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COLLEGE OF ARTS AND SCIENCES

PHYLOGEOGRAPHY OF THE SIGMODONTINE RODENT, *PHYLLOTIS*
XANTHOPYGUS, AND A TEST OF THE SENSITIVITY OF NESTED CLADE
ANALYSIS TO ELEVATION-BASED ALTERNATIVE DISTANCES

By

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I am dedicating this thesis to my family: a group that includes those bound to me either by kinship or by friendship. This extended clan includes the FSU E&E graduate students, and my friends of BYOE just as much as it does my parents and siblings. Particularly close to my heart are my close friends Dave Low, Sarah Tso, and Toni Sturtevant and my “big sister” Jill Holliday.

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ABSTRACT

I undertook a phylogenetic and phylogeographic study of the South American rodent, *Phyllotis xanthopygus*. My goals were to obtain general information about the history of the species and to explore some issues of Nested Clade Analysis (NCA) sensitivity in an empirical framework. The mitochondrial marker, cytochrome-*b* was sequenced to produce an intraspecific maximum-likelihood phylogeny of *P. xanthopygus*. The sensitivity of NCA to alternative distances incorporating elevation information was assessed using *P. xanthopygus* as an empirical test case. Two alternative distance frameworks incorporating physiognomic information were explored. One was based on constraining dispersals across 1000 meter isoline intervals and the other was based on imposing a lower elevation limit to dispersals. Both alternatives produced congruent results, but they differed from the standard distance measurement, or great-circle distance. Only three out of nine phylogeographic inferences were the same for the great-circle and alternative distances.

INTRODUCTION

Phylogeography is the study of the geographic history of a species or group of related species. Information about the geographic distribution of gene lineages is used to make inferences about the past (Avice, 2000). In this study I use phylogeographic methods to explore the history of the South American leaf-eared mouse, *Phyllotis xanthopygus* (Waterhouse, 1837). We also used *P. xanthopygus* as a model-system to assess the effects of incorporating elevation information in the framework of a Nested Clade Analysis (NCA) (Templeton *et al.*, 1995) is a common statistical phylogeographic technique which has grown in popularity over recent years (Creer *et al.*, 2001; Cruzan & Templeton, 2000; Mardulyn, 2001; Templeton, 1998). Despite its popularity there has been only one attempt to test its ability to incorporate alternative distance information (Fetzner & Crandall, 2003) to explore any sensitivity issues which might arise. No previous study has attempted to use distances derived from elevation.

Phyllotis xanthopygus

Phyllotis xanthopygus, originally collected by Darwin during his voyage on the *HMS Beagle* and described as *Mus xanthopygus* by Waterhouse (1837), is a wide-ranging South American rodent with a broad distribution in Peru, Bolivia, Chile, and Argentina. In earlier systematic revisions, Pearson (1958) dealt with the genus *Phyllotis* and Hershkovitz (1962) dealt with both the genus level taxonomy and with the tribe Phyllotini. Both treated the subspecies we now recognize as being members of *P. xanthopygus* as geographic races of *Phyllotis darwini*. It wasn't until Spotorno and Walker's cytogenetic and breeding studies (Spotorno & Walker, 1983; Walker *et al.* 1984) that there was evidence of hybrid sterility. The populations along the Chilean coast were placed in *Phyllotis darwini* and *Phyllotis xanthopygus* was restricted to the remaining members of the far-ranging species. When Stepan (1995) undertook a recent revision to the tribe, he followed Spotorno and Walker's recommendations and treated *P. darwini* as a separate species.

As a montane species, *P. xanthopygus*' range encompasses an extensive elevation gradient from high elevations in the central Andes (5000 meters) down to sea-level in the Pacific slopes in the west and to low isolated rock outcroppings in the eastern extent of their range (Hershkovitz, 1962). This distribution pattern provides an excellent natural experiment for exploring the effects of mountain topography on phylogeography and speciation. Although the genus is found throughout the central and southern Andes, most species of *Phyllotis* have a very limited distribution — *P. xanthopygus* is unique in having such a broad range.

The phyllotines are a tribe wholly endemic to South America, and are members of the New World subfamily Sigmodontinae within the family Cricetidae and the very speciose superfamily Muroidea (Stepan *et al.*, 2004). The species of leaf-eared mice of the genus *Phyllotis* are distributed throughout the Andes from Ecuador to Chile. Seven subspecies of *P. xanthopygus* have historically been recognized, and six of those

subspecies are still currently assigned to: *P. x. chilensis*, *P. x. posticalis*, *P. x. ricardulus*, *P. x. rupestris*, *P. x. vaccarum* and, *P. x. xanthopygus*. Related species include *Phyllotis bonaeriensis*, *Phyllotis darwini*, *Phyllotis limatus*, and *Phyllotis magister*.

The primary habitat associated with *P. xanthopygus* is the montane puna grassland of the Andean altiplano and rocky and brushy habitats (Hershkovitz, 1962). The altiplano is a large plateau between the eastern and western cordilleras of the Andes in southern Peru and Bolivia extending into northernmost Chile and Argentina. There are summits with greater elevation throughout the main cordilleras of the Andes, but, at over 4,000 meters above sea level, it is the largest contiguous expanse of high elevation habitat in the Andes. Comprised predominantly of a mixture of fescue (*Festuca spp.*) and couch grass (*Calamagrostis spp.*), this vegetation zone of the puna is characterized by having cold and xeric conditions (Hershkovitz, 1962). Phyllotines can survive in marginal desert environments and on islands of suitable habitat isolated on rock outcroppings scattered in the otherwise unsuitable Pampas grasslands.

Steppan (1998) documented the presence of several discrete mitochondrial gene lineages which seemed to reflect deep partitioning of the species' phylogeny associated with geography. In this study I elaborate with much improved taxon sampling within *Phyllotis xanthopygus* and elucidate some novel findings.

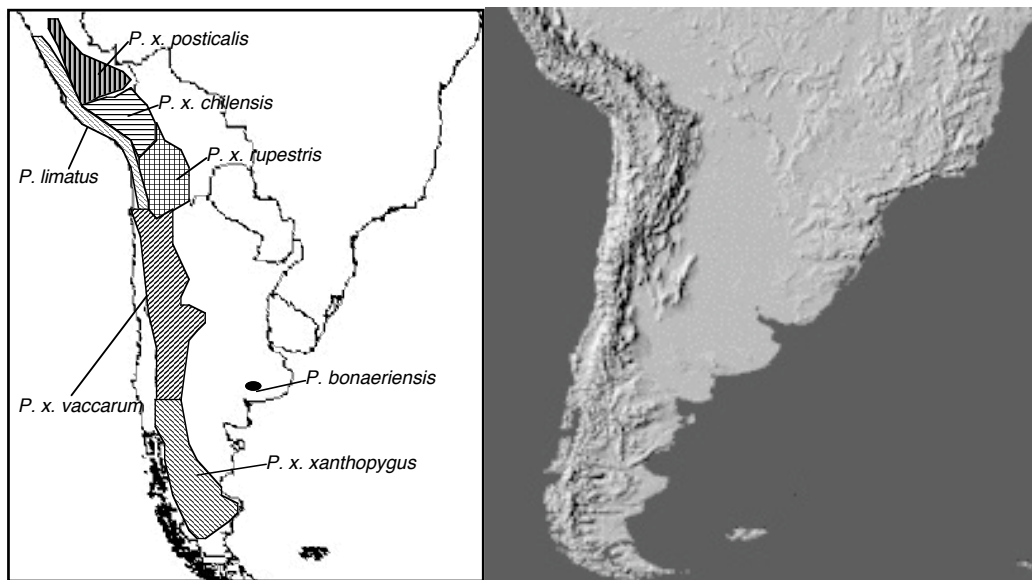


Figure 1. Maps Showing the Ranges of Species and Subspecies in the *P. xanthopygus* Complex and the Topography of the Andes. The map on the left shows the ranges of the taxa included in this study. The map on the right is a physical relief map showing the southern Andes.

Phylogeography

The field of phylogeography is a relatively recent discipline in which investigators seek to uncover the processes resulting in geographical patterns of

genealogical lineages within species and among closely allied species (Avice, 2000). As a field of study integrating aspects of biogeography and phylogenetics, phylogeography involves combining historical hypotheses with spatial distributions of gene lineages. The discipline has developed over the last twenty years as the ability to assess genetic variation within populations through molecular techniques has improved. Since it involves within and, occasionally, among species variation, phylogeography is at the cusp of macroevolutionary phylogenetic studies and intraspecific microevolutionary processes (Avice, 2000). Traditionally, phylogeographers have gleaned their inferences from phylogenetic observations. In this paradigm, researchers test hypotheses about historical population movements and distribution patterns based on observed correlations of geography and the phylogeny. For instance barriers to dispersal may result in vicariance events that could be detected as a pattern of divergences on the phylogeny coinciding with the location (and, if known, timing) of the barriers. On the other hand dispersal corridors can allow for the spread of individuals to a new region, and this pattern on an intraspecific phylogeny would reflect the wide spread of a few individuals haplotypes. If very few founding individuals were involved then there would also be a stochastic sampling of the original population.

Coalescence Theory and Nested Clade Analysis

Wright (1943) introduced the inbreeding coefficient, F_{ST} , as a measure of genetic differentiation over subpopulations. F-statistics in general are derived from measures of the coefficient of inbreeding in a population, and measures of relatedness of populations allow for estimations of gene flow. Though this metric provides information on genetic subdivision and gene flow, it does not incorporate any temporal element. This makes it useful for problems in population genetics, but less helpful in phylogeography, where the goal is to extract the historical information about population structure and movements. The requisite innovation was the development of coalescence theory.

Coalescence theory is a field of mathematical and statistical treatments of gene genealogies at both the interspecific and intraspecific levels, which emerged in the 1970s (Kingman, 1982). Coalescent theory is predicated on the assumption that homologous haplotypes in different individuals are identical (or derived) by common descent. “Coalescence” refers to the fact that one can trace the alleles present in a population to a common ancestor, where the gene lineages are said to coalesce. This is a straightforward consequence of stochastically differential success in gene propagation. All variations must be derived from a common ancestor version followed by vertical transmission and mutational changes

Nested Clade Analysis (NCA) was designed by Templeton *et al.* (1995) to assess population level phylogeography, unlike traditional approaches by using a statistical framework. The statistical techniques available to look at the relationship of geography to population structure, such as spatial autocorrelation (Sokal *et al.*, 1989), lack historical information. Such techniques search for a correlation between a variable in reference to spatial location of that variable. Instead, Templeton *et al.* (1995) sought to use a coalescent and cladistic-based approach to obtain information about population history.

The advantage of NCA (Templeton *et al.*, 1995) is that it provides a historical component by assessing the branching pattern of tip clades, which are recent derivations

of internal clades on a phylogeny. Note that by “clade” we refer to the methodology of the procedure of looking for monophyletic genealogical groups for a particular gene tree without implying that these are true “clades” in the classical sense. The “clades” can be individuals, haplotypes, or populations. Since genetic variation within and among populations may reflect historical events rather than ongoing population level processes, this can provide considerable information that would otherwise be ignored. The other advantage of this approach is that it provides a means to quantify qualitative inferences with statistics. Since its inception, phylogeography has been a relatively qualitative science based on observing correlated patterns in a phylogeny. Essentially, everything NCA can demonstrate can be determined in a qualitative fashion from inspection of a phylogeny, but it allows one to decide how much faith should be put in these inferences. Another advantage of NCA is that it searches for multiple overlaying patterns within the data set. There is nothing about the hypotheses of restricted gene flow, fragmentation events etc that makes them mutually exclusive hypotheses for different levels in a tree.

Alternative Distance Metrics

Over the past several years, nested clade analysis has become one of the most popular phylogeographic approaches (Templeton, 1998) but the sensitivity and accuracy of this technique are untested. In the original presentation of the technique (Templeton *et al.*, 1995), an empirical example, that of the salamander *Ambystoma tigrinum*, was used to illustrate the operation and capabilities of NCA. Later, Templeton (1998) followed up with several more examples of the use of NCA taken from the literature. These worked examples are helpful but do not deal with the issue of detecting biases and sensitivity issues under a full range of conditions. To date the only published study to present a simulation study is that of Knowles and Maddison (2002). They concluded that NCA made several incorrect inferences and discussed alternative approaches to statistical phylogeography. Templeton (2004) responded by pointing out that the mistakes made were all in cases he had earlier discussed as being potentially sensitive and that with those caveats a competent researcher could draw sound conclusions from the areas where NCA performs more robustly.

One of the key issues in NCA is the determination of geographical measures of clade distance (Templeton *et al.*, 1995). These are the values used to assess the geographic spread of haplotype and nested clades. The two distances are the within clade distance, D_C , and the nested clade distance, D_N . The former, D_C , is simply a measure of how widespread a haplotype clade is (the average distance of clade members from the geographical center of their range). The latter, D_N , is a measurement of how far members of the given clade are from all other members of the larger clade they are nested within. In both of these measurements the center of a haplotype’s distribution is determined geographically and then weighted by the frequency of the haplotype at the sampling localities. These measurements allow discrimination between short and long-distance movements, and can be tested against the coalescent-based predictions. A third metric, the I-T contrast, is a comparison of the distances for internal and tip clades, and this measurement is essential to adding the historical element to NCA. Ancestral (internal) haplotypes will tend to be concentrated near the original population center, with a subset of tip haplotypes finding their way to more distant locales. Exceptions, such as when tip

clades are more widespread than internals, mean that there has been a differential spread of haplotypes, perhaps evidence for a range expansion in just those individuals. Newer mutations should be located near their center of origin (nested clade) if there is little gene flow. Furthermore, long distance movements of small pioneer populations will have a limited sampling of the available haplotype pool.

The D_C , D_N , and I—T distance metrics used to estimate the geographic spread of haplotype clades are based on a simple assumption that plants and animals are able to disperse freely, and the shortest distance between two points, the great-circle distance, is assumed to be a fair approximation of the biological distance. This is likely to be a reasonable assumption in many cases where the environment is uniform, but it is not always biologically realistic. For instance, the shortest distance between two points in a riverine or riparian habitat may be across unsuitable terrestrial habitats and may even entail travel between completely separate drainage systems. Because the great-circle distance is the shortest possible path between points, constrained distances will always be longer. Templeton *et al.* (1995) mentioned that alternatives could be employed, but they did not explore the implications of using these distances.

Some authors have begun to use river distances for riverine, coastal, and riparian species (Turner *et al.*, 2000). However there has only been one study to explicitly explore the effects of an alternative distance framework. Fetzner and Crandall (2003) performed a comparison of the performance of NCA using river distances and great-circle distances. Because their study organisms were crayfish with very limited terrestrial dispersal capabilities, river distances were undoubtedly more biologically realistic estimates. They found that conclusions drawn from analyses using the river distance were similar to those drawn from the great-circle distances, but that there were important exceptions. At lower, less inclusive clade levels the differences were fairly great, but at higher levels the different analyses produced largely congruent results.

A principle aim of this study is to empirically investigate the effects of alternative distance metrics. Because *Phyllotis* lives in a topographically complex region with habitat zones varying across elevation gradients, the use of distances constraining movement across elevations is likely to be more biologically realistic than simply disregarding elevation. In this study we make use of two elevation-based distances. They are both based on the idea of choosing paths that limit the amount of movement across elevation lines.

To what degree do these metrics capture a more faithful picture of reality than great-circle distances? One fairly obvious improvement of both of the distance measurements, is that they take the coastline of South America into account. The shortest distance between northwestern Peru and Chile would be across a section of the Pacific Ocean. It is easy to see how sea-crossing might be an unrealistic scenario for montane animals. This is analogous to Fetzner and Crandall's (2003) use of riparian distances as a distance metric. We do not have a good deal of information about the degree of vertical movement in these mice. Like many rodent groups, the phyllotines have been known to have outbreak population cycles (Pearson, 1975). It is possible that most long-distance dispersals of individuals take place in times of stress such as these.

Phylogeographic Hypotheses

Because phylogeography is predicated on the interplay of geography and history, it is important to consider both elements when developing *a priori* hypotheses for species phylogeography. The early Pleistocene was characterized by warm and wet conditions in the pampas and eastern Andes ((Tonni *et al.*, 1992; Verzi *et al.*, 2002; Vucetich *et al.*, 1997) and possibly even in the area of the Atacama desert (Betancourt *et al.*, 2000). This statement is supported by both the biotic record (fossils of mammal species, such as *Clyomys*, (Tonni *et al.*, 1992; 1999a) provide excellent indicators for habitat and paleoclimate) and lithology. However, this period was followed by cooling and drying effects, which were coincident with Pleistocene glaciation events. Tonni *et al.* (1999a) produced a thorough survey of the effects this had on the mammalian assemblages of central Argentina. The zoogeographic history and geological effects of cooling in the Buenos Aires region, particularly in the Ensenadan period (which ended approximately 0.78 million years ago) are especially well resolved (Nabel *et al.*, 2000; Tonni *et al.*, 1999b). The summary of these studies is that during the late Pleistocene there was a period when climatic conditions became generally colder and dryer, and this was associated with range expansions of species that prefer these conditions. In many regions evidence from rodent middens indicates that aridification has lasted without remission through to the present. In other regions, similar evidence points to periods of warmer and more humid environments. This is the case for midden built by *P. limatus* in the Atacama desert (Kuch *et al.*, 2002). Not only was this evidence of a more moist period in the Atacama, but it also represents the only available fossil evidence of a range shift in *Phyllotis*. The location where the midden was found slightly further south (50 km) than any extant populations of *P. limatus* have been recorded. It does not seem that actual glaciers expanded very far north in Chile and Argentina, so these should not have provided dispersal barriers in the lowlands. This hypothesis can be rejected if inferred dispersals are restricted to the lower latitudes in the north with very little trans-Andean migration in Chile and Argentina.

A study by Lamb *et al.* (1997) provides a ready parallel to the situation in *P. xanthopygus*. *Sciurus aberti* is the tassel-eared squirrel of the American southwest. This example is of particular importance because of the similarities in conditions to the situation with *Phyllotis xanthopygus* in Argentina. The squirrels were located on disjunct mountains with a historical period of more widespread suitable habitat during which range expansion was possible. This situation is similar to our *a priori* expectations for *P. xanthopygus* although on a smaller scale. We predicted that *P. xanthopygus* should show a similar pattern of range expansion in the Pleistocene based on the paleoclimate of the Andes and on the habitat requirements of *Phyllotis*. Lamb *et al.* tested hypotheses about the origin of disjunct populations of squirrels. Vicariance biogeography was employed in the context of a scattered montane conifer forest habitat, with conclusions about paleoclimate invoked to explain the present distribution of these systems. The researchers were primarily interested in distinguishing between an origin via vicariance of a widely distributed ancestral population leaving relic populations and the alternative of pre-Pleistocene dispersal across unsuitable habitat. The genetic variance partitioned into two distinctive phylogroups, a western and an eastern clade, indicating a deep

vicariant event. Northern outliers of the eastern population at the tips of the phylogeny indicate that subsequent dispersal events followed.

The Pleistocene glaciations would have increased the geographic range suitable for phyllotines as cold and dry habitat dominated by xeric-adapted low shrubs and bunch-grasses spread with vegetation shifts to lower elevations. One of our expectations is that these Pleistocene climate shifts would have resulted in a more widespread distribution for *P. xanthopygus*. Subsequent retraction would have left peripherally isolated populations on suitable mountain habitat islands, as may have been the case for *P. x. vaccarum* populations in Argentina and for *P. bonaeriensis* in the Sierra de la Ventana mountains. Because the central and southern Andes consist of areas well within the preferred habitat zone of *Phyllotis* and because the large altiplano region is a plateau albeit one with a significant amount of relief of its own, we expect these areas to be insulated from the effects of habitat shifts to lower elevations. Therefore they would presumably be dominated to a greater degree by isolation by distance with continual gene flow than the southeastern populations in the Pampas and Patagonia, and this pattern can be tested with NCA.

Andean Biogeography

Relatively little attention has been paid to the role of the Southern Andes as a major biogeographic entity. Studies in South America have tended to focus on the Amazon basin (Colwell, 2000; Patton, da Silva, 1998), or the forested eastern slopes (Patton, Smith, 1992; Wilmott *et al.*, 2001). Patton and Smith (1992) tested to see whether patterns of endemism and coordinated character change were due to vertical ecological gradients or the effects of refugia in *Akodon* mice in the eastern Andes. They found no evidence that gradients were involved in speciations. In high Andean birds; Vuilleumer and Simberloff (1980) discussed the importance of considering what they termed ecological limits (such as species competition) in biogeography before constructing historical hypotheses involving other habitat limitations or possible dispersal barriers. One of the main conclusions of Vuilleumer and Simberloff was that the ecological effects on biogeography could not be considered without also looking at the historical influences. At the time, they found it difficult to separate the two patterns, but the phylogeographic approaches which have developed since then, including NCA, have provided a way to estimate the history.

At the northern end of *P. xanthopygus*' range the sampling scheme included eight sample localities in Peru and seven sample localities in Bolivia. This area is the high central Andes and includes the large volcanic plateau of the altiplano surrounded by two main cordilleras that converge in northern Peru and in northern Chile. In the southern extent of the Andes, there were nine localities in Chile and fifteen in Argentina, including a locality in the disjunct Sierra de la Ventana range in eastern Argentina near the Atlantic coast (Locality 1). The physiognomy of the southern portion of the Andes includes a central Andean cordillera running along the border of Chile and Argentina. The Atacama desert is located in northern Chile and the Monte deserts of the eastern foothills in Argentina merge with the Pampas grasslands in the east and Patagonia in the south.

There are also a number of questions about Andean biogeography in general that recovering the phylogeographic history of *Phyllotis xanthopygus* may help us resolve.

For instance, the role of the Andes in speciation is a critical question. The amount of communication across the central Andean cordillera will vary with species, and *P. xanthopygus* provides an opportunity to test for trans-Andean dispersal in a mid to high elevation specialist. To what degree has the Atacama desert affected the dispersal of animals and plants into northern Chile? If we see a deep break suggestive of a vicariant fragmentation in *Phyllotis* and in other Andean mammals then this would be indicative of a disruption. On the other hand, evidence of gene flow across this region would weaken this hypothesis.

METHODS

Sampling Scheme

The sampling scheme was designed to maximally cover the range of *Phyllotis xanthopygus*. The sampling sites include localities in all of the major subspecies ranges and in the zones of contact between ranges. In this way we captured the pattern of diversity both in the high Andes, where most of the subspecific diversity is concentrated and on the lower elevation areas at the edges of the species' habitat tolerance. Table 1 contains a description of the collection localities. Figure 2 is a map showing the locations of all sampling localities.

The number of individuals collected at each locality ranged from 1—7 except in the case of the specimens from Arequipa, Peru. In this instance there was an elevational transect with relatively few individuals from several localities separated by a few kilometers. We chose to combine these sites into a single locality and therefore had 20 specimens in total. The sampling was most thorough near the intersection of Peru, Bolivia, Chile, and Argentina, but the entire distribution of *P. xanthopygus* was covered.

We collected individuals from all but one of the historically recognized subspecies of *P. xanthopygus*. This subspecies, *P. x. ricardulus*, is restricted to northern Argentina just east of the range of *P. x. vaccarum*. In addition we collected individuals from two related species, *P. limatus*, along the Peruvian and Chilean coasts, and *P. bonariensis*, in the Sierra de la Ventana.

Mice were trapped and a tissue sample, typically a portion of liver, was removed. We also obtained material through loans from museum collections (see Appendix 1). The tissue samples were either frozen or preserved in ethanol.

DNA Extraction and Amplification

Tissue extractions took place in a room free of PCR products and other possible contaminants. Standard phenol-chloroform methods were used to extract DNA from either frozen or ethanol-preserved tissue (Sambrook *et al.*, 1989). The tissue was then pulverized and incubated with first with proteinase-K and then with RNAase, followed by hot phenol-chloroform and chloroform separation of organic components. Ethanol-precipitated total genomic DNA was then used for polymerase chain reaction (PCR) amplification.

All 1144 base pairs of the mitochondrial gene, cytochrome-*b*, were amplified by standard PCR techniques. The primer pair used consisted of S62 (MV214) and S63 (MV25) at a volume of 2.5 μ l and concentrations of 10 μ M. The primer sequences are:

Forward S62 (MV214) 5'-GGTCTTCATCTYHGCTTACAAGAC-3'

Reverse S63 (MV25) 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'

The PCR reaction cocktail consisted of 15.65 μ l H₂O, 2.5 μ l 10 x reaction buffer, 2 μ l MgCl₂ (25mM), 1.25 μ l DMSO, 0.15 μ l dNTP (25mM), and 0.15 μ l *taq* polymerase (5 U/ μ l) in a volume of approximately 25 μ l. The polymerase chain reaction had an initial denaturation step at 94° C for 12 minutes. This was followed by 40 cycles consisting of

denaturation at 94° C for 45 min, primer annealing at 51° C for 45", and extension at 72° C for 1'15" for 40 replicates followed by a final extension of 72°C for 6 minutes and finishing with a final hold at 4°C (∞). Positive and negative controls with λ -DNA and positive and negative controls for the *cyt-b* gene were used. PCR products were then assayed using standard agarose gel electrophoresis.

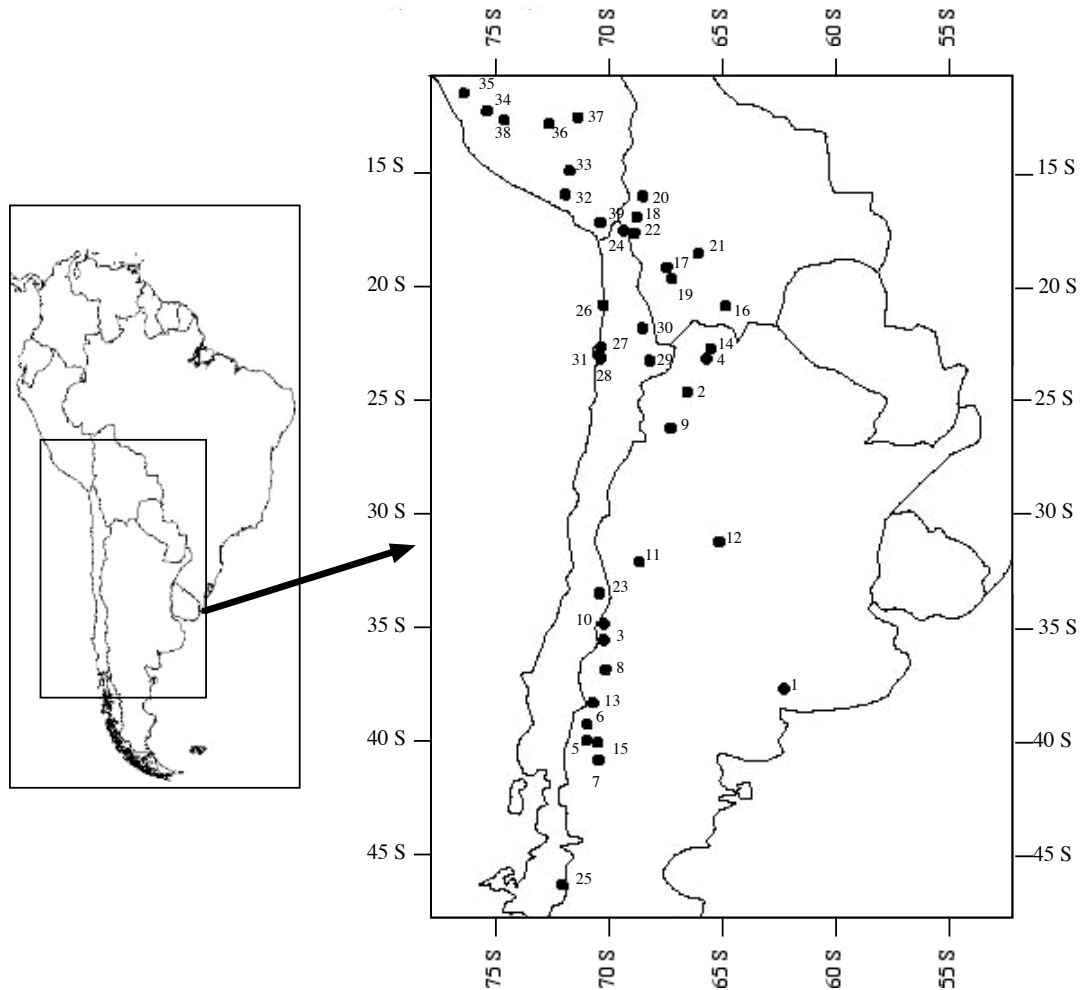


Figure 2 Sampling Localities Map. Only locality numbers are shown on the map. Location names are given in Table 1.

PCR products were purified by either PEG precipitation or the Exo-SAP-IT method. Automated sequencing was carried out at the Florida State University Sequencing Facility using an Applied Biosystems 3100 Genetic analyzer with capillary electrophoresis and ABI Prism dye terminator sequencing chemistry. The resulting sequences were edited and aligned in Sequencher 3.0 (Gene Codes Corp.). Sequences are deposited in GenBank.

Phylogenetic Analyses

We performed maximum parsimony and maximum likelihood searches on the consensus sequences using PAUP* 4 beta 10 (Swofford, 2002). We used the multinode PETAL computer cluster operated by the School for Computational Sciences at Florida State University. For the parsimony analysis we used equally weighted parsimony in a heuristic search with random-addition replicated and Tree Bisection and Reconnection (TBR) branch swapping. 100-replicate nonparametric bootstrap analyses were employed for both maximum parsimony and maximum likelihood to assess robustness (Felsenstein, 1985). Maximum-likelihood analyses were conducted using the gamma-distributed rates (GTR+ Γ +I) substitution model with parameters estimated from the data (Yang, 1994). We had evidence from genus-wide phylogenetic analyses (Steppan, 1998) that *Phyllotis darwini* is closely related yet reciprocally monophyletic with *P. xanthopygus*, so we used four individuals from this species as an outgroup. All specimens were given subspecies designations based off their locations compared with historical usage. As expected within a species, these subspecies should not be considered as clear boundaries between lineages or clades. We also did not attempt to make a morphological diagnosis for every specimen, but these subspecies do represent geographic units.

In order to estimate important divergence dates we also assessed the depth of four cladogenetic events on the phylogeny under a molecular clock framework. Molecular divergences were estimated directly from branch lengths. We did not test for the suitability of a molecular clock or apply any kind of rate smoothing or within phylogeny calibration. Steppan *et al.* (2004) showed that the mammalian genetic divergence rate of ~2% per million years is an underestimate of molecular divergence for muroids. Their recalibration of 7.3 %/million years for mitochondrial genes used an estimate for the origin of Murines of 12-Mya This new rate was used to obtain the divergence dates.

Nested Clade Analyses

The haplotype network was generated using Templeton *et al.*'s (1992) statistical parsimony approach within the program TCS 1.13 (Clement *et al.*, 2000). Most of the deeper divergences exceeded the maximum allowable number of mutational steps as outlined in Templeton *et al.* (1992). In other words, the divergence exceeded the probability for parsimony for pair-wise differences exceeds the 0.95 cut-off level. In those cases the program was therefore not able to join the entire network together. In order to bridge the gaps between sub-networks, we used the maximum-likelihood phylogeny generated in PAUP as a guide to deep level topology and branch lengths. This did not affect the distribution of haplotypes in sub-networks derived from TCS. Necessary mutational steps between sampled haplotypes are treated as unsampled haplotypes. Individuals with identical haplotypes are combined into haplotype-level clades. We then utilized the nesting algorithm described in Templeton and Sing (1993) to organize the haplotype network into a series of nested clades. In this scheme the lowest level clades correspond to the tip haplotypes in the network. Higher nesting levels incorporate new sampled haplotypes at a distance of one mutational step away while working towards the interior of the network. For all inferences we used the most recent version of the Templeton inference key (Templeton, 2004).

Table 1. Sampling Localities. The locality number, latitude and longitude, and sample size (N) are included.

Number	Location Name and Province	Latitude	Longitude	N
Argentina localities				
1	Abra de la Ventana, Buenos Aires Province	38.00 S	62.00 W	2
2	Alto Cachi, Salta Province	25.03 S	66.22 W	2
3	Bardas Blancas, Mendoza Province	35.83 S	69.88 W	3
4	Camino a Garganta del Diablo, Tilcara, Jujuy Province	23.59 S	65.37 W	1
5	Cerrito Piñon, Collón Curá., Neuquén Province	40.25 S	70.63 W	3
6	Las Coloradas, Neuquén Province	39.56 S	70.59 W	1
7	Comallo, Río Negro Province	41.10 S	70.10 W	1
8	Corcel Negro, Buta Ranquil, Neuquén Province	37.15 S	69.81 W	3
9	Laguna Blanca, Catamarca Province	26.61 S	66.94 W	3
10	Laguna de la Niña Encantada, Malargue, Mendoza Province	35.16 S	69.87 W	2
11	Mendoza, Mendoza Province	32.46 S	68.30 W	1
12	Pampa de Achala, San Luis, Córdoba Province	31.58 S	64.83 W	5
13	La Porteña, Las Lajas, Neuquén Province	38.61 S	70.35 W	2
14	Sierra de Zenta, Humahuaca, Jujuy Province	23.13 S	65.20 W	1
15	Yuncón, Piedra de Aguila, Neuquén Province	40.35 S	70.13 W	2
Bolivia localities				
16	Abra Condor, Tarija Province, Tarija Department	19.59 S	65.22 W	2
17	Escuela Seccional Villa Ventilla, Oruro Department	19.60 S	67.12 W	1
18	Huancarama, Huancarama Province Oruro Department	17.40 S	68.41 W	1
19	Laguna Colorada Daniel Campos Province, Potosí Dept.	20.08 S	66.90 W	1
20	La Paz, La Paz Province, La Paz Department	16.50 S	68.15 W	1
21	Potosí, Potosí Province, Potosí Department	19.50 S	65.90 W	2
22	Sajama, Sajama Province, Oruro Department	18.10 S	68.54 W	1
Chile localities				
23	Baños Morales, Región Metropolitana	33.83 S	70.05 W	5
24	Chapiquina, Arica, Region I (de Tarapaca)	18.29 S	70.20 W	1
25	Chile Chico, Región XI	46.55 S	71.70 W	1
26	Desembarcadura Río Loa, Región II	21.25 S	69.89 W	1
27	Mejillones, Región II	23.08 S	70.00 W	1
28	Los Molles, Región II	23.58 S	70.00 W	1
29	Talabre, Región VI	23.67 S	67.85 W	1
30	Q.J. Toconao, Región II	22.27 S	68.18 W	3
31	El Yeso, Región II	23.42 S	70.05 W	2
Peru localities				
32	Arequipa, Arequipa Province, Arequipa Department	16.42 S	71.53 W	20
33	Chivay, Caylloma, Province Arequipa Department	15.38 S	71.36 W	1
34	Huancavelica, Huancavelica Province, Huancavelica Dept.	12.77 S	74.98 W	3
35	Huarochiri, Huarochiri Province, Lima Department	12.00 S	76.00 W	3
36	Ollenta, Cuzco Province, Cuzco Department	13.33 S	72.25 W	1
37	Pisac, Calca Province, Cuzco Department	13.05 S	71.00 W	2
38	Ramichaca, Ayacacho Province, Ayacacho Department	13.17 S	74.22 W	1
39	Tarata, Tacna Province, Tacna Department	18.02 S	70.25 W	1

To assist in geographic analyses, we created a GIS file in ArcView 3 (ESRI GIS) with the sample localities mapped in South America. Elevation information was added in the form of hypsographic points that were then connected into topographic contour lines. The measuring tool in ArcView was used to measure the distance along lines chosen to reflect constrained dispersal routes. Both of these measurements use *a priori* criteria to better approximate plausible dispersal options of living mice. The rationale of this approach is to make the estimates of haplotype and clade spread more biologically realistic.

The first alternative distance, henceforth referred to as “1000 meter interval”, was based on minimizing the amount of elevation change the mice would experience in moving between locations. The biological assumption was that vertical movement over large altitude intervals should be more difficult than movements over the same altitude interval. This does not mean that phyllotines lack the physiological and ecological flexibility to live at these different elevations. It is just assumed that individuals would preferentially limit their vertical dispersal into different ecological zones.

The algorithm we used to generate the new distances is relatively simple. Hypsographic information was obtained from ESRI’s 1993 version of the Digital Chart of the World (DCW) project and was added to the sample locality map contoured into isolines of one kilometer. These isolines were used as a way to break a continual elevation gradient into discrete units. Paths between localities were constrained so that the fewest number of contour lines would be crossed. For instance, in Figure 3 the shortest path, corresponding to the Great-Circle distance crosses six isolines while the 1000-meter interval path only crosses two isolines. We assumed that vertical movement across isolines was a rare event as a simplification in order to make the problem more tractable. These distances were computed for every locality pairing, and a distance matrix was generated.

It should be pointed out that using a different contour interval would result in different distance estimates. This is because the amount of topographic detail varies with the choice of isoline mapping. Physiognomic features with elevation changes of less than a kilometer will be invisible to this technique. Also, we know the contours extend over the observed distribution of *Phyllotis* so it is obvious that for the species as a whole the ecological and environmental changes associated with elevation do not exceed the species’ tolerance limits. However, taking elevation into account at all increases the complexity of the distance-determination model over a simple great-circle distance. Furthermore, contour intervals of one kilometer seem likely to be reasonable given the level of vagility of *Phyllotis*. Elevation shifts of less than one kilometer are likely to be so short in terms of ecological distance and the actual dispersal distances of the mice that these short distances should make little impact on movement corridors.

The second distance, referred to as above 1000 meter, used a different set of criteria. The biological assumption here was based on imposing a lower elevation limit for movements. There was no constraint to crossing elevation lines above 1000 meters, but the paths cannot cross below a minimum elevation of 1000 meters. In those cases where the migration paths had to cross low-elevation areas, the shortest path across the lowlands area was chosen whether or not this was the shortest distance to the next locality. One way in which this method may be an improvement over the 1000-meter interval distance is that it’s taking into account a more reliable proxy for habitat suitability. At lower elevations *Phyllotis* habitat becomes more rare, and therefore setting a lower bounds is one way to look at a known environmental barrier.

The largely north—south orientation of the Andes means that most of our sampling localities are distributed in a linear fashion from north to south. Since, for the most part, the topographic lines were oriented along the same axes, there was relatively little difference in the distances based on elevation versus strict great-circle distances (see Appendices 3 and 4). These distance metrics make simplistic and somewhat arbitrary assumptions about biological dispersal. However they are an improvement over great-

circle distances because great-circle distances makes the larger assumption that animal dispersal is not affected by topographic features.

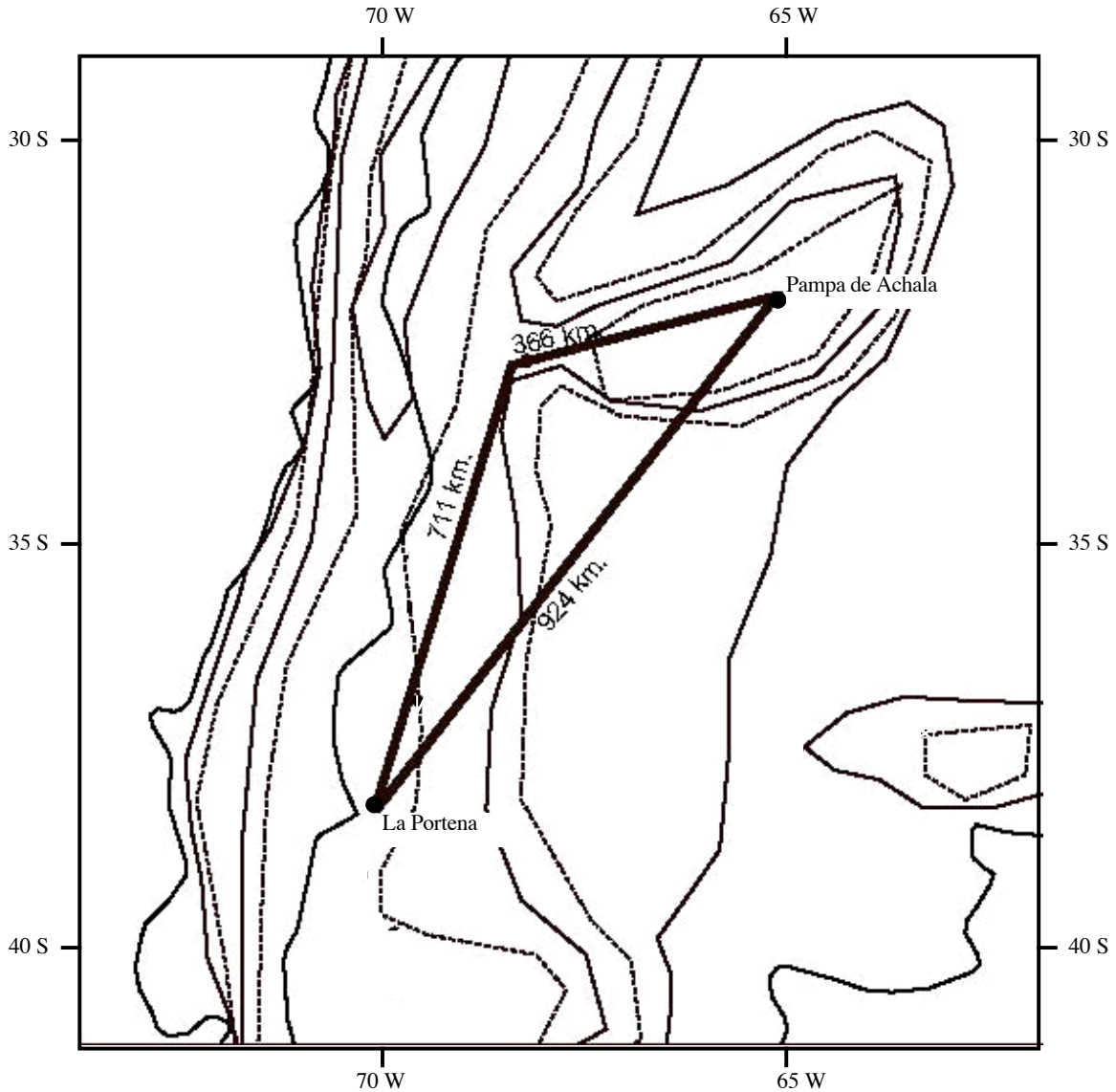


Figure 3. An Alternative Distance Measurement. The great-circle distance between Pampa de Achala and La Portena is 924 km., but when elevation changes are minimized the new constrained distance is 711 km. + 386 km.= 1097 km. The isolines correspond to contour lines of 1 km.

To carry out the analyses of nested clades, the program Geodis 2.0 (Posada *et al.*, 2000) was used. We performed three separate analyses based on the three alternative distance frameworks utilized in this study. The first analysis was a standard NCA run using the latitude and longitude points to define D_c and D_n values. The second analysis employed the 1000 meters interval distances, and the third analysis used the above 1000 meters distances. The distances between all localities were included in matrix format under the “user-defined distances” option of Geodis.

RESULTS

Phylogenetic Results

The cytochrome-*b* maximum likelihood phylogeny was well-resolved and had good bootstrap support (most major nodes are over 50%, and most major nodes close to 100%). This tree confirms previous findings for *Phyllotis* and shows several novel results that have only become clear with the increased taxon sampling of the present study. Figure 4 shows the phylogeny, with maximum likelihood branch lengths and bootstrap support values. The tree topology shows great haplotype diversity and very deep divergences within *P. xanthopygus*. These deep divisions tend to be of geographically related clades with little overlap between regions. There is a basal division into two major clades (Figure 4: Altiplano and North—South). These two large clades coincide with the high-elevation plateau of the altiplano and the main north-south range of the Andes. Additional support for this pattern comes from nuclear gene phylogenies (Steppan, in press) that show the same basal division into Altiplano and N—S clades, although the resolution at the tips is poor in the nuclear gene trees.

Another major observation is that of the paraphyly of *Phyllotis xanthopygus* with respect to the species *Phyllotis limatus* and *Phyllotis bonariensis*. Of course the subspecific designations are not necessarily indicative of monophyletic gene lineages, but paraphyly does indicate when historically recognized geographic populations are not discrete entities. There is strong bootstrap support (94%) of the node placing *P. limatus* within the N—S clade of *P. xanthopygus*. More specifically, *P. limatus* is found to be the sister group to the clade containing the non-altiplano populations of *P. x. rupestris* and the main western population of *P. x. vaccarum*. The precise placement of *P. bonariensis* (usually as the sister group to the *P. x. vaccarum* of the disjunct eastern Andes) is less well-supported, but the best-supported trees consistently show *P. bonariensis* falling within the N—S clade of *P. xanthopygus* as well.

The complete haplotype network is shown with the individuals within haplotypes (Figure 5). The groupings of tip clades based on statistical parsimony and the tip clades from the maximum likelihood analysis are largely congruent. There are slight differences though. For instance, in the haplotype network, UP 411 and 439 are closer to each other than to UP 440. In the rooted phylogeny, UP 439 and 440 are sister to each other. The bootstrap support in the phylogeny for the 411-439-440 clade is 87% and the support for the sister group relationship between 439 and 440 is 64%. Moreover, Templeton's haplotype networks are able to incorporate multiple connection pathways. They are not constrained to tree-like bifurcations. One such case in this particular network is in the same clade of UP 411, 439, and 440; there are two alternative connections between the three haplotypes included in the network (Figure 5). Two things the network shows very well is that there are few shared haplotypes, and no geographically wide-spread ones.

NCA Results

The same set of nine clades with significant p-values for the initial chi-square test was found using the three different distance metrics (as shown in Tables 2–5). Most of these examples were clustered at the higher (more inclusive) clade levels, although there was one clade found to be significant at the relatively low 2-level. The higher clade levels included one clade each at the 6, 7, 8 and 9 levels. Three of the level-10 clades had significant results, and one of the level-11 clades was significant. Also, in many cases, the same lower level clades are included in the more inclusive higher clade levels, and this is shown by the connections in Tables 2 and 3.

The principal differences found between the three analyses were in the D_C , D_N , and I-T P-values, and, to a lesser degree, in the inferences drawn from them. The two alternative distance datasets did not produce significantly different values of D_C , D_N , and I-T, and the inferences drawn were entirely congruent. There were, however, differences between the alternative distance metrics as a group and the standard great-circle analysis in both the values of D_C , D_N , and I-T and in the conclusion drawn from them.

Table 2 contrasts the raw D_C , D_N and I–T values from the standard and alternative distance NCA analyses using great-circle distances for those clades with both significant D_C and D_N and an I–T contrast. If the tip/interior status was unresolved then no conclusions could be drawn even if D_C and D_N were significant. Significantly smaller and larger D_C and D_N values than expected are indicated with a superscript ^S for small and ^L for large. Notice that in general the 1000-meter analysis had the same or fewer significant values than the great-circle analysis. The one exception is the case of 2-1. In the great-circle case there were no significant values beyond the initial chi-square test, but the 1000-meter results include significantly small values for the within clade and nested clade I-T contrasts.

Some of the differences in D_C and D_N shown in Table 2 are quite large, and are greater than what would be expected given the fact that the pairwise differences between localities was not of the same magnitude. The reason for this is that the D_C and D_N metrics are measures for an entire clade and the individual locality differences can therefore be magnified greatly.

Table 3 contains the inferences drawn from the great-circle analysis and also shows what localities were included in each nested clade. The inferences drawn from the 1000-meter interval analysis are included in Table 4.

Major differences in outcome were found in clades 2-1, 6-1, 7-1, 10-3, and 11-2. In the first case, clade 2-1, the great-circle analysis had an inconclusive outcome but the alternative distance metrics returned a result of contiguous range expansion. The great-circle analysis of clade 6-1 produced the inference chain 1(No)-2(No)-11(Yes)-12(No). The inference that is drawn from this chain is that of a contiguous range expansion, but in the alternative distance scheme the chain follows the path 1(No)-2(Yes)-3(No)-4(No), which is consistent with restricted gene flow with isolation by distance. For 7-1, the great-circle inference chain is 1(No)-2(No)-11(Yes)-12(Yes)-13(No)-14(No) which results in a conclusion of long-distance colonization and/or past fragmentation, while the alternative distances produced the chain 1(No)-2(No)-11(Yes)-12(No), or contiguous range expansion.

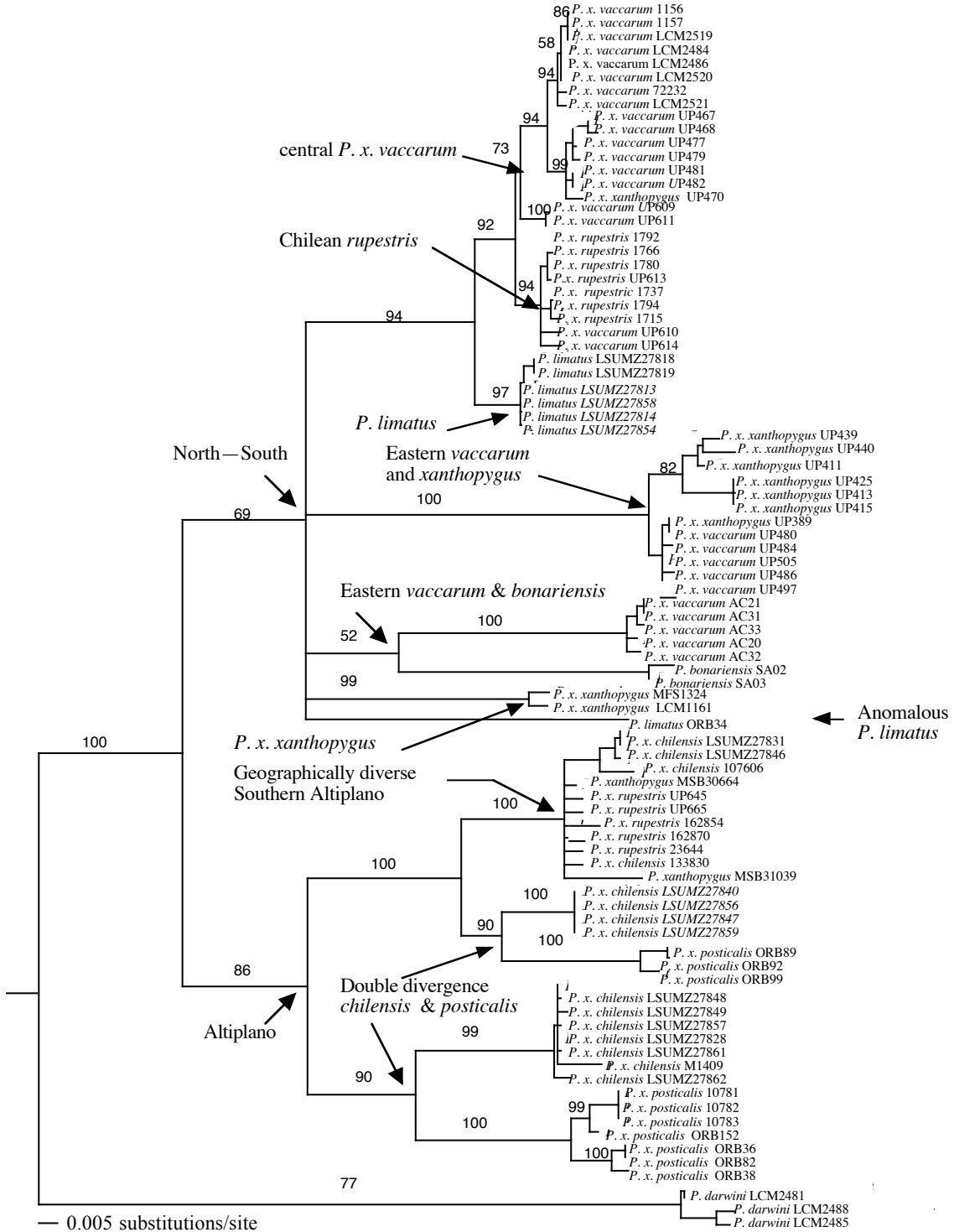


Figure 4. Maximum Likelihood Cytochrome-*b* Phylogeny with Maximum Likelihood Bootstraps. Discussed nodes are labeled.

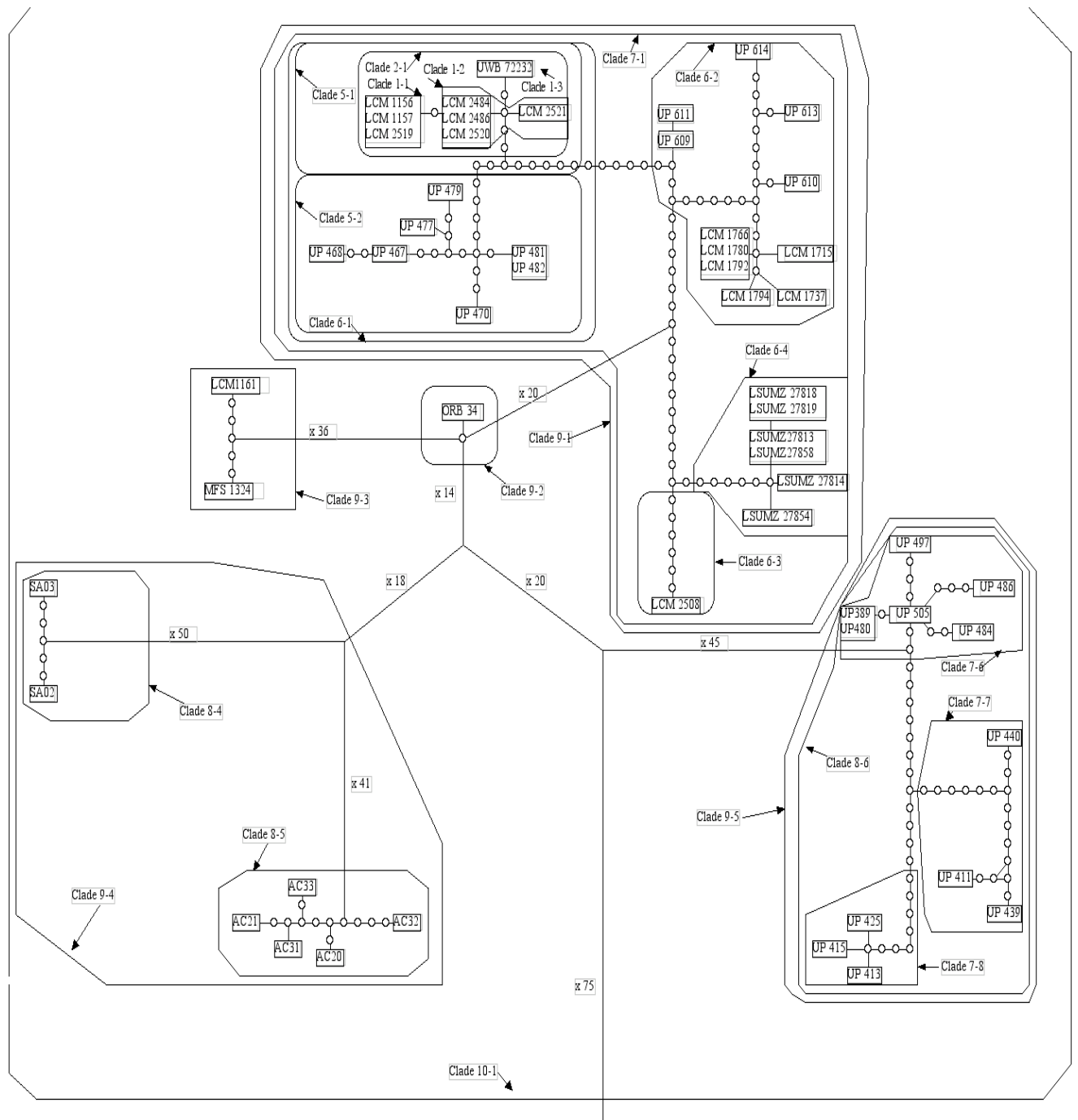


Figure 5. The North—South Clade of the Statistical Parsimony Haplotype Network With Individual Specimens Identified and Significant Nesting Clades Indicated.

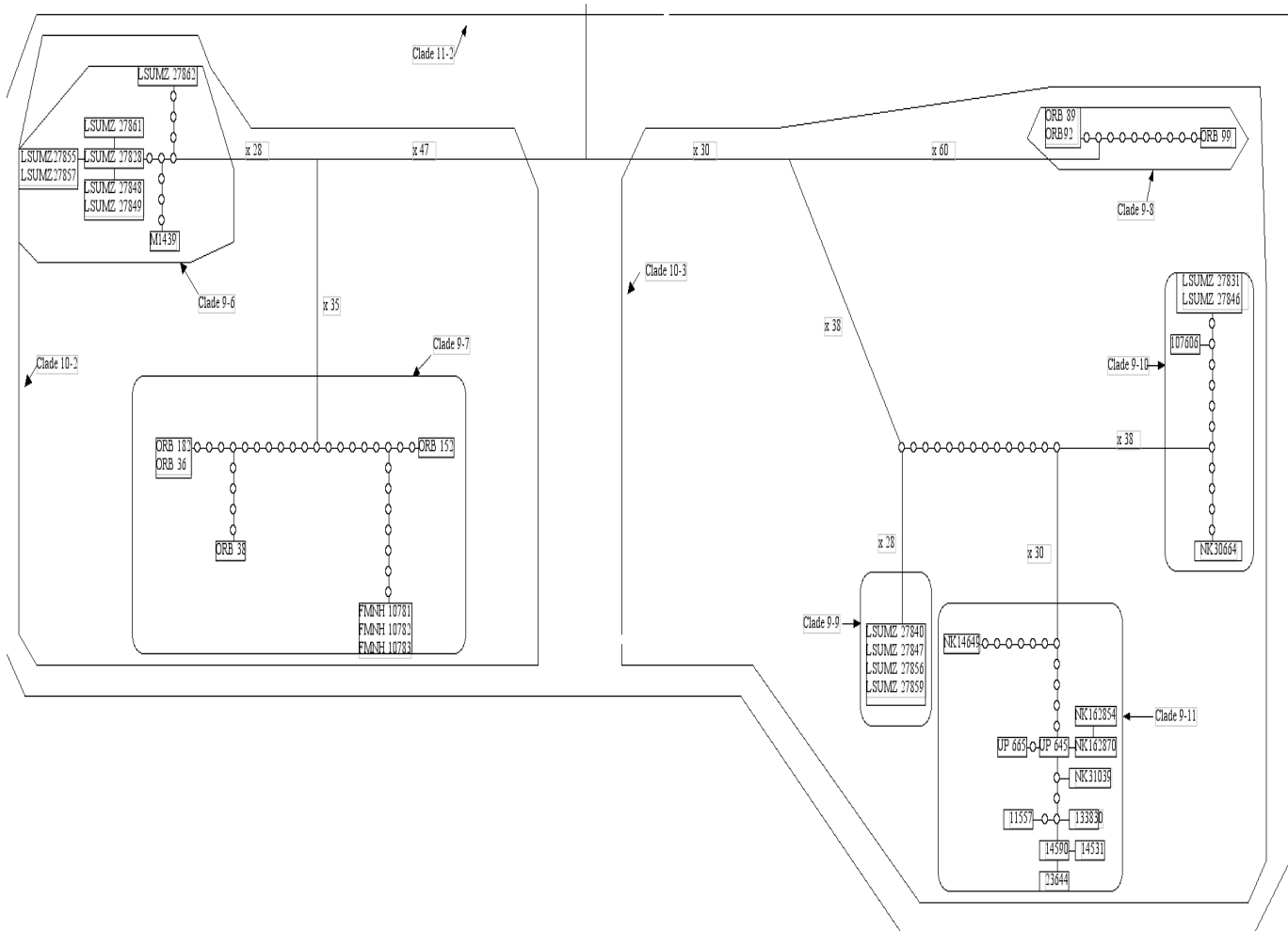


Figure 6. The Altiplano clade of the Statistical Parsimony Haplotype Network With Individual Specimens Identified and Significant Nesting Clades Indicated.

10-3 and 11-2 both have the same differences. In the case of the great-circle data set the conclusion drawn is long-distance colonization and/or past fragmentation (1-No-2-No-11-Yes-12-Yes-13-No-14-No) while in the elevation-based analyses the conclusion is that of contiguous range expansion (1-No-2-No-11-Yes-12-No). Clades 8-6, 9-4, 10-1, and 10-2 produced essentially the same results with all three analyses. In these cases the inference key proved fairly robust to small changes in clade distance measures, and it was sometimes the case that an early deviation in the inference key would ultimately return the same result despite a very different path. For instance, in clade 8-6 the inference chain of the great-circle analysis is 1(No)-2(Yes)-3(No)-4(No) resulting in a conclusion of restricted gene flow with isolation by distance, while in the alternative distance analyses the chain was 1(No)-2(No)-11(No)-17(Yes)-4(No), ending with the same conclusions.

In the case of clade 10-1, the inferences were nearly identical, but differed in one additional detail. The alternative distance analyses yielded the chain 1(No)-2(Yes)-3(No)-4(No), which as discussed above is restricted gene flow with isolation by distance. The great-circle analysis of the same clade level produced the inference chain 1(No)-2(Yes)-3(Yes)-5(No)-6(No)-7(Yes) and results in the conclusion of restricted gene flow with the addition of some long-distance dispersal.

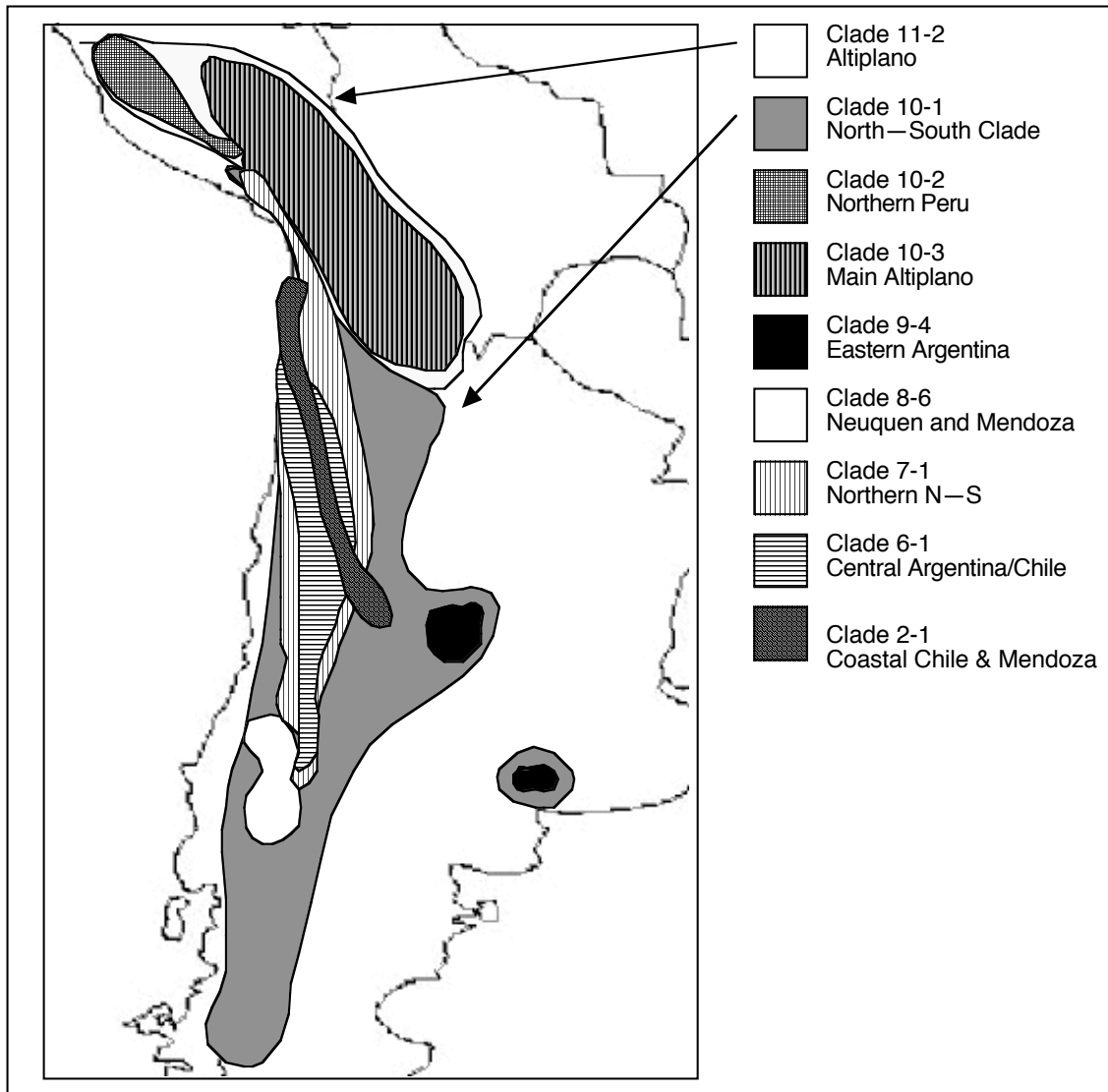


Figure 7. Significant Nested Clades Overlaid on Geography. Note that the boundaries of clades are not exact. Inclusive clades are drawn larger for visual clarity.

Table 2. NCA Results For Clades Producing Geographic Conclusions. The results for Great-Circle and the Alternatives are compared. Note the “T” indicates a tip clade, “I” indicates an internal clade, “S” indicates a significantly small result, and “L” indicates a significantly large result.

Great-Circle Distance				Alternative Distances			
Clade	D _c	D _n	Nesting Clade	Clade	D _c	D _n	Nesting Clade
1-1 ^T	17.1	51.6		1-1 ^T	936.0	846.9	
1-2 ^I	0.0	92.6	2-1	1-2 ^I	0.0 ^S	467.5	2-1
1-3 ^T	0.0	131.3		1-3 ^T	0.0	545.9	
I-T	-12.9	21.1		I-T	-702.0 ^S	-304.1 ^S	
5-1 ^T	89.1	103.3		5-1 ^T	689.8	711.0 ^L	
5-2 ^I	164.2 ^L	147.1	6-1	5-2 ^I	104.7 ^S	496.9	6-1
I-T	-75.2 ^S	-43.8		I-T	585.1 ^L	214.1	
6-1 ^T	142.9 ^S	695.7		6-1 ^T	610.8 ^S	1114.2	
6-2 ^I	217.5 ^S	511.3 ^S		6-2 ^I	473 ^S	936 ^S	
6-3 ^T	0	488.0	7-1	6-3 ^T	0.0	0.12	7-1
6-4 ^T	0 ^S	1299.3		6-4 ^T	0.0	1449.9	
I-T	119.9	-339.6 ^S		I-T	56.8	-257.6 ^S	
7-6 ^I	289.3	346.0 ^L		7-6 ^I	240.7	330.7 ^L	
7-7 ^T	68.9	118.5 ^S	8-6	7-7 ^T	147.2	258.7 ^S	8-6
7-8 ^T	36.8 ^S	210.5		7-8 ^T	65.6	284.4	
I-T	236.5 ^L	181.6 ^L		I-T	134.3	59.2 ^L	
8-4 ^T	0.0	378.4 ^S		8-4 ^T	0.0	1463.6 ^L	
8-5 ^I	0.0	380.1 ^L	9-4	8-5 ^I	0.0 ^S	805.0 ^S	9-4
I-T	0.0 ^S	1.7 ^L		I-T	0.0	-658.6 ^S	
9-1 ^T	606.0 ^S	677.6		9-1 ^T	1103.9 ^S	1252.3	
9-2 ^I	0.0	2359.2 ^L	10-1	9-2 ^I	0.0	2535.0 ^L	10-1
9-3 ^T	309.4	1292.5 ^L		9-3 ^T	621.0	1562.3	
9-4 ^T	379.2	659.4		9-4 ^T	1038.7	1376.0	
9-5 ^T	257.8 ^S	641.8		9-5 ^T	302.6 ^S	1069.6 ^S	
I-T	-488.2 ^L	1669.2 ^L		I-T	-888.6	1298.9	
9-6 ^I	0.01 ^S	506.6		9-6 ^I	0.0 ^S	438.2 ^L	
9-7	83.1 ^S	93.0 ^S	10-2	9-7	125.3 ^S	449.1 ^L	10-2
I-T	-83.1	413.6 ^L		I-T	-125.3 ^S	-10.9	
9-8 ^T	43.4 ^S	686.8 ^L		9-8 ^T	110.4 ^S	694.8	
9-9 ^I	0.01 ^S	400.2		9-9 ^I	0.0 ^S	651.5	
9-10 ^T	184.4	293.5	10-3	9-10 ^T	336.6 ^S	567.7	10-3
9-11 ^T	253.9 ^S	317.2		9-11 ^T	410.4 ^S	702.1	
I-T	-202.4	30.9		I-T	-343.6	-14.0	
10-2 ^I	143.4 ^S	722.9 ^L		10-2 ^I	443 ^S	660.61	
10-3 ^T	364.9 ^S	391.2	11-2	10-3 ^T	674.1	714.3	11-2
I-T	-221.5 ^S	331.7 ^L		I-T	-230.2	-53.7	

DISCUSSION

Phylogenetic Conclusions and Systematic Implications

Phyllotis xanthopygus is a species with a significant amount of geographic variation that has been parsed into discrete subspecies. The divergences within *P. xanthopygus* are quite deep and are comparable to the amount of molecular divergence found between species for the genus as a whole. This suggests that at the very least, local populations have been diverging in relative isolation for a considerable amount of time.

In general there was little correlation with historical subspecies definitions and most groups were paraphyletic. For instance, *Phyllotis xanthopygus vaccarum* and *P. x. rupestris* fall out in multiple geographically nonconcordant clades. Although the criteria by which subspecies have been described (typically morphological features that vary consistently within regions of a species range) does not necessarily require monophyly, it is typically assumed that there is some degree of shared evolutionary history giving an at least passing identity to subspecies. Those members of *P. x. vaccarum* in the central Andean cordillera through Chile and Argentina are not closely related to *P. x. vaccarum* in the somewhat disjunct range further east. *P. x. rupestris* is divided into a clade within the N—S group and other clades in the altiplano group. *P. x. chilensis* and *P. x. posticalis* show patterns of apparently parallel diversification where member of *P. x. chilensis* are sister to members of *P. x. posticalis* in two separate clades which are themselves sister to each other.

The paraphyly of *P. xanthopygus* with respect to *P. limatus* and *P. bonariensis* has implications for both the systematics of the group and for their pattern of speciation. The individuals currently recognized as *P. limatus* consist of populations originally in the subspecies *P. x. limatus* and the northern Pacific slope populations of *P. x. rupestris*. Steppan (1998) argued for the species status of *Phyllotis limatus* on morphological and molecular grounds. This species is unique within the genus in having quite narrow and deep incisors. *P. limatus* also has a slightly longer tail and a generally lighter pelage color than *P. xanthopygus*. The evidence for *P. bonariensis* being a separate species is less strong, and largely stems from the isolation of the Sierra de la Ventana range in eastern Argentina. These findings suggest either that the two species are either really just subspecific varieties of *P. xanthopygus*, or that *P. xanthopygus* is a paraphyletic but biologically justifiable species, or that there are in fact multiple species.

In the case of a geographically widespread species it is possible that peripheral populations might diverge enough to speciate, and if these divergences are recent enough than reciprocal monophyly will not be achieved. This would be an example of a phenomenon known as centripetal speciation (Frey, 1993); not the classical model described by Mayr (1963), but it is analogous if the amount of gene flow between the peripheral areas is low enough. In this way individuals of the central species would be more closely related to individuals from the nested species than to their own conspecifics. So it is possible that there could be two daughter species nested within a larger species.

Note that, for the most part, *P. limatus* does form a monophyletic group, but that there is one anomalous individual, ORB 34. This individual was collected in Huarochiri, Peru, in a region associated with *P. limatus* but that there is some uncertainty as to its identity. This individual is a juvenile and, as such, does not clearly show the dental morphology and other unique character of *P. limatus*.

The other systematic problem in the group is the possibility of there being at least two, possibly more, cryptic species. The major division of the tree is into the North—South and Altiplano clades. Previous studies had too little data to suggest this divide, and it was not suspected prior to increased sampling molecular work within this species. Furthermore, the mitochondrial phylogeny can be divided into approximately 10—11 clades separated from other each other by long branches. These divisions include *P. limatus* and *P. bonariensis*, which have comparable branch lengths to the rest. It is possible that each of these lineages might represent a separate species. If that were the case, then *P. limatus* and *P. bonariensis* would be just another pair of closely related species within the species complex of *P. xanthopygus sensu lato*. Although the amount of morphological variation, mainly localized pelage differences, does not suggest the presence of these species, the molecular data is highly suggestive. We recommend performing karyotyping and breeding experiments to explore the species limits.

We used a very approximate estimation employing a molecular clock to date important nodes. In this way we obtained dates for the divergence of *P. limatus* from the northern members of the North—South Clade, for the split between *P. bonariensis* and the eastern members of *P. x. vaccarum*, and for the origin of the Altiplano Clade/North—South Clade divide. *Phyllotis limatus* appears to have had an independent lineage for ~140,000 years. This timescale seems appropriate for a recent speciation during the Pleistocene, and is also in accord with Kuch *et al.*'s findings that 11,700-year old paleo-DNA shows little divergence from modern *P. limatus* sequences. The division of *P. bonariensis* and eastern *P. x. vaccarum* is characterized by a greater branch length that indicates a divergence ~820,000 years ago. This date is also within the late Pleistocene. This estimate provides an upper bound on the isolation of *P. bonariensis* (either by vicariance or colonization) because the genetic divergence would necessarily predate the onset of geographic isolation. The origin of the Altiplano and North—South clade is dated to 1.4 Mya, in the early Pleistocene. The date we obtained for the split between *P. darwini* and the *P. xanthopygus* complex falls in the late Pliocene at 2.1 million years ago. The branch lengths strongly indicate that *P. xanthopygus* is an old taxon. Also, the depth of the earliest branching events suggests that although hypotheses based on Pleistocene paleoclimate are suitable for the more recent events, the basal divisions require explanations other than Pleistocene glaciations.

Phylogeographic Conclusions

As mentioned before, the most basal division is between the Altiplano clade and the North—South clade. We have already discussed the systematic implications of this division, but the phylogeographic implications are just as intriguing. There is very little to suggest that there should be a major geographic barrier between the Altiplano and the

rest of the Andes, particularly because the northern extent of the Altiplano clade continues into areas north of the true Altiplano. It is possible that the basic division represents an ancestral fragmentation and that the two, still largely allopatric, clades have expanded and come into secondary contact.

Within the North—South clade, working from the “top” of the phylogeny down, we recover seven clades of particular phylogeographic interest (see Figure 4); the northern populations of *P. x. vaccarum*, western populations of *P. x. rupestris*, *P. limatus*, southern *P. x. vaccarum*, the *P. x. vaccarum* and *P. bonariensis* clade, and *Phyllotis x. xanthopygus*.

The northern populations of *P. x. vaccarum* can be split roughly into a group along the Chilean coast, and others extending to the south and crossing to the east into western Argentina.

The western populations of *P. x. rupestris* is an informative clade in that it includes individuals in northern Chile (coastal and inland) and individuals in Salta, Argentina on the other side of the Atacama Desert where there is no evidence of *Phyllotis*. The absence of *Phyllotis* in the Atacama suggests that this clade loops down to the southeast before coming extending back to the north. But there obviously is, or has been, some degree of trans-Andean movement in this region to explain this distribution pattern. These individuals have helped to refine our knowledge of the boundaries between the Altiplano and N—S clades because just north of Salta, in Jujuy Province are members of the Altiplano clade. The fact that the Salta individuals are more closely related to those on the other side of the Atacama than they are to the much more proximate populations in Jujuy is highly suggestive of a restriction on trans-Andean.

Phyllotis limatus represents the northern-most extent of the North—South clade. They range from northern Chile up along the coast into southern Peru, but do not reach further inland into the Altiplano. The sister group relationship of *P. limatus* to the northern members of the North—South clade in Chile is reasonable, and suggests that *P. limatus* is located in the general region in which it diverged, and probably speciated, from *P. xanthopygus sensu strictu*. Although fossil midden evidence (Kuch *et al.*, 2002) from the Atacama region indicates that the southern limits of *P. limatus* have shifted northward.

The clade of southern *P. x. vaccarum* is restricted to Neuquen and Mendoza provinces in central-western Argentina. Within the clade there is very little geographic structuring. Individuals from the northern and southern ends of these provinces are scattered throughout the clade.

The next group of interest is the *P. bonariensis*+*P. vaccarum* clade. All of these eastern *P. x. vaccarum* individuals are restricted to the eastern ranges of the Andes, east of Mendoza in the Pampa de Achala. *Phyllotis bonariensis* is localized in the disjunct Sierra de la Ventana even further to the east, and the south. This area is closer to the Atlantic coast than to the main chain of the Andes. The southern *P. x. vaccarum* group of Neuquen and southern Mendoza provinces is located slightly closer to *P. bonariensis*, albeit further west than the eastern *P. x. vaccarum* individuals of Pampa de Achala, they are not the sister group. It seems likely that the center of origin for *P. bonariensis* (either through range expansion followed by secondary fragmentation or via long-distance colonization) was to the north in Pampa de Achala. However, *P. bonariensis* is clearly

sister to the eastern clade of *P. x. vaccarum* and not nested within the clade, which suggests that the origin was not a recent long-distance colonization.

Phyllotis xanthopygus xanthopygus is the most southern subspecies. The fact that all of the southernmost members of *P. x. vaccarum* cluster together and not with this clade confirms that the edge of the range for this group is to the south (between Rio Negro Province and Neuquen Province).

Within the Altiplano clade there are three major clades of interest: the central group of *P. x. rupestris* and *P. x. chilensis* in the main southern Altiplano, the twin clades of *P. x. chilensis* and *P. x. posticalis*.

The diverse altiplano clade contains individuals recognized as *P. x. chilensis* and *P. x. rupestris* from a number of localities from nearly the entire altiplano. It is a remarkable clade because unlike other comparable clades in *P. xanthopygus* it does not show very localized pattern seen elsewhere. It is possible that the topography of the altiplano plateau results in more dispersal and gene flow and allows for more widespread haplotypes.

The next two clades are quite interesting, and seem to represent a parallel division in separate clades. The specimens of *P. x. chilensis* in these clades are located in the region of Arequipa in southern Peru. There are two major *P. x. chilensis* clades, and in each case, the sister group consists of members of *P. x. posticalis*. The *P. x. posticalis* specimens come from different locations in northern Peru. It suggests that what we recognize as the subspecies *P. x. posticalis* might represent two separate movements into northern Peru from a source population in southern Peru. One of the *P. x. posticalis* clades seems to be more of a western population and the other seems to be more central north.

One of our hypotheses was that the Altiplano plateau might facilitate dispersal because of its decreased amount of physiognomic relief. This hypothesis is supported by two lines of evidence. One is that although most clades are very localized, the dominant clade in the Altiplano is very widespread. Most clades contain between one and three localities. This clade has ten from all over the Altiplano. Furthermore, the NCA results suggest that population movements (either long-distance dispersal or range expansion) are the dominant feature with little evidence of restricted gene flow. Several locations in the North—South clade show evidence of restricted gene flow.

The NCA analysis is divided into roughly comparable clades, but using the Templeton naming scheme (Templeton, 1995). Clade 2-1 is the coastal populations of the northern *P. x. vaccarum* clade, and Clade 6-1 is generally the entire northern *P. x. vaccarum* clade. Clade 7-1 consists of the northern members of the North—South clade, extending from Arequipa in the north down along the Chilean coast and down to Neuquen province in central Chile. Clade 8-6 is just the southern *P. x. vaccarum* clade of Neuquen and Mendoza Provinces. Clade 9-4 is the *P. bonariensis*+*P. x. vaccarum* clade. Clade 10-1 is the entire N—S group. Clade 10-2 is the northern group of the Altiplano clade, and Clade 10-3 is the diverse main Altiplano group.

Although we were initially conservative in determining tip and interior clades, we did run the NCA with great-circle distances and both alternative tip/interior estimates for the total cladogram. With the Altiplano clade as the interior the inference drawn from NCA was that of contiguous range expansion. With the North—South clade treated as the interior the conclusion was that of restricted gene flow with isolation by distance.

The analysis based on great-circle distance suggests that in the region of Chile and west-central Argentina that there was a range expansion. This is not inconsistent with predictions of restricted dispersal into Chile through the south and not across the Atacama desert. In southern Peru, Arequipa appears to be a zone of contact between the two major clades since individuals from this area fall out in both the Altiplano and the North—South Clade.

The relationship of *P. bonaeriensis* in the Sierra de la Ventana to the eastern populations of *P. x. vaccharum* is shown by NCA to be allopatric fragmentation rather than long-distance colonization. This inference supports the idea that *Phyllotis* was more widespread during the Pleistocene and that there has been a range reduction causing isolation of the disjunct regions to the east.

Both distance models suggest that restricted gene flow with isolation by distance is the dominant phylogeographic pattern in central-western Argentina (Mendoza and Neuquen Provinces). The overall pattern for the N—S clade as a whole is also restricted gene flow but with some long-distance dispersal. This makes sense if there is a continuum of gene flow and movement along the cordillera of the Andes.

The part of the North-South clade extending from southern Peru (Arequipa) to northern Peru (Huancavelica) shows evidence of either long distance colonization followed by subsequent fragmentation or of fragmentation followed by a range expansion. This is suggestive since it is the northern extreme of the N—S clade.

The alternative distance metrics differ from the great-circle distances consistently for the Altiplano. Great-circle distance analysis produces an inference of long-distance colonization and/or past fragmentation as the main pattern. The alternative metrics suggest that the pattern is one of contiguous range expansion.

Sensitivity Issues of Nested Clade Analysis

Many of the nested clades did not produce significant results, but this lack of significance is itself a valid result worth exploring. In this example there are two phenomena that reduce the number of significant clades. One is the highly localized diversity pattern of this species. At most of the lower, less inclusive, clade levels the haplotypes have too little geographic spread to produce a spatial pattern. *Phyllotis xanthopygus* is characterized by having very few shared haplotypes — most individual haplotypes are unique to certain localities. Because of the deep divergences between groups this remains a problem for the lowest level clades because few new individuals can be joined at those levels. At higher, more inclusive, nested clade levels this is no longer a problem because several clades are joined and this results in wider geographic spread.

Table 3. Inferences Drawn From NCA Using Great-Circle Distances. The top six clades are members of the North—South Clade and the bottom three are members of the Altiplano Clade.

	Nesting Clade	Nested Clades	Localities (by number)	General Geographic Description	Inference Drawn
North—South Clade	2-1	1-1 1-2 1-3	23, 31 23 11	Eastern Chile Eastern Chile Central Argentina	Inconclusive Outcome
	6-1	5-1 5-2	11, 23, 31 3, 8	Eastern Chile, central Argentina Central Argentina	Contiguous range expansion
	7-1	6-1 6-2 6-3	3, 8, 11, 23, 31 2, 9, 26, 27, 29, 30 28	Coastal Chile, western Argentina Northern Argentina, northern Chile Northern Chile	Long-distance colonization and/or past fragmentation
	8-6	7-6 7-7 7-8	3, 10, 15 5, 13 5, 6	Central/western Argentina Central/western Argentina Central/western Argentina	Restricted gene flow with isolation by distance
	9-4	8-4 8-5	1 12	Eastern Argentina Central Argentina	Allopatric fragmentation
	10-1	9-1 9-2 9-3 9-4 9-5	3, 8, 9, 11, 23, 26, 27, 28, 29, 30, 31, 32 35 7, 25, 1, 12 3, 5, 6, 10, 13, 15	Argentina, Chile, Peru Peru Southern Argentina Central and eastern Argentina Central/western Argentina	Restricted gene flow/dispersal but with some long-distance dispersal
Altiplano Clade	10-2	9-6 9-7	32 34, 35, 38	Southern Peru Central Peru	Long-distance colonization with subsequent fragmentation <i>or</i> past fragmentation followed by range expansion
	10-3	9-8 9-9 9-10 9-11	36, 37 32 20, 33, 39 4, 14, 16, 17, 18, 19, 21, 22, 24	Central Peru South/central Peru Southern Peru, Bolivia Argentina, Bolivia, Peru	Long-distance colonization and/or past fragmentation
	11-2	10-2 10-3	32, 34, 35, 38 4, 14, 16, 17, 18, 19, 20, 21, 22, 24, 32, 36, 37	Peru Argentina, Bolivia, Peru	Long-distance colonization and/or past fragmentation

The other reason why there are not more significant results is that in this network it is often impossible to define certain clades as being interior so there can be no tip/interior contrasts. This lack of interiors stems from the deep divergences between clades which means that each clade represents a long period of isolation and very few clades will come out as ancestral. This aspect of the network dominated at higher clade levels, where there were often significant values for the overall clade and for D_C and D_N but no I-T value. This was, for instance, the reason why no inferences could be drawn for the entire network. All values were highly significant, but the two major clades, the Altiplano and North—South, represented a basal division. One could not be said to be ancestral to the other. Nested clade analysis requires a tip-interior contrast to include temporal polarity.

Table 4. Inferences Drawn From NCA Using 1000-meter Intervals Distances. Inferences that differ from those produced using great-circle distances are indicated with an asterisk

	Nesting Clade	Nested Clades	Localities (By number)	General Geographic Description	Inference Drawn
North—South Clade	2-1	1-1 1-2 1-3	23, 31 23 11	Eastern Chile Eastern Chile Central Argentina	Contiguous range expansion *
	6-1	5-1 5-2	11, 23, 31 3, 8	Eastern Chile, central Argentina Central Argentina	Restricted gene flow with * isolation by distance
	7-1	6-1 6-2 6-3	3, 8, 11, 23, 31 2, 9, 26, 27, 29, 30 28	Coastal Chile, western Argentina Northern Argentina, northern Chile Northern Chile	Contiguous range expansion *
	8-6	7-6 7-7 7-8	3, 10, 15 5, 13 5, 6	Central/western Argentina Central/western Argentina Central/western Argentina	Restricted gene flow with isolation by distance
	9-4	8-4 8-5	1 12	Eastern Argentina Central Argentina	Allopatric fragmentation
	10-1	9-1 9-2 9-3 9-4 9-5	3, 8, 9, 11, 26, 27, 28, 29, 30, 31, 32 35 7, 25 1, 12 3, 5, 6, 10, 13, 15	Argentina, Chile, Peru Peru Southern Argentina Central and eastern Argentina Central/western Peru	Restricted gene flow with * isolation by distance
	Altiplano Clade	10-2	9-6 9-7	32 34, 35, 38	Southern/central Peru Northern Peru
10-3		9-8 9-9 9-10 9-11	36, 37 32 20, 33, 39 4, 14, 16, 17, 18, 19, 22, 24	Central Peru South/central Peru Southern Bolivia, Peru Argentina, Bolivia, Peru	Contiguous range expansion *
11-2		10-2 10-3	32, 34, 35, 36 4, 14, 16, 17, 18, 19, 20, 21, 22, 24, 32, 36, 37	Peru Argentina, Bolivia, Peru	Contiguous range expansion *

In the case of Clade 2-1 (of central Chile and Mendoza, Argentina), the great-circle distance analysis produced a significant chi-square result for the whole clade, but the D_C and D_N were not significant. The two alternative distance metrics had a single D_C measure with a marginal p-value of 0.058 and highly significant I-T contrasts. The difference in this example stems from the increase of the D_N distance for this one clade from ~92 km in the great-circle example to ~467 km in the alternative distances.

For Clade 6-1 the reason why the great-circle analysis returned a result of contiguous range expansion while the alternative distances produced an inference of restricted gene flow with isolation by distance is because the D_C and D_N patterns are effectively reversed in the two analyses. For the tip clade the alternative distances increased the nested clade distance enough so that the results switched from a significantly large to a significantly small D_C . One of the things decision point 2 on the inference key is asking for is the relative value of D_C for tips and internals. If tips are more widespread than interiors then this is taken to be evidence of a range expansion. Neither conclusion, restricted gene flow in the alternative distances, or contiguous range expansion in the great-circle analysis represents a biologically implausible scenario for this clade.

The inferences drawn from the analyses of Clade 7-1 were relatively similar to each other. The possible conclusion of long-distance colonization from the great-circle analysis and the conclusion of contiguous range expansion in the alternative distance analyses are both subsets of the general category of range expansions. The difference stems from a lack of significant D_N values in the alternative distance analyses. The within clade distances increased more than the nested clade distances in the alternative distance schemes and this meant that the D_N were no longer significantly large. It was the pattern of significant reversal, large D_N s and small D_C s that caused the great-circle analysis to return a result of long-distance colonization and/or past fragmentation. It is interesting that the implications for this clade level are that there was likely some degree of range expansion in the northern North—South clade, because this is not inconsistent with the pattern for the subset of 6-1.

The example of Clade 8-6 is a very interesting one, because it shows how alternative pathways can produce the same inference with Templeton's key, especially for restricted gene flow with isolation by distance. The reason for the initial deviation in the inference heuristic is that there are no significant D_C values in the alternative distances which causes decision point 2 to move to step 11, the determining step for range expansions. Decision point 11 checks to see if the reason for choosing to move to that point was based on a violation of 2 consistent with range expansion or not. If not, then the next move is to 17 which determines that the initial move from 2 was due to a lack of significant D_C s and the next move is to decision 4. At this point, the great-circle and alternative distances are on the same heuristic path again, and the same inference is chosen. This is reassuring because the overall pattern of I-T and D_N values is the same in all cases. The only difference is the addition of a single significant D_C in the great-circle analysis.

All three metrics produce the same result Clade 9-4, that of allopatric fragmentation. The clades (eastern *P. x. vaccarum* and *P. bonariensis*) within the nesting clade do not overlap. This is the first step in Templeton's more recent inference keys. The next step is 19. Stating that the species is absent in the area between sampling localities at step 19 determines that the lack of overlap between the clades represents allopatric fragmentation. If the species is said to be present and was not sampled then the key indicates that sampling was inadequate. This pattern is reinforced by the long-branch between the two clades. Alternatively, if the gulf between localities is not found to be large, then the key will return the result of contiguous range expansion.

There was very little difference between the inferences produced in Clade 10-1. All of the distance measurements produced a result of restricted gene flow. The only difference hinged entirely on a marginally significant D_N value ($p=0.058$) in the great-circle analysis. This difference meant that one tip clade had a significantly large D_N . This was enough to modify the result to restricted gene flow with some long-distance dispersal.

All of the analyses agreed that Clade 10-2 represented either long-distance colonization and past fragmentation or past fragmentation followed by range expansion. The observed pattern of significant within-clade and nested-clade distances was very similar and the inference chain was identical.

For Clades 10-3 and 11-2 the differences paralleled those in Clade 7-1. Just as in Clade 7-1 the great-circle analyses produced long-distance colonization and/or past

fragmentation instead of contiguous range expansion because there were some significantly large D_N s that were not significant in the alternative distance analyses and this produced a pattern of “significant reversal” in the great-circle analyses only.

There are a few key points in the inference key where we have noticed that it is particularly sensitive to exceptions to assumptions. Decision point 2 in the inference key is a particularly critical early step and there is one exception that arose on numerous occasions. The key is trying to determine if there is a deviation from the pattern of widespread internals and relatively localized tips. One way to achieve this is if the tips have significantly small D_C s and if the D_C s for some but *not all* interiors are significantly small. However, it was often the case that there was only one internal clade. In those examples even if the one internal is small, by definition *all* internal clades are small. Typically this made the difference between a range expansion estimate and restricted gene flow.

There were several differences between the analyses using the alternative distances and the great-circle distance. Overall two-thirds of the inferences for significant clades were different. Three clades produced identical results: restricted gene flow with isolation by distance for 8-6, allopatric fragmentation for 9-4, and some combination of a fragmentation and range expansion event in 10-2. One of the cases, 10-1, was very nearly identical, with the only difference being the addition of a qualifying term of some long-distance dispersal. The remaining cases differed to a greater degree. Three were somewhat similar in general category, but different in the particulars—contiguous range expansion versus long-distance colonization. And two produced quite different conclusions. The fact that there was congruence in only three out of nine results indicated that NCA is not very robust to changes in distance metric. When we examined the pattern for just the more inclusive clade levels, we found a fairly consistent pattern. In the case of the great-circle analysis, the most common results were long-distance colonization with past fragmentation. In the alternative frameworks, the dominant pattern at all levels, but particularly the more inclusive levels, was consistently contiguous range expansion. This pattern was driven by the nested clade distances. The alternative distance metrics increased within clade distances more than they increased nested clade distances. It is not immediately clear why this bias should exist.

We did not observe the consistent increase in the number of significant D_C and D_N values described by Fetzner and Crandall (2003). We also did not detect a different magnitude of effect between lower and higher clades as they reported, but it is difficult to generalize from this because we did not have many significant lower level clades.

The sensitivity of NCA to differing distance metrics suggests that blindly using great-circle distances is not advisable. But which answer should we believe? One approach is to only accept the conclusions that were supported by both elevation-based and great-circle distances. Alternatively, we could assume that the great-circle is preferable based on *a priori* preferences, but this does not take the actual inferences into account. The fact that the alternative distances at more inclusive levels show a pattern of range expansion does seem to be consistent with general expectations for the history of a widespread species. We suggest that simulations using known phylogeographic models may be the most effective way to test whether alternative distances reflect reality better.

Suggestions for Future Research

There are now hundreds of studies employing nested clade analysis in phylogeographic analyses (Templeton, 1998). It is increasingly important that we continue to revise and evaluate this technique in order to assure that we are obtaining reasonably good estimates of the population history of studied organisms. Empirical applications, such as this present study, can be used to test some of the underlying assumptions. Further insight can be gleaned through simulation-approaches where the true history is known, and can be compared to the conclusions from NCAs.

Biological context is critical. The inferences derived from phylogeographic analyses should be interpreted in a taxon-specific biological context. Considerations such as size (small organisms are not able to disperse as far and have shorter generation times and different rates of gene sorting) and behavior and life history traits (philopatry, central breeding sites) can have a large impact on biological interpretation.

We recommend further investigation and refinement of biologically plausible distance measurements. If detailed information about ecology is available this could be used instead of elevation isolines in order to create a less arbitrary estimate of barriers to vertical dispersal. It is also possible to create and apply relative costs to crossing isolines and build a more sophisticated model. Although it may prove operationally intractable, it is possible that a different distance model could be entirely nonlinear, with costs to dispersal increasing geometrically rather than arithmetically. In both theoretical and applied approaches it may prove promising to expand the distance metric parameters to extremes beyond the biologically realistic in order to see where NCA starts to break down.

Biological information should be used in order to determine whether great-circle distance measurements are likely to be appropriate. We agree with the suggestion of Fetzner and Crandall (2003) that one should use linear riverine distances in rivers and riparian habitats. We would also add that any physiognomically complex habitat may require alternative distance methods. If the study organism has known constraints to movement or if the environment is complex then researchers should use both great-circle and an alternative method. But we do caution that this is not an excuse to try two approaches and choose the preferred inferences from both. Consistency is critical when choosing which conclusion to draw. The move towards greater objectivity that NCA provides is a laudable goal, but it must not become a mindless heuristic. Researchers must continue to keep the biology of their study organisms in mind and to think critically about the operation and conclusions of this approach.

APPENDIX

Specimen Species Designations and Voucher Numbers

The voucher number corresponds to the museum voucher number of the lending institution except when there was no voucher number available because the sample came from the collection of an individual and not an institution. Those cases are indicated with an asterisk. The following abbreviations are used: Field Museum of Natural History, FMNH; Laboratorio Citogenetica Mammiferos, LCM; Louisiana State University Museum, LSUMZ; Museum of Field Studies, MFS; Museum of Southwestern Biology, MSB; Museum of Vertebrate Zoology, MVZ; Museo de Historia Natural, UNSM; Burke Museum- Zoology Section, UWBM.

<u>Species and Subspecies</u>	<u>Voucher Number</u>
<i>Phyllotis bonariensis</i>	SA 02*
<i>Phyllotis bonariensis</i>	SA 03*
<i>Phyllotis darwini</i>	LCM 2481
<i>Phyllotis darwini</i>	LCM 2485
<i>Phyllotis darwini</i>	LCM 2488
<i>Phyllotis darwini</i>	LCM 2493
<i>Phyllotis limatus</i>	LSUMZ 27813
<i>Phyllotis limatus</i>	LSUMZ 27814
<i>Phyllotis limatus</i>	LSUMZ 27818
<i>Phyllotis limatus</i>	LSUMZ 27819
<i>Phyllotis limatus</i>	LSUMZ 27854
<i>Phyllotis limatus</i>	LSUMZ 27858
<i>Phyllotis limatus</i>	ORB 34*
<i>Phyllotis xanthopygus chilensis</i>	FMNH 107606
<i>Phyllotis xanthopygus chilensis</i>	FMNH 133830
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27828
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27831
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27840
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27846
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27847
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27848
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27849
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27852
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27855
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27856
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27857
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27859
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27861
<i>Phyllotis xanthopygus chilensis</i>	LSUMZ 27862
<i>Phyllotis xanthopygus posticalis</i>	ORB 36*
<i>Phyllotis xanthopygus posticalis</i>	ORB 38*

<i>Phyllotis xanthopygus posticalis</i>	ORB 82*
<i>Phyllotis xanthopygus posticalis</i>	ORB 89*
<i>Phyllotis xanthopygus posticalis</i>	ORB 92*
<i>Phyllotis xanthopygus posticalis</i>	ORB 99*
<i>Phyllotis xanthopygus posticalis</i>	ORB 152
<i>Phyllotis xanthopygus posticalis</i>	UNSM 10781
<i>Phyllotis xanthopygus posticalis</i>	UNSM 10782
<i>Phyllotis xanthopygus posticalis</i>	UNSM 10783
<i>Phyllotis xanthopygus rupestris</i>	FMNH 162854
<i>Phyllotis xanthopygus rupestris</i>	FMNH 162870
<i>Phyllotis xanthopygus rupestris</i>	LCM 1737
<i>Phyllotis xanthopygus rupestris</i>	LCM 1766
<i>Phyllotis xanthopygus rupestris</i>	LCM 1780
<i>Phyllotis xanthopygus rupestris</i>	LCM 1792
<i>Phyllotis xanthopygus rupestris</i>	LCM 1794
<i>Phyllotis xanthopygus rupestris</i>	MSB 55350
<i>Phyllotis xanthopygus rupestris</i>	MSB 67261
<i>Phyllotis xanthopygus rupestris</i>	MSB 70563
<i>Phyllotis xanthopygus rupestris</i>	MSB 75255
<i>Phyllotis xanthopygus rupestris</i>	MVZ 1715
<i>Phyllotis xanthopygus rupestris</i>	UP 613*
<i>Phyllotis xanthopygus rupestris</i>	UP 614*
<i>Phyllotis xanthopygus rupestris</i>	UP 645*
<i>Phyllotis xanthopygus rupestris</i>	UP 665*
<i>Phyllotis xanthopygus vaccarum</i>	AC 20*
<i>Phyllotis xanthopygus vaccarum</i>	AC 21*
<i>Phyllotis xanthopygus vaccarum</i>	AC 31*
<i>Phyllotis xanthopygus vaccarum</i>	AC 32*
<i>Phyllotis xanthopygus vaccarum</i>	AC 33*
<i>Phyllotis xanthopygus vaccarum</i>	LCM 1156
<i>Phyllotis xanthopygus vaccarum</i>	LCM 1157
<i>Phyllotis xanthopygus vaccarum</i>	LCM 2484
<i>Phyllotis xanthopygus vaccarum</i>	LCM 2486
<i>Phyllotis xanthopygus vaccarum</i>	LCM 2519
<i>Phyllotis xanthopygus vaccarum</i>	LCM 2520
<i>Phyllotis xanthopygus vaccarum</i>	LCM 2521
<i>Phyllotis xanthopygus vaccarum</i>	UP 467*
<i>Phyllotis xanthopygus vaccarum</i>	UP 468*
<i>Phyllotis xanthopygus vaccarum</i>	UP 477*
<i>Phyllotis xanthopygus vaccarum</i>	UP 479*
<i>Phyllotis xanthopygus vaccarum</i>	UP 480*
<i>Phyllotis xanthopygus vaccarum</i>	UP 481*
<i>Phyllotis xanthopygus vaccarum</i>	UP 482*
<i>Phyllotis xanthopygus vaccarum</i>	UP 484*
<i>Phyllotis xanthopygus vaccarum</i>	UP 486*
<i>Phyllotis xanthopygus vaccarum</i>	UP 497*

Phyllotis xanthopygus vaccarum UP 505*
Phyllotis xanthopygus vaccarum UP 609*
Phyllotis xanthopygus vaccarum UP 610*
Phyllotis xanthopygus vaccarum UP 611*
Phyllotis xanthopygus vaccarum UWBM 72232
Phyllotis xanthopygus xanthopygus LCM 1161
Phyllotis xanthopygus xanthopygus MFS 1324
Phyllotis xanthopygus xanthopygus UP 389*
Phyllotis xanthopygus xanthopygus UP 411*
Phyllotis xanthopygus xanthopygus UP 413*
Phyllotis xanthopygus xanthopygus UP 415*
Phyllotis xanthopygus xanthopygus UP 425*
Phyllotis xanthopygus xanthopygus UP 439*
Phyllotis xanthopygus xanthopygus UP 440*
Phyllotis xanthopygus xanthopygus UP 470*

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BIOGRAPHICAL SKETCH

Background and Education:

James Albright was born in Vero Beach, Florida on December 7, 1977. He received his undergraduate education at the Florida State University (FSU) where he earned a Bachelor's of Science with a major in the biological sciences and a minor in Spanish language. He commenced his graduate studies in evolutionary biology at FSU in 2001.

Research Experience:

His work and volunteer experience include a variety of research projects. In 1995—1996 he performed a volunteer two-year census of breeding colonies of osprey (*Pandion haliaetus*) at two sites in Indian River County; Blue Cypress Lake and the Oslo Riverfront Conservation Area on the Indian River Lagoon. He worked for the St. John's Water Management District as a hydrology intern in 1996. James was able to work with High Pressure Liquid Chromatography (HPLC) techniques in an honor's chemistry project in 1997. In 1998—2000, he assisted in a research project monitoring behavioral interactions of red-bellied woodpeckers (*Melanerpes carolinus*) and red-cockaded woodpeckers (*Picoides borealis*) in the Apalachicola National Forest with Dr. F.C. James of FSU. He also worked on the molecular biology of mosquitoes at the Florida Medical Entomology Laboratory of the University of Florida in 2000—2001. In related endeavors, he provided scientific illustration assistance in 2003 for a paper on the evolution of hypercarnivory.

Teaching Experience:

James has taught a range of subjects to a diversity of age groups. He has taught marine biology for elementary school students (1995) and limnology to high school students (1997). He has also been a teaching assistant for a number of classes at the undergraduate level, including; an animal diversity survey course, introductory biology (for majors), comparative vertebrate anatomy, and evolution,