Provincial 27	4	4	1	43°49′S 67°45′W
Paso de Indios	(15) Ruta Provincial 12, 6 km S Cerro Cóndor, 72 km S Paso del Sapo	5	1	1	43°23′S 69°10′W
Languineo	(16) Pampa de Agnia	3	1	1	43°45′S 69°38′W
Biedma	(17) Puerto Madryn	4	2	2	42°47′S 64°58′W
Biedma	(18) Ruta Provincial 42, 11 km N Puerto Madryn, El Doradillo beach	2	2	1	42°39′S 64°59′W
Biedma	(19) Ruta Provincial 42, 7 km N Punta Flecha	2	2	1	
Telsen	(20) Ruta Provincial 8, Quelé Cura (Sierra Colorada)	3	1	1	42°13′S 66°21′W
Gastre	(68) Ruta Provincial 12, near Cerro Gorro Frigio, 53 km S Paso del Sapo	1	1	1	43°08′S 69°17′W
Neuquén					
Collón Cura	(69) Ruta Nacional 40, 2 km S Cerrito Piñón, 20 km S La Rinconada	1	1	1	40°15′S 70°38′W
fitzingerii complex					
Chubut					
Tehuelches	(21) Ruta Nacional 40, 7.9 km SW junction Ruta Provincial 20	4	2	1	44°21′S 70°29′W
Tehuelches	(22) Ruta Provincial 20, 3 km N La Laurita, 20 km S Nueva Lubecka	2	2	1	44°44′S 70°12′W
Río Senguer	(23) Ruta Provincial 20, 19 km W Los Manantiales	2	2	1	45°27′S 69°42′W
Sarmiento	(24) 24 km SE Sarmiento, Bosque Petrificado	2	2	1	45°47′S 69°04′W
Río Senguer	(25) Ruta Nacional 40, 2 km S Rio Mayo	1	1	1	45°42′S 70°15′W
Escalante	(33) Ruta Nacional 26, Km 42, 27 km W Pampa del Castillo	3	1	1	45°42′S 68°19′W

APPENDIX 2 *Continued*

Province/department	Locality	mtDNA gene regions			Coordinates
		Cytochrome *b*	ND4	12S	
Florentino Ameghino	(34) Playa Elola, Bahía Camarones	6	7	4	44°50′S 65°43′W
Mártires	(35) Ruta Provincial 29, 45 km W Garayalde	4	3	1	44°33′S 67°04′W
Paso de Indios	(36) Ruta Provincial 29, junction Ruta Provincial 27, 106 km W Garayalde	4	1	1	44°36′S 67°48′W
Florentino Ameghino	(37) Cabo Raso	5	4	1	44°19′S 65°15′W
Florentino Ameghino	(38) Ruta Provincial 1, 12 km S Estancia Dos Naciones	3	3	3	43°32′S 65°20′W
Rawson	(39) Bahía Isla Escondida	4	3	1	43°40′S 65°20′W
Florentino Ameghino	(40) Ruta Provincial 1, 1 km S Dos Pozos	2	2	2	43°54′S 65°24′W
Florentino Ameghino	(41) Ruta Provincial 1, 10 km S Dos Pozos	5	5	3	43°58′S 65°25′W
Florentino Ameguino	(42) Ruta Provincial 32, 4 km W junction Ruta Provincial 1	1	1	1	44°01′S 65°30′W
Florentino Ameghino	(43) Ruta Provincial 1, 20 km S junction Ruta Provincial 1	1	1	1	44°10′S 65°25′W
Santa Cruz					
Lago Buenos Aires	(26) Ruta Provincial 43, 19 km W Perito Moreno	4	3	1	46°36′S 71°69′W
Lago Buenos Aires	(27) Ruta Provincial 43, El Pluma	3	1	1	46°28′S 70°02′W
Deseado	(28) Ruta Provincial 43, 16 km E Las Heras	3	3	1	46°33′S 68°40′W
Deseado	(29) Ruta Nacional 3, Km 1923, 10 km S Caleta Olivia	4	2	1	46°32′S 67°27′W
Deseado	(30) Ruta Nacional 3 and Río Deseado	4	1	1	47°11′S 67°15′W
Deseado	(31) 1 Km W Tellier	4	1	1	47°39′S 66°03′W
Deseado	(32) Ruta Nacional 3, Km 2107, 7 km N Tres Cerros	3	2	2	48°03′S 67°36′W
donosobarrosi group					
Mendoza					
Malargüe	(85) Ruta Provincial 186, 20 km W Mina Ethel	1	1	1	35°58′S 69°01′W
Malargüe	(86) 5 km NE La Salinilla	1	1	1	36°13′S 68°31′W
Malargüe	(98) Ruta Provincial 180, 90 km S El Nihuil	3	2	2	35°48′S 68°40′W
Neuquén					
Zapala	(72) 2 km SE La Amarga	1	2	2	39°06′S 69°34 W
Zapala	(73) 6 km NW La Amarga	7	7	2	39°04′S 69°37′W
Zapala	(74) Mina de Bentonita, 40 km S Zapala por Ruta Nacional 40	1	1	1	39°12′S 69°56′W
La Pampa					
Chical Có	(99) Huella a Chos Malal, 54 km W empalme Ruta Nacional 151	3	1	1	36°42′S 67°57′W
cuyanus complex					
La Pampa					
Puelén	(44) 7 km NE Casa de Piedra	1	1	1	38°07′S 67°06′W
Curacó	(45) 1 km N junction Ruta Provincial 23 and road to 25 de Mayo	1	1	1	37°52′S 67°06′W
La Rioja					
Castro Barros	(46) Anillaco	1	1	1	28°49′S, 66°57′W
General Lamadrid	(53) Ruta Nacional 76, 3 km N Villa Castelli	1	1	1	29°59′S 68°12′W

Felipe Varela	(55) Ruta Nacional 76, Km 158, 18 km S Pagancillo	1	1	1	29°41′S 68°01′W
Famatina	(56) Ruta Nacional 40, Km 657, 9 km E Pituil	2	1	1	28°32′S 67°22′W
Famatina	(58) Road to Antinaco, 3,8 km E Ruta Nacional 40	3	1	1	28°50′S 67°24′W
Arauco	(59) Aimogasta	3	1	1	28°32′S 66°45′W
Catamarca					
Tinogasta	(47) Ruta Nacional 60 and Río La Puerta	4	2	1	28°14′S 67°27′W
Tinogasta	(49) 10 km N Medanitos, road to Tatón	5	2	1	27°28′S 67°35′W
Tinogasta	(50) Ruta Provincial 34, 16 km S Palo Blanco	1	1	1	27°26′S 67°40′W
Tinogasta	(57) Ruta Nacional 60, 4 km W Salado	1	1	1	28°18 S 67°18′W
San Juan					
Ullúm	(48) Matagusanos, on Ruta Nacional 40	6	4	2	31°14′S 68°38′W
Ullúm	(54) Ruta Nacional 40, 8.5 km N de Talacasto	1	1	1	31°01′S 68°38′W
Valle Fértil	(52) Ruta Provincial 510, Km 88, 2 km E Baldecitos	1	1	1	30°12′S 67°40′W
Mendoza					
La Paz	(51) Ruta Nacional 146, Km 276, E Monte Comán	1	1	1	34°17′S 67°14′W
boulengeri complex					
Río Negro					
25 de Mayo	(1) Ruta Provincial 6, 64 km NE Ingeniero Jacobacci	3	3	2	40°53′S 69°17′W
25 de Mayo	(3) Ruta Nacional 23, 14 km W Aguada de Guerra	6	3	2	41°09′S 68°30′W
25 de Mayo	(7) Cantera Las Lajas, 7.5 km W Los Menucos	4	3	1	40°51′S 68°10′W
25 de Mayo	(4) Cari Lauquen Lagoon, 7 km N Ingeniero Jacobacci	2	1	1	41°13′S 69°24′W
25 de Mayo	(60) 40 km SE Maquinchao, road to El Ca'n	3	3	1	41°30′S 68°33′W
Ñorquinco	(61) Ruta Provincial 6, 31 km N Ñorquinco	1	1	1	47°46′S 70°37′W
Ñorquinco	(67) Ruta Provincial 6, 7 km NE Mamuel Choique	3	3	1	41°42′S 70°07′W
Chubut					
Languineo	(64) Ruta Nacional 25, 5 km W Pampa de Agnia	1	1	1	43°44′S 69°48′W
Río Senguer	(70) Ruta Provincial 20, 23 km W Los Manantiales	2	2	1	45°27′S 69°43′W
Santa Cruz					
Deseado	(28) Ruta Provincial 43, 16 km E Las Heras	2	1	1	46°33′S 68°40′W
Deseado	(65) Ruta Nacional 281, 45 km NW Tellier	3	2	2	47°28′S 66°33′W
Deseado	(66) Ruta Nacional 3, Tres Cerros	1	1	1	48°03′S 67°36′W
darwinii group					
Liolaemus koslowskyi					
Catamarca					
Tinogasta	(47) Ruta Nacional 60 and Río La Puerta	2	2	1	28°14′S 67°27′W
Liolaemus olongasta					
San Juan					
Ullúm	(48) Matagusanos, on Ruta Nacional 40	1	1	1	31°14′S 68°38′W
Liolaemus darwinii					
Río Negro					
General Roca	(87) 18 km NE Villa Regina	1	1	1	39°02′S 66°56′W

APPENDIX 2 *Continued*

Province/department	Locality	mtDNA gene regions			Coordinates
		Cytochrome *b*	ND4	12S	
Liolaemus sp. *3*					
Catamarca					
Santa Maria	(88) Ruta Provincial 47, 20 km S Punta Balasto	1	1	1	27°07′S 66°13′W
Liolaemus chacoensis					
La Rioja					
Capital	(89) Ruta Provincial 9, 37.3 km E Anillaco, Sierra de Mazán	1	1	1	28°52′S 66°38′W
Liolaemus abaucan					
Catamarca					
Tinogasta	(90) Ruta Provincial 36, 16 km S Palo Blanco	1	1	1	27°26′S 67°40′W
Liolaemus albiceps					
Salta					
Rosario de Lerma	(91) Santa Rosa de Tastil	1	1	1	24°27′S, 65°57′W
Liolaemus cf. *ornatus*					
Salta					
La Poma	(92) Ruta Nacional 40, 2 km N La Poma	1	1	1	24°41′S 66°11′W
Liolaemus sp. *1*					
Salta					
Guachipas	(93) Ruta Nacional 68, 44.1 km NE Cafayate	1	1	1	25°52′S 65°42′W
Liolaemus sp. 2					
Tucumán					
Tafi del Valle	(94) Ruta Provincial 307, 21.7 km E Amaicha del Valle	1	1	1	26°40′S 65°48′W
Liolaemus quilmes					
Catamarca					
Santa Maria	(95) Santa Maria	1	1	1	26°40′S 66°02′W
Liolaemus uspallatensis					
Mendoza					
Las Heras	(96) Ruta Nacional 7, 4 km W Uspallata	1	1	1	32°36′S 69°24′W
Liolaemus irregularis					
Salta					
Los Andes	(97) 5 km NW San Antonio de los Cobres. Paraje Pompeya	1	1	1	24°14′S 66°19′W
Liolaemus laurenti					
La Rioja					
Famatina	(100) Ruta Nacional 40, Km 657, 9 km E Pituil	1	1	1	28°32′S 67°22′W
rothi complex					
Río Negro					
Bariloche	(75) San Carlos de Bariloche	4	2	1	41°09′S 71°09′W
Ñorquinco	(77) Ojo de Agua	3	3	1	41°32′S 69°51′W

Ñorquinco	(61) Ruta Provincial 6, 31 km N Ñorquinco	1	1	1	41°46′S 70°37′W
25 de Mayo	(76) Ruta Provincial 76, 57 km S Ingeniero Jacobacci	2	2	1	41°45′S 69°21′W
25 de Mayo	(62) Ruta Provincial 8, 17 km S San Antonio del Cuy	2	2	2	40°17′S 68°27′W
Valcheta	(63) Meseta de Somuncurá	3	1	2	41°11′S 66°53′W
Neuquén					
Aluminé	(78) Ruta Provincial 13. Pampa de Lonco Luán, 12 km E Río Litrán	3	3	3	38°53′S 70°58′W
wiegmannii group					
Liolaemus salinicola					
Catamarca					
Tinogasta	(49) 10 Km N Medanitos, road to Tatón	1	1	1	27°28′S 67°35′W
Liolaemus wiegmannii					
Buenos Aires					
Bahía Blanca	(80) Bahía Blanca	2	1	1	38°38′S 62°18′W
La Pampa					
Utracán	(81) Ruta Nacional 35, 6 km N Padre Buodo	3	1	1	37°14′S 64°17′W
Liolaemus wiegmannii 1					
Mendoza					
La Paz	(51) Ruta Nacional 146, Km 276, E Monte Coman	2	1	1	34°17′S 67°14′W
Catamarca	(82) Agua de las Palomas	1	1	1	27°37′S 66°07′W
Liolaemus scapularis					
Catamarca					
Santa Maria	(83) Ruta Nacional 40, 5 km E Los Nacimientos	1	1	1	27°08′S 66°40′W
Liolaemus multimaculatus					
Buenos Aires					
Municipio Urbano de Monte Hermoso	(84) Atlantic shore, between Monte Hermoso and Pehuén Có towns	1	1	1	38°59′S 61°23′W
Liolaemus pseudoanomalus					
La Rioja					
Felipe Varela	(79) Ruta Provincial 26, 3 km N Pagancillo	1	1	1	29°30′S 68°07′W
Outgroups					
Liolaemus vallecurensis	San Juan. Dto. Iglesia. Llanos de la Lagunita				29°24′S 69°25′W
Liolaemus famatinae	La Rioja. Dto. Famatina. Near Station 8, Mina La Mejicana				29°00′S 67°44′W
Liolaemus ruibali	San Juan. Dto. Iglesia. Ruta Provincial 436. Alto del Colorado				30°37′S 69°05′W
Liolaemus bibroni	Río Negro. Dto. 25 de Mayo. Ruta Nacional 23, 14 km W Aguada de Guerra				41°09′S 68°30′W
Liolaemus kingii	Chubut, Dto. Río Senguer. Ruta Nacional 40, 2 km S Río Mayo				45°42′S 70°15′W
Liolaemus lineomaculatus	Río Negro, Dto. Bariloche, PN Nahuel Huapi, faldeo NW Piedra del Cóndor, Cerro Catedral				41°12′S 71°17′W
Liolaemus petrophilus	Chubut. Dto. Paso de Indios. Valle de los Mártires, Ruta Nacional 25, Km 249, 19 km E intersección Ruta Provincial 27				43°49′S 67°45′W
Liolaemus ramirezae	Tucumán. Dto. Tafí del Valle. Ruta Provincial 307, 21.7 km E Amaichá del Valle				26°40′S 65°48′W
Liolaemus robertmertensi	Catamarca. Dto. Tinogasta. Ruta Nacional 60 and Río La Puerta, Km 1298				28°14′S 67°27′W
Phymaturus indistinctus	Chubut. Dto. Río Senguer. Ruta Provincial 20, 19 km W Los Manantiales, Sierra de San Bernardo				45°27′S 69°42′W

APPENDIX 3

SPECIMENS OF REFERENCE WITH MUSEUM VOUCHER NUMBERS LISTED BY LOCALITY

Melanops complex
Loc. 1: LJAMM 2974-75; Loc. 2: LJAMM 2966, 2960, 2964-65, 4880*, 4881*; Loc. 3: LJAMM 2977-78*, 2979*-80; Loc. 4: LJAMM 2947*-48-49; Loc. 5: MLP.S 2471*; Loc. 6: BYU 47304-05-06, MLP.S 2477*-8*, 4771-4773; Loc. 7: LJAMM 4767*; Loc. 8: BYU 47307*, LJAMM 2959*, 47(fn), 4777, 2951-52, MLP.S 2475-6; Loc. 9: LJAMM 2560-61*, 2562-63*; Loc. 10: LJAMM 2458*, 2461, MLP.S 2456, 2457*; Loc. 11: LJAMM 2422*-23, 2481-82-83, MLP.S 2455, 2454*, 2465*; Loc. 12: LJAMM 2429*, 2431*-32; Loc. 13: FML 13047*, LJAMM 2230-31-32; Loc. 14: FML 13053*, LJAMM 2226, MLP.S 2467-8*; Loc. 15: BYU 47280-81*, LJAMM 2926, 2930, MLP.S 2472; Loc. 16: BYU 47283*-84, MLP.S 2470*; Loc. 17: LJAMM 2942*-43, 2944*-45; Loc. 18: LJAMM 2617*-18*. Loc. 19: LJAMM 2415*-16*; Loc. 20: LJAMM 2934*, MLP.S 2473-4*; Loc. 68: LJAMM 2927*: Loc. 69: SDSU 4284*.

Fitzingerii complex
Loc. 21: LJAMM 2921*-22, BYU 47308-91**; Loc. 22: LJAMM 2888*, 4890*; Loc. 23: FML 13050*, MLP.S 2462*; Loc. 24: FML 13049*, MLP.S 2459*; Loc. 25: LJAMM 2889*; Loc. 26: BYU 47286-87, LJAMM 2875-76*; Loc. 27: BYU 47292*, LJAMM 2913*-14; Loc. 28: BYU 47293, LJAMM 2882-83*; Loc. 29: BYU 47299-300*, LJAMM 2895-96*; Loc. 30: 319(fn), BYU 47285*, LJAMM 2891-92; Loc. 31: 303(fn), BYU 47297*-98, 2919*-20; Loc. 32: BYU 47295-96*, LJAMM 2872; Loc. 33: BYU 47288, LJAMM 2879, 4888*; Loc. 34: FML 13052*, LJAMM 2462, 2464*-65*, 2500*, 2502, MLP.S 2469; Loc. 35: BYU 47290, 47289*, LJAMM 2907-08; Loc. 36: BYU 46769*, 47282, LJAMM 2911-12; Loc. 37: LJAMM 2491-92*, 2493-94, MLP.S 2464; Loc. 38: FML 13051*; MLP.S 2463*, 2458*; Loc. 39: LJAMM 2485-86-87*, MLP.S 2461*; Loc. 40: LJAMM 2284*-85*; Loc. 41: LJAMM 2425-26, 2427*-28*, MLP.S 2528*; Loc. 42: FML 13048*; Loc. 43: MLP.S 2460*.

Donosobarrosi group
Loc. 44: LJAMM 2178*; Loc. 45: LJAMM 2988*; Loc. 46: LJAMM (2000)*; Loc. 47: LJAMM 2316*-17-18-19*; Loc. 48: BYU 47315*; LJAMM 2386-87-88-89, 2391*; Loc. 49: BYU 47316, LJAMM 2323-24-25, 2327*; Loc. 50: LJAMM 2340*; Loc. 51: LJAMM 4023*; Loc. 52: LJAMM 4077*; Loc. 53: LJAMM 4094*; Loc. 54: LJAMM 4096*; Loc. 55: LJAMM 4136*; Loc. 56: BYU 47312*, LJAMM 4156; Loc. 57: LJAMM 4172*; Loc. 58: BYU 47313*, LJAMM 4204-05; Loc. 59: BYU 47314*, LJAMM 4319*, 4321; Loc. 72: LJAMM 2542-43*; Loc. 73: BYU 47301*-02-03, LJAMM 2642*-43-44-45; Loc. 74: LJAMM 4473*. Loc. 85: LJAMM 4006*; Loc. 86: LJAMM 4145*; Loc. 101: LJAMM 4213-14-15*; Loc. 102: LJAMM 4230-31-32*.

Boulengeri complex
Loc. 1: LJAMM 2852*-54-55*; Loc. 3: LJAMM 2846*-47-48*, 2850-51, 4790*; Loc. 4: LJAMM 2818-19*; Loc. 7: LJAMM 2812, 4795*, 4796, 4799; Loc. 28: LJAMM 2840*-41; Loc. 60: LJAMM 2816*-17, 165(fn)*; Loc. 61: LJAMM 2165*; Loc. 64: LJAMM 2845*; Loc. 65: LJAMM 2842, 2843*, 4805; Loc. 66: PMC 0399(fn)*; Loc. 67: LJAMM 2172-74-75*; Loc. 70: LJAMM 2186*-87*.

Darwinii group
Loc. 47: *Liolaemus koslowskyi*: LJAMM 2328*, 2330*; Loc. 48: *Liolaemus olongasta*: LJAMM 2378*; Loc. 87: *Liolaemus darwinii*: LJAMM 2409*; Loc. 88: *Liolaemus* cf. *quilmes*: MLP.S 2530*; Loc. 89: *Liolaemus chacoensis*: MLP.S 2508*; Loc. 90: *Liolaemus abaucan*: LJAMM 2372*; Loc. 91: *Liolaemus albiceps*: LJAMM 2648*; Loc. 92: *Liolaemus* cf. *ornatus*: MLP.S 2531*; Loc. 93: *Liolaemus* cf. *quilmes* 1: MLP.S 2526*; Loc. 94: *Liolaemus* cf. *quilmes* 2: LJAMM 4417*; Loc. 95: *Liolaemus quilmes*: MLP.S 2527*; Loc. 96: *Liolaemus uspallatensis*: LJAMM 4459*; Loc. 97: *Liolaemus irregularis*: LJAMM 2628*; Loc. 103: *Liolaemus laurenti*: LJAMM 4161*.

Montanus group
Loc. 98: *Liolaemus vallecurensis*: LJAMM 2698*; Loc. 99: *Liolaemus famatinae*: FML 9446*; Loc. 100: *Liolaemus ruibali*: LJAMM 2369*.

Rothi group
Loc. 61: LJAMM 2163*; Loc. 62: LJAMM 2489*-90*; Loc. 63: LJAMM 4457*-58, 4461; Loc. 75: LJAMM 3091*-92-93, 3095; Loc. 76: LJAMM 3065*, 214(fn)*; Loc. 77: LJAMM 2134, 2236-37*; Loc. 78: 2548-49-50*.

Wiegmannii group
Loc. 49: *Liolaemus salinicola*: LJAMM 2375*; Loc. 51: MLP.S 2499*, LJAMM 4040; Loc. 80: *Liolaemus wiegmannii*: MLP.S 2480*, LJAMM 3100; Loc. 81: LJAMM 3096*, 3200* MLP.S 2479*; Loc. 82: LJAMM 4300*; Loc. 83: *Liolaemus scapularis*: MLP.S 2529*; Loc. 84: *Liolaemus multimaculatus*: LJAMM 4464*. *Liolaemus pseudoanomalus*: Loc. 79: LJAMM 2300*.

Outgroups
Liolaemus bibroni: BYU 47183*; *Liolaemus kingii*: LJAMM 2308*-09*; *Liolaemus lineomaculatus*: SDSU 4268*; *Liolaemus petrophilus*: LJAMM 2125*; *Liolaemus ramirezae*: BYU 47180*; *Liolaemus robertmertensi*: LJAMM 1961*; *Phymaturus indistinctus*: LJAMM 2393*.

Individuals used for the phylogeny represented in Figure 2

Liolaemus boulengeri (LJAMM 2175), *Liolaemus cuyanus* BYU 47312, *Liolaemus darwinii* (LJAMM 2410/2409 c-mos), *Liolaemus fitzingerii* (LJAMM 2876 [cytochrome *b*, GAPDH, c-mos], MLP.S 2462 [ND4], FML 13050 [12S]), *Liolaemus kingii* (BYU 47281], *Liolaemus melanops* (LJAMM 2947), *Liolaemus montanus* (LJAMM 2034), *Liolaemus petrophilus* (BYU 47098), *Liolaemus pseudoanomalus* (LJAMM 2300), *Liolaemus rothi* (LJAMM 3091), *Liolaemus wiegmannii* (MLP.S 2480), *Phymaturus indistinctus* (LJAMM 2124)

APPENDIX 4

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

Haplotype number	Locality number	Number of individuals
cuyanus complex		
1	47	1 (LJAMM 2318)
2	47	2 (LJAMM 2317, 2319)
	56	2 (BYU 47312, LJAMM 4156)
	57	1 (LJAMM 4172)
	58	3 (BYU 47313, LJAMM 4204-5)
3	49	3 (BYU 47316, LJAMM 2323, 2325)
4	49	1 (LJAMM 2324)
5	50	1 (LJAMM 2340)
6	49	1 (LJAMM 2327)
7	55	1 (LJAMM 4136)
8	52	1 (LJAMM 4077)
9	59	1 (LJAMM 4319)
10	59	2 (BYU 47314, LJAMM 4321)
11	46	1 (LJAMM 2000)
12	48	3 (LJAMM 2386-87-88)
13	54	1 (LJAMM 4096)
14	48	1 (BYU 47315)
15	48	1 (LJAMM 2389)
16	53	1 (LJAMM 4094)
17	44	1 (LJAMM 2178)
18	45	1 (LJAMM 2988)
19	51	1 (LJAMM 4023)
melanops complex		
melanops south		
1	13	3 (FML 13047, LJAMM 2230-1)
	14	1 (FML 13053)
	15	5 (BYU 47280-81, LJAMM 2926, 2930, MLP.S 2472)
	16	2 (BYU 47283, MLP.S 2470)
2	17	1 (LJAMM 2944)
3	18	1 (LJAMM 2617)
4	16	1 (BYU 47284)
5	17	1 (LJAMM 2943)
6	19	1 (LJAMM 2416)
7	20	2 (LJAMM 2934, MLP.S 2474)
8	11	1 (LJAMM 2422)
9	11	1 (MLP.S 2455)
10	68	1 (LJAMM 2927)

APPENDIX 4 *Continued*

Haplotype number	Locality number	Number of individuals
11	11	2 (LJAMM 2423, 2482)
	17	1 (LJAMM 2942)
	20	1 (MLP.S 2473)
12	18	1 (LJAMM 2618)
13	19	1 (LJAMM 2415)
14	11	1 (MLP.S 2454)
15	17	1 (LJAMM 2945)
melanops north		
1	1	1 (LJAMM 2974)
2	1	1 (LJAMM 2975)
	2	2 (LJAMM 2960, 2966)
	3	2 (LJAMM 2977-8)
	4	3 (LJAMM 2947-48-49)
3	2	2 (LJAMM 118fn, 2964)
4	8	1 (LJAMM 2952)
5	6	1 (MLP.S 2477)
6	6	1 (BYU 47305)
7	8	2 (LJAMM 2951, BYU 47307)
8	8	1 (LJAMM 47fn)
9	6	1 (MLP.S 2478)
10	3	1 (LJAMM 2980)
11	6	1 (BYU 47304)
12	10	1 (MLP.S 2456)
13	8	1 (MLP.S 2476)
14	10	2 (LJAMM 2458, 2461)
15	10	1 (MLP.S 2457)
16	6	1 (BYU 47306)
17	7	1 (LJAMM 94fn)
18	2	1 (LJAMM 4880)
19	5	1 (MLP.S 2471)
20	9	1 (LJAMM 2562)
21	9	1 (LJAMM 2561)
22	9	1 (LJAMM 2560)
23	8	1 (MLP.S 2475)
24	8	1 (LJAMM 4777)
25	6	2 (LJAMM 4771-72)
26	6	1 (LJAMM 4773)
27	11	3 (LJAMM 2481, 2483, MLP.S 2465)
28	12	1 (LJAMM 2429)
29	2	1 (LJAMM 2965)
30	12	1 (LJAMM 2431)
31	12	1 (LJAMM 2432)
32	69	1 (SDSU 4284)
fitzingerii complex		
1	22	1 (LJAMM 2888)
2	27	1 (LJAMM 2914)
3	24	1 (MLP.S 2459)
4	30	1 (LJAMM 2891)
5	23	1 (MLP.S 2462)
6	31	2 (BYU 47297-8)
7	31	2 (LJAMM 2920, 303fn)
	32	3 (BYU 47295-6, LJAMM 2872)
8	34	1 (LJAMM 2465)
9	24	1 (FML 13049)

APPENDIX 4 *Continued*

Haplotype number	Locality number	Number of individuals
	21	2 (BYU 47308, LJAMM 2921)
	22	1 (LJAMM 4890)
	25	1 (LJAMM 2889)
	26	3 (BYU 47286-7, LJAMM 2876)
	27	1 (LJAMM 2913)
	28	2 (LJAMM 2882-3)
	29	4 (BYU 47299-30, 2895-6)
	30	3 (BYU 47285, LJAMM 2892, 4875)
	33	3 (BYU 47288, LJAMM 2879, 4888)
10	27	1 (BYU 47292)
11	26	1 (LJAMM 2875)
12	23	1 (FML 13050)
13	21	2 (BYU 47291, LJAMM 2922)
14	41	1 (LJAMM 2427)
15	34	4 (FML 13052, LJAMM 2462, 2464, 2502)
16	38	1 (MLP.S 2458)
17	38	1 (FML 13051)
18	35	1 (BYU 47289)
19	36	1 (LJAMM 2911)
20	35	3 (BYU 47290, LJAMM 2907-8)
	36	2 (BYU 47282, 46769)
21	36	1 (LJAMM 2912)
22	37	1 (LJAMM 2493)
23	43	1 (MLP.S 2460)
	41	1 (LJAMM 2426)
	37	2 (LJAMM 2492, 2494)
24	41	1 (LJAMM 2428)
25	40	1 (LJAMM 2284)
26	42	1 (FML 13048)
27	39	2 (MLP.S 2461, 2485)
28	41	1 (MLP.S 2528)
	38	1 (MLP.S 2463)
29	40	1 (LJAMM 2285)
	39	1 (LJAMM 2486)
30	39	1 (LJAMM 2487)