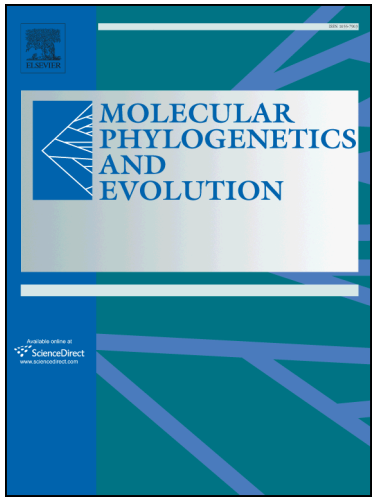

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The Mouse-colored Tyrannulet (*Phaeomyias murina*) is a species complex that includes the Cocos Flycatcher (*Nesotriccus ridgwayi*), an island form that underwent a population bottleneck

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Abstract

Simultaneous examination of evolutionary history in island forms and closely related mainland relatives can provide reciprocal insight into the evolution of island and mainland faunas. The Cocos Flycatcher (*Nesotriccus ridgwayi*) is a small tyrant flycatcher (Tyrannidae) endemic to Cocos Island, an oceanic island in the eastern Pacific Ocean. We first established its close relationship to the mainland species Mouse-colored Tyrannulet (*Phaeomyias murina*) using a phylogeny from genome-wide ultraconserved elements and exons. We then used mitochondrial DNA to explore the relationships between *Nesotriccus* and *Phaeomyias* populations from across its distribution in Central and South America. We found that *Nesotriccus* is nested within the *Phaeomyias* evolutionary tree, and that *Phaeomyias* represents a complex of at least four evolutionarily distinct species that differ in plumage, voice, and habitat association. *Nesotriccus* underwent a population bottleneck subsequent to its divergence from Central American and northern South American *Phaeomyias* populations in the middle Pleistocene. The 46 UCE loci containing alleles that are fixed between the two species are widely distributed across the genome, which suggests that selective or neutral processes responsible for divergence have occurred genome-wide. Overall, our simultaneous examination of *Phaeomyias* and *Nesotriccus* revealed divergent levels of genetic diversity and evolutionary histories between island and mainland forms.

Keywords: phylogeny, Cocos Island, phylogeography, ultraconserved elements, exons, coalescent methods

1. Introduction

Evolutionary biologists have long recognized the utility of islands for studying the evolution of organisms (Wallace, 1880). Due to the discrete geographical nature of islands, populations on islands are isolated from high levels of gene flow typical on continents, providing unique opportunities to study adaptation and speciation (Grant and Grant, 1996; Losos and Ricklefs, 2009). Island species have been used to examine modes of speciation (Barrett, 1996; Cameron et al., 1996; Gittenberger, 1991; McDonald and Smith, 1990; Stuessy et al., 1990), adaptive radiations (Carlquist, 1995, 1974; Grant and Grant, 1994; Grant, 1984; Tarr and Fleischer, 1995; Vincek et al., 1997), and taxon cycles (Greenslade, 1968; Klein and Brown, 1994; Ricklefs and Cox, 1978, 1972; Roughgarden and Pacala, 1989; Wilson, 1961, 1959). Despite extensive study, our knowledge of the genetic diversities and evolutionary histories of many island species is limited (Barrett, 1996; Franks, 2010). Population genetic information from island species can provide information on genetic diversity and population size (Frankham, 1997), demographic history including bottlenecks and founder effects (Clegg et al., 2002a), adaptation and natural selection (Barton, 1996), and the impacts of inbreeding (Frankham, 1998). Comparative studies between closely related island and mainland taxa are especially useful because the typically larger mainland populations provide a reference for patterns and processes inferred on islands (Barrett, 1996; Woolfit and Bromham, 2005).

Simultaneous examination of island and mainland relatives may also provide insight into the evolution of the continental species. Particularly in tropical regions, continental species may have deep evolutionary histories and contain high levels of cryptic diversity (Bickford et al., 2007; Gehara et al., 2014; Hebert et al., 2004; Janzen et al., 2005; Lecocq et al., 2013; Willig et

al., 2003). Island forms can serve as evidence of historical diversity and distributions for closely related populations or taxa on the mainland (Gotelli and Graves, 1990; Miller et al. in review; Olson, 1997, 1993; Snow, 1985). Genetic information from island populations can be used to assess the monophyly of mainland populations (Crews et al., 2010; Fernández-Mazuecos and Vargas, 2011; Phillimore et al., 2008; Wilson et al., 2015). In cases where the geological history of the island is well-known, island populations can be used to calibrate divergence time estimates among mainland populations (Almeida et al., 2005; Runemark et al., 2012; Smith and Klicka, 2013; Tollis and Boissinot, 2014), and infer histories of selection (Blondel et al., 1999; Clegg et al., 2002b; Edwards, 1993; Griffith et al., 1999) or demographic or distributional changes in the mainland populations. Reciprocal insight into both mainland and island evolution is therefore possible by examining closely related insular and mainland taxa simultaneously.

Cocos Island is a 24 km^2 oceanic island roughly 550 km off the Pacific coast of Costa Rica. Like the Galápagos Islands, Cocos arose volcanically as recently as 2 Mya and probably was never connected with the mainland by a land bridge (Castillo et al., 1988; Dalrymple and Cox, 1968). A few phylogenetic studies provide insight into the relationships and biogeographic history of terrestrial organisms on Cocos Island. The plant species found on the island arrived via independent colonizations from other regions, mostly Central American and northwestern South America (Igea et al. 2015). Both the Yellow Warbler (*Setophaga petechia*) and the Cocos Finch (*Pinaroloxis inornata*), however, are most closely related to populations on the Galápagos Islands, and the finch especially appears to represent a colonization from that archipelago (Petren et al., 1999; Chaves et al., 2012). More detailed studies of the population and demographic history of Cocos Island's terrestrial species, however, are lacking.

The Cocos Flycatcher (*Nesotriccus ridgwayi*) is the only member of its genus, and is endemic to Cocos Island. *Nesotriccus* is a small, brownish member of the Tyrannidae (flycatchers) with a curiously long bill, and its taxonomic affinities were historically unclear (Fitzpatrick, 2004; Stiles and Skutch, 1989). Similarities of the nasal septum and of the supporting elements of the syrinx, however, led Lanyon (1984) to suggest the nearest relatives of *Nesotriccus* are two other flycatcher species, Mouse-colored Tyrannulet (*Phaeomyias murina*) and Yellowish Tyrannulet (*Capsiempis flaveola*). *Phaeomyias murina* is also the only member of its genus, and is distributed widely in the Neotropics from Panama south through lowland northern South America to Argentina (Fitzpatrick, 2004). Like *Nesotriccus*, *Phaeomyias* is brown with a grayish-olive to dark brownish-olive breast. There is considerable variation in plumage and voice across the distribution of *Phaeomyias* (Fitzpatrick, 2004), and populations west of the Andes in South America are sometimes considered a separate species, the Tumbes Tyrannulet (*Phaeomyias tumbezana*; Ridgely and Greenfield, 2001). The Yellowish Tyrannulet (*Capsiempis flaveola*) is an olive-colored flycatcher with bright yellow underparts and, like *Phaeomyias*, is distributed widely in mainland Central and South America.

To elucidate the evolutionary affinities of *Nesotriccus*, we evaluated its position in a phylogeny containing hypothetical close relatives using sequence data from genomic exons and ultraconserved elements. We then further investigated the relationship between *Nesotriccus* and *Phaeomyias*, as well as diversity within *Phaeomyias*, by collecting mitochondrial data from range-wide samples of the two species. Finally, we used exon and ultraconserved element data from *Nesotriccus* and a closely related population of *Phaeomyias* to estimate the demographic history of these populations.

2. Methods

2.1. Sampling and DNA isolation

We sampled 46 individuals from across the distribution of the Mouse-colored Tyrannulet (*Phaeomyias murina*; Table S1), including all named subspecies (Fitzpatrick, 2004). All genetic samples were obtained from fresh tissue, with the exception of one toe pad from a study skin from Colombia. We also sampled toe pads from two individuals of Cocos Flycatcher (*Nesotriccus ridgwayi*) from Cocos Island (Table S1) and single tissues from the closely related *Capsiempis flaveola* and Planalto Tyrannulet (*Phyllomyias fasciatus*). Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue extraction kit (Qiagen, Valencia, California), following the manufacturer's protocol. Extractions from toe pads of museum study skins were conducted in a lab space separate from other tissue extractions to minimize contamination risk. We obtained an ND2 sequence of *Capsiempis flaveola* (DQ294563) from Genbank for use as an outgroup for mitochondrial analyses.

2.2. PCR amplification and mitochondrial DNA sequencing

We used polymerase chain reaction (PCR) to amplify the entire second subunit of the NADH dehydrogenase mitochondrial gene (ND2; 1041 bp). Target DNA fragments were amplified using primers L5215 (Hackett, 1996) and H6313 (Johnson and Sorenson, 1998) for fresh tissues, and 3 pairs of internal primers (Table S2) for toe pad samples. We designed the custom PCR

primers using an alignment of existing *Phaeomyias* sequences from GenBank and the PrimerQuest Tool from Integrated DNA Technologies (http://www.idtdna.com/primerquest). Each pair covers a fragment of about 200 bp, and they are staggered so as to potentially recover a ~600 bp region. PCR amplifications were performed in 25 μL reactions using the following protocol: denaturation at 94°C for 2:15 min, 34 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 1 min, followed by 7 min elongation at 72°C. DNA from toe pads was similarly amplified, via polymerase chain reaction (PCR) in 25 μL reactions. However, we used Qiagen hot-start plus Taq (Qiagen, Valencia, California) and the following protocol: denaturation at 95°C for 5 min, 34 cycles of 94°C for 45s, 50°C for 45s, and 72°C for 1 min, followed by 10 min elongation at 72°C.

PCR products were sent to Beckman-Coulter (Danvers, MA) for SPRI purification and sequencing using BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) on a PRISM 3730xl Genetic Analyzer (Applied Biosystems). Raw sequence data from both strands were inspected, edited, and aligned using Geneious v5.4 (Drummond et al., 2011). Sequences obtained in this study were deposited in GenBank (accession numbers pending).

2.3. Analyses of mitochondrial sequence data

We used Akaike's Information Criterion (AIC) implemented in MrAIC.pl (Nylander, 2004) to determine the best-fit model of nucleotide substitution for ND2 (JC69) and used this model for subsequent analyses. We estimated mitochondrial haplotype networks using the TCS method in the program PopArt (Leigh and Bryant, 2015). We used MrBayes v3.2.2 (Ronquist et al., 2012) to estimate a Bayesian phylogenetic tree using 100 million generations, four chains, two replicate

runs, and a 10% burn-in. We evaluated convergence and stationarity in Tracer v1.5 (Rambaut and Drummond, 2007). We used BEAST v2.0.2 (Drummond et al., 2012) to estimate divergence times among clades using a standard ND2 rate of 2.5% per million years based on published calibrations (Smith and Klicka, 2010). We used a relaxed clock, a lognormal distribution for the clock prior, and a coalescent (constant size) tree prior. We ran the analysis for 100 million generations, sampling every thousand, but thinned to 10% of the sampled trees and used a 10% burn-in to estimate a maximum clade credibility consensus tree in TreeAnnotator v.2.0.2 (distributed as part of the BEAST package).

2.4. UCE and exon sequencing

We used sequence capture to target ultraconserved elements (UCEs) and exons from across the genome from one *Nesotriccus* individual and two *Phaeomyias* from the most closely related population (Central and northern South America; see Results). We also conducted sequence capture on the *Capsiempis flaveola* and *Phyllomyias fasciatus* samples. The sampling was part of a larger phylogenomic study to be published elsewhere. We modified existing probe sets for UCEs (Faircloth et al., 2012) in order to obtain additional sequence from the more variable UCE flanks that might be useful for estimating shallow population histories. In UCE loci targeted with a single probe, we designed two probes extending further into the UCE flanks. The 120-mer probes were tiled such that they had 50% overlap (60 bp) in the middle of the locus and covered 180 bp total. Probe sequences were based on the chicken (*Gallus gallus*) genome release ICGSC Gallus_gallus-4.0 (Hillier et al., 2004). We also targeted conserved exons adjoining variable introns that have been used in prior avian phylogenetic studies (Kimball et al., 2009; Smith et al.,

2013; Wang et al., 2012). Although conserved, these exons are potentially more variable than UCEs and might therefore provide useful information at the population level. Probes were designed off the chicken genome sequence and were again tiled such that they covered the entire exon sequence at 2x coverage (50% overlap between adjoining probes). The final probe set included 4,715 probes targeting 2,321 UCEs and 96 exons.

We sent all samples to Rapid Genomics (Gainesville, FL) for sequence capture and sequencing following the general protocol described in Faircloth et al. (2012) and Smith et al. (2014). Samples were multiplexed at 160 samples per lane on a 100 bp paired-end Illumina HiSeq 2500 run. Rapid Genomics demultiplexed raw reads using custom scripts and strict barcode matching. We cleaned reads with Illumiprocessor (Faircloth, 2013). For the phylogenetics analysis, we obtained consensus sequences for *Nesotriccus* and close relatives using the Phyluce pipeline (Faircloth, 2015).

In order to obtain diploid sequence representing both alleles in each *Nesotriccus* and *Phaeomyias* individual for population genetics analyses, we developed a second pipeline (https://github.com/mgharvey/seqcap_pop) to process and assemble datasets as follows. We used Velvet (Zerbino and Birney, 2008) and the wrapper program VelvetOptimiser (Gladman and Seemann, 2009) exploring hash lengths of between 67 and 71 to assemble reads across all individuals into contigs *de novo*. We mapped contigs to UCE probe sequences using Phyluce (Faircloth, 2015). For each individual, we mapped reads to contigs that aligned to UCEs using bwa (Li and Durbin, 2009). We explored thresholds that allowed anywhere from 1 to 7 mismatches between reads for mapping and settled on allowing 4 mismatches per read for each assembly. We converted sam files to bam format using samtools (Li et al., 2009) and cleaned bam files by soft-clipping reads outside the reference contigs with PICARD

(http://broadinstitute.github.io/picard/). We added read groups for each individual using PICARD and merged the bam files across individuals with samtools. We realigned reads to minimize mismatched bases using the RealignerTargetCreator and realigned indels using IndelRealigner in the Genome Analysis Toolkit (GATK; McKenna et al., 2010). We called single nucleotide polymorphisms (SNPs) and indels using the GATK UnifiedGenotyper, annotated SNPs with VariantAnnotator, and masked indels using VariantFiltration. We removed SNPs with a quality score below Q30 and conducted read-backed phasing using the GATK. We output SNPs in vcf format and used add_phased_snps_to_seqs_filter.py (from the seqcap_pop pipeline) to insert SNPs into reference sequences and produce alignments for each locus across individuals. SNPs on the same locus for which phasing failed were inserted using the appropriate IUPAC ambiguity codes. We collated sequences and produced final alignments using MAFFT (Katoh et al., 2005).

2.5. Analyses of ultraconserved elements and exons

We used the consensus UCE and exon sequences from Phyluce for each individual of *Nesotriccus* and close relatives, based on preliminary data from a larger phylogenetic study, to estimate a phylogenetic tree. The tree was estimated using a concatenated dataset partitioned by locus in ExaML (Kozlov et al., 2015) and support was assessed using 500 bootstrap replicates. We estimated a tree using only UCE data, one using only exon data, and one with both classes of markers combined.

Using the seqcap_pop dataset containing both alleles from UCEs and exons for each individual, we calculated basic summary statistics and mapped the consensus sequence from

each recovered contig to the Zebra Finch (*Taeniopygia guttata*; Warren et al., 2010) genome to determine their chromosomal positions. We estimated the demographic history of *Nesotriccus* and the closely related (Panama + Colombia + Guyana) clade of *Phaeomyias* (based on mitochondrial analyses, see Results) using the UCE and exon data in G-PhoCS v.1.2.1 (Gronau et al., 2011). We used a two-population model and estimated the divergence time between *Nesotriccus* and *Phaeomyias*, theta for both *Nesotriccus* and *Phaeomyias* populations, and theta for the ancestral population. We examined a model with no migration between populations subsequent to divergence as well as a model allowing for migration. Due to the possibility that deep divergence between the two *Phaeomyias* samples would complicate demographic analysis, we ran a separate analyses in which the Guyana sample (the further sample from Cocos Island geographically) was removed. For each analysis, we used G-PhoCS v.1.2.1 (Gronau et al., 2011) with gamma (α, β) priors of (1, 5000) for theta and tau and (1, 3) for migration and two replicate runs of 800,000 (sampling every 100). We also explored the impact of theta and tau priors of (1, 50).

There are no standardized substitution rates for combined UCE and exon datasets. In lieu of internal fossil or geologic calibrations we converted population size and migration rate estimates to real values by standardizing the divergence time to that from the mitochondrial tree dated in BEAST based on a standard estimates of ND2 substitution rate (following Smith et al., 2014). The divergence time from analysis of mitochondrial data in BEAST was based on a model that did not allow for migration and was subject to the vagaries of single-gene coalescence, but using this date was the best available strategy for converting parameter estimates from the demographic analyses of UCE and exon data. From this, the estimated UCE and exon

206 substitution rate was 1.10×10^{-6} substitutions/site/My (s/s/My) for the analysis without migration
207 and 1.14×10^{-6} s/s/My for the analysis with migration.
208
209
210 **3. Results**
211
212 *3.1. Genomic variation and divergence among populations*
213
214 We obtained high-quality ND2 sequence averaging 1,030 bp long from tissues and 519 bp
215 long from toe pads. The ND2 alignments included 138 SNPs, 7 of which had substitutions fixed
216 between the two *Nesotriccus* and all *Phaeomyias* individuals. We recovered 1,930 loci from the
217 UCE and exon probe set averaging 389 bp (sd = 107) in length and containing 1,444 SNPs in
218 total. Genotypes were missing from 64.4% of SNPs for the *Nesotriccus* individual sampled from
219 a toe pad versus 0.1% for the *Phaeomyias* sample from a tissue and 14.8% for the *Phaeomyias*
220 sample from a toe pad. Heterozygosity was much lower in *Nesotriccus* than *Phaeomyias*: only
221 7.4% of successfully genotyped SNPs (i.e., after removing missing genotype data) were
222 heterozygous in the *Nesotriccus* individual versus 47.5% and 43.5% in the two *Phaeomyias*
223 individuals. Median read depths of homozygous alleles were similar across the three samples
224 (1,021x for *Nesotriccus*, 1,020x for the two *Phaeomyias*), suggesting that lower read depth is not
225 responsible for the lower heterozygosity observed in *Nesotriccus*). The two *Phaeomyias*
226 individuals shared more unique alleles (16.3% of successfully genotyped sites) than the
227 *Nesotriccus* individual shared with either *Phaeomyias* (3.3% and 5.3%). Amongst the 470 SNPs
228 that had complete genotypes across samples, 48 had fixed alleles between the *Nesotriccus* and

the two *Phaeomyias* individuals. The fixed SNPs were distributed across 46 UCE loci (two loci had two fixed SNPs), 3 of which mapped to the Zebra Finch Z chromosome, 42 to the autosomes, and 1 to an unplaced scaffold (Fig. S1). The number of fixed SNPs between the *Nesotriccus* and the two *Phaeomyias* individuals was higher than between either *Phaeomyias* individual and the other two individuals (26 and 24 fixed SNPs).

3.2. Relationships among populations

The MrBayes mitochondrial tree reveals that *Nesotriccus* is nested within *Phaeomyias* with complete support, and is sister to a *Phaeomyias* clade containing individuals from Central America, northern Colombia, and Guyana (PP = 0.94; Fig. 1a). UCE and exon data confirm that the close relationship between *Nesotriccus* and *Phaeomyias* is not a result of horizontal gene flow or deep coalescence of mitochondrial alleles. In the ExaML tree of concatenated UCEs and exons, the two *Phaeomyias* individuals were monophyletic (98% bootstrap support; Fig. S2) and were sister to the *Nesotriccus* sample (100% bootstrap support). The same relationship was recovered in the trees based on only UCEs and only exons. These were sister to the *Phyllomyias fasciatus* individual (100% bootstrap support), and *Capsiempis flaveola* was sister to that entire group (100% bootstrap support). Based on the BEAST analysis of mitochondrial data, *Nesotriccus* diverged from the Central American *Phaeomyias* population ~1.2 Mya (HPD = 0.49-2.46 Mya; Fig. 1b).

We also observed deep mitochondrial structure across *Phaeomyias* with populations from the western Amazon south to Argentina, the Guianan region and eastern Brazil, Central America through Colombia to Guyana, the Marañon Valley and Tumbesian highlands, and coastal

Tumbesian region all exhibiting isolation for 0.25 My or longer (Fig. 1b). The haplotype network provides further support for deep phylogeographic structure and clade membership across individuals (Fig. 2).

3.3. Demographic history

Converted effective population size estimates from the model implemented in G-PhoCS for *Nesotriccus* averaged one (analysis with both *Phaeomyias* samples) or two (analysis with Guyana *Phaeomyias* sample removed) orders of magnitude smaller than those for the (Panama + Colombia + Guyana) clade of *Phaeomyias* (Table 1). In both analyses, the *Nesotriccus* effective population size was also much smaller than that of the population ancestral to both *Nesotriccus* and the (Panama + Colombia + Guyana) *Phaeomyias* population. In the analysis with both *Phaeomyias* samples included, the ancestral population size was extremely large, but when the Guyana *Phaeomyias* sample was removed the ancestral population size was similar to that of the contemporary size of the (Panama + Colombia + Guyana) *Phaeomyias* population. Migration rate estimates in the model with migration parameters were very low, and effective population sizes and substitution rates from the analyses with and without migration were similar (see Methods; Table 1).

4. Discussion

The Cocos Flycatcher (*Nesotriccus ridgwayi*) is not as distinct as its placement in a monotypic genus would suggest, but rather is phylogenetically nested within populations of the Mouse-colored Tyrannulet (*Phaeomyias murina*). Because Cocos Island is an oceanic island, *Nesotriccus* presumably colonized from mainland populations at some time subsequent to the formation of the island. The mean estimated divergence date between *Nesotriccus* and the closest *Phaeomyias* population from our analyses (1.2 Mya), assuming it accurately reflects the timing of island colonization, is consistent with arrival subsequent to the island's formation about 2 Mya (Castillo et al., 1988). Many island populations have decreased genetic diversity consistent with a population bottleneck (Frankham, 1997). The lower heterozygosity of the *Nesotriccus* sample relative to the *Phaeomyias* individuals with UCE data is consistent with a severe founder effect (Nei et al., 1975), and the relatively small effective population size in *Nesotriccus* from demographic modeling further confirms the existence of a historical population bottleneck in this species. The low rate of migration recovered from the demographic model with migration parameters, and the minimal impact of the addition of migration to the estimates of other parameters, suggest that recent gene flow between *Nesotriccus* and mainland *Phaeomyias* populations has been negligible.

Several features of the demographic reconstructions deserve further discussion from a methodological perspective. The effective population sizes are generally very large. This could be due to the use of the mitochondrial divergence time for calibrating substitution rates. In cases of no gene flow subsequent to divergence, the mitochondrial gene tree should always coalesce at an earlier time than population divergence (Edwards and Beerli, 2000), and this inflated divergence time would result in spuriously high substitution rate estimates and large effective population sizes. Using mitochondrial divergence time to calibrate demographic parameter

297 estimates is questionable for these reasons, but until standard substitution rate estimates are
298 available for UCEs and conserved exons, other calibration strategies are unavailable. In analyses
299 in which both the Colombia and Guyana samples from *Phaeomyias* were included in
300 demographic analyses, the deep divergence between these samples is the probable cause of the
301 very large effective population size estimate of the ancestral population. Alleles that are not
302 shared by the two *Phaeomyias* samples inflate the inferred number of coalescence events in the
303 ancestral population and result in a large inferred population size. When only the Colombia
304 sample was included, the ancestral effective population size was similar to the contemporary
305 population size of the *Phaeomyias* population.

306 Some of the divergence between *Nesotriccus* and mainland *Phaeomyias* populations may be
307 related to adaptive evolution. Early colonists to islands are thought to broaden their niches to
308 become more generalist (Lack, 1976). Sherry (1985) found that, although the diversity of prey in
309 *Nesotriccus* was no more than mainland relatives, the number of insect guilds and the diversity
310 of foraging tactics were greater in *Nesotriccus*. The long bill of *Nesotriccus* relative to all
311 *Phaeomyias* populations on the mainland (Fig. S3; Sherry, 1985) is also evidence that it has
312 undergone adaptations to a novel environment. Further investigation of the genetic
313 underpinnings of these adaptations is warranted. The 46 loci containing fixed SNPs observed
314 between *Nesotriccus* and *Phaeomyias* may be in regions associated with important adaptations in
315 either species. Their wide dispersion across the genome, however, suggests that adaptations are
316 either scattered across the genome, or that many of the fixed alleles are a result of neutral
317 processes, potentially including founder effects. Moreover, some of the putative fixed SNPs may
318 not actually be fixed between the species, but may be artifacts resulting from the very small
319 sample sizes of individuals examined in the UCE and exon datasets.

Five to six clades within *Phaeomyias*, as currently recognized, represent divergences as deep or deeper than the split of *Nesotriccus* from *Phaeomyias*. Deep divergences within *Phaeomyias* are consistent with previous suggestions that the species may represent more than one species, although prior studies generally only regarded the populations east and west of the Andes as putative species-level taxa (Rheindt et al., 2008; Ridgely and Greenfield, 2001). A split into three species is necessary to maintain monophyly of *Phaeomyias* taxa with respect to *Nesotriccus*. The first proposed species, sister to *Nesotriccus*, is distributed from Central America (currently *eremonoma*) through northern Colombia to Guyana (currently *incomta*). The second occurs west of the Andes in the Tumbesian region as well as in the Marañon valley and on adjacent Andean slopes in northwestern South America (currently *tumbezana*, *inflava*, and *maranonica*). The last is widespread east of the Andes in the Guianas and the Amazon Basin south to Argentina (currently *incomta*, *murina*, and "*ignobilis*"). Interestingly, this last clade appears to overlap with the clade from Central America and northern South America in Guyana. Specimens assigned to either clade (LSUMZ 48589 and 48557) were collected less than 15 km apart and without any obvious intervening habitat or landscape barrier (S. Claramunt, pers. comm.). The specimens differ, however, in plumage (Fig. S4), and it is possible that populations in one or both clades are migratory, thus they may not breed syntopically. Larger samples of markers and individuals, as well as additional information on movement behavior, are desirable to better characterize *Phaeomyias* diversity in this region.

In addition, in northwestern Peru we recovered two deeply divergent clades of *Phaeomyias*: a coastal Tumbes population and a highland population distributed in the Tumbes mountains and Marañon Valley. There are three named subspecies of *Phaeomyias* from northwestern Peru: *tumbezana* was described from the coastal city of Tumbes (Taczanowski, 1877), *inflava* from

Virú near the coast of La Libertad to the south (Chapman, 1924), and *maranonica* from Jaen in the Marañon Valley (Zimmer, 1941). The divergent lowland genetic clade recovered in our analyses includes samples from near the city of Tumbes and likely corresponds to *tumbezana*. We did not observe any divergence within coastal birds south to Lambayeque, but lack samples near the type locality of *inflava*. Our montane Tumbes samples from Cerros de Amotape and the west slope of the Andes are genetically similar to Marañon samples, and birds from these areas may best be combined under the name *maranonica*. Plumage and vocal characters are also similar between montane Tumbes and Marañon populations, but the coastal Tumbes populations differ markedly in plumage and voice (Pratolongo et al., 2012; Schmitt et al., 2013). Lowland *tumbezana* and montane populations matching *maranonica* in plumage, voice, and mitochondrial DNA occur within about 10 km of each other on the lower slopes of the western Andes, where they appear to segregate by habitat and elevation (Pratolongo et al., 2012; Schmitt et al., 2013; F. Angulo P., D. Lane, pers. comm.). Vocal, morphological, and genetic data divergence between *tumbezana*/*inflava* and *maranonica* (including montane Tumbes populations), combined with their nearly sympatric distributions, suggest the two merit recognition as separate species. Further work is needed to ascertain if interbreeding or introgression occurs in this region.

A final split within the widespread eastern clade between birds from northeastern South America (Guyana and eastern Brazil) and those in central and southern South America (Peru, Bolivia, and Argentina) may be warranted, but divergence is not as deep as the other splits. Additional sampling is needed from the vast area of forest and cerrado between eastern Bolivia, the right bank of the Madeira River, and northeastern Brazil to resolve the relationships between these populations.

We used simultaneous examination of island and mainland populations to study the evolution of both the insular endemic *Nesotriccus* and its mainland relative *Phaeomyias*. Although relationships could be resolved using mitochondrial data, genomic data from a subset of samples allowed us to estimate contemporary genetic diversity and historical demography with greater precision. Additional studies of closely related island and mainland populations based on genomic datasets are desirable to determine whether population bottlenecks are truly pervasive in island populations and to better understand the biogeographic and demographic histories of both island and mainland taxa.

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Figure 1. Bayesian phylogenies of relationships within *Phaeomyias*, including *Nesotriccus* (marked in red), from ND2 data using MrBayes (a) and BEAST (b). The BEAST tree is time-calibrated and gray bars indicate the limits of the high posterior density estimate of divergence time at each node.

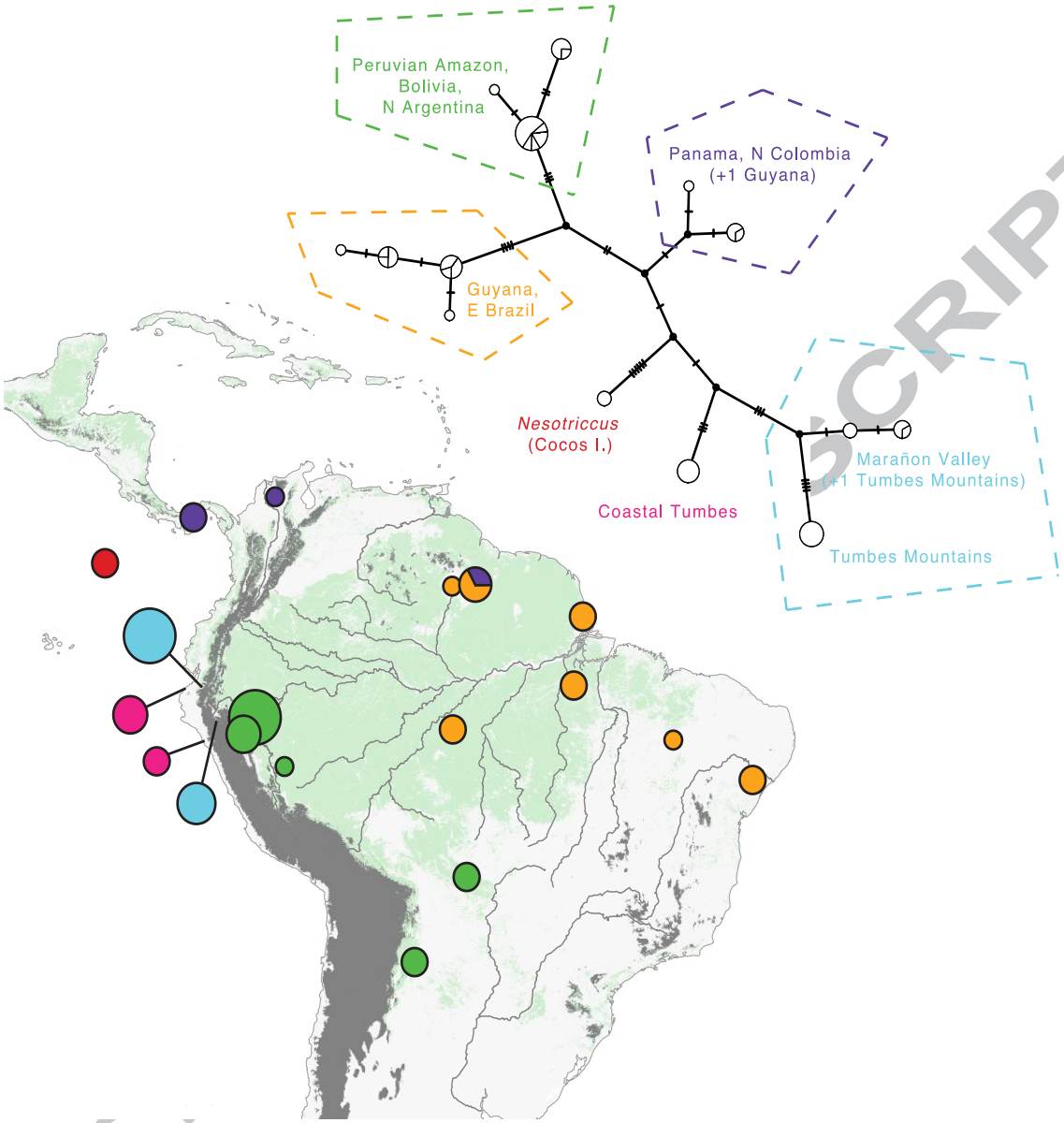


Figure 2. A network showing ND2 haplotype differentiation across the distribution of *Phaeomyias* and *Nesotriccus* based on the TCS method in PopArt. Circle size corresponds to the number of individuals represented. The map shows the distribution of each haplotype group, and the circle colors match the colors of the dashed line surrounding a particular group in the network. The +1 Guyana sample clustering with Panama and Colombia samples is LSUMZ B-48589.

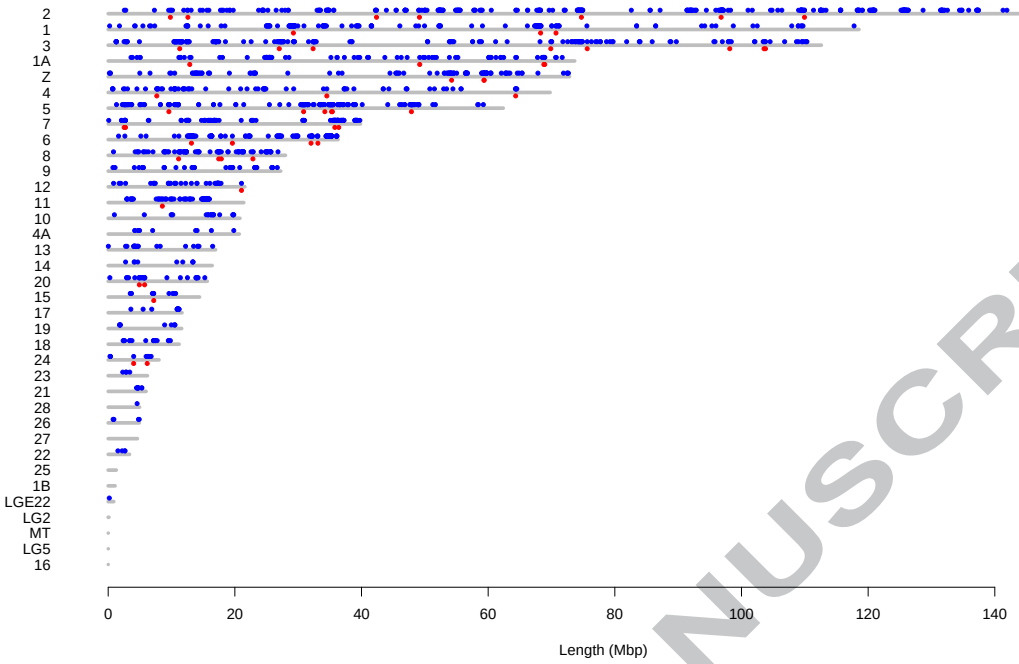


Figure S1. The distribution of all UCEs and exons recovered (blue circles) and those containing SNPs fixed between *Nesotriccus* and *Phaeomyias* (red circles) on the assembled chromosomes in the Zebra Finch (*Taeniopygia guttata*) genome.

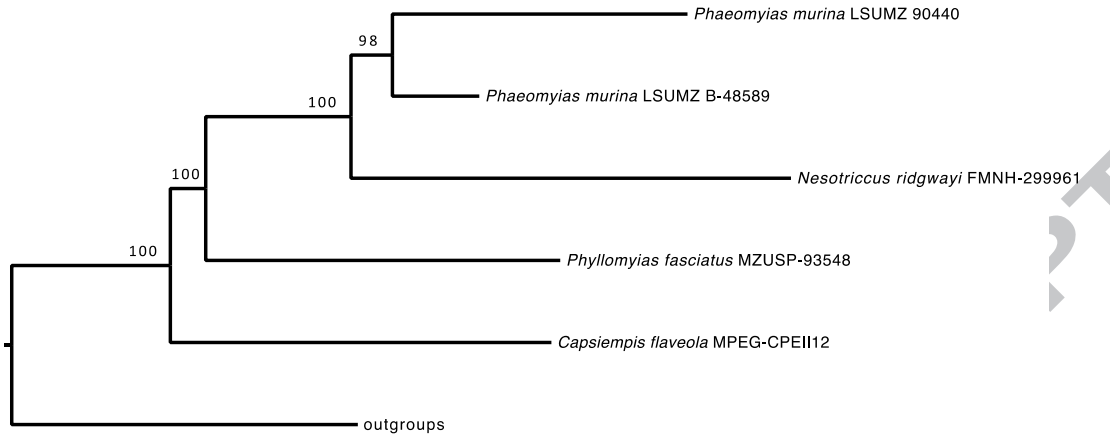


Figure S2. Maximum-likelihood phylogenetic tree of *Nesotriccus ridgwayi* and close relatives from ExaML. Values at nodes indicate bootstrap support across 500 replicates.

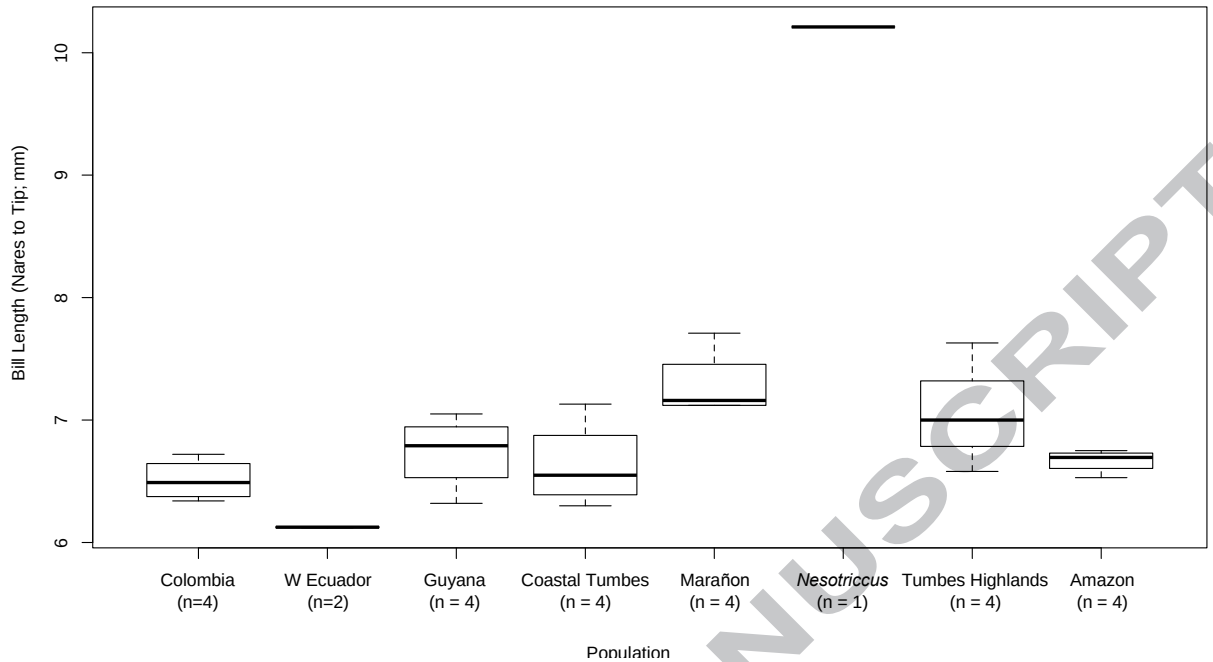


Figure S3. Bill length (nares to tip of the maxilla) in male specimens of *Nesotriccus* and *Phaeomyias* from various populations.

Figure S4. Photograph of the two specimens from Guyana that were collected about 15 km apart but differ in plumage (especially ventral coloration) and mitochondrial haplotype. The top specimen (LSUMZ 175437) corresponds to tissue LSUMZ B-48589, and the bottom specimen (LSUMZ 175436) corresponds to tissue LSUMZ B-48557.

Table S1. Sample information.

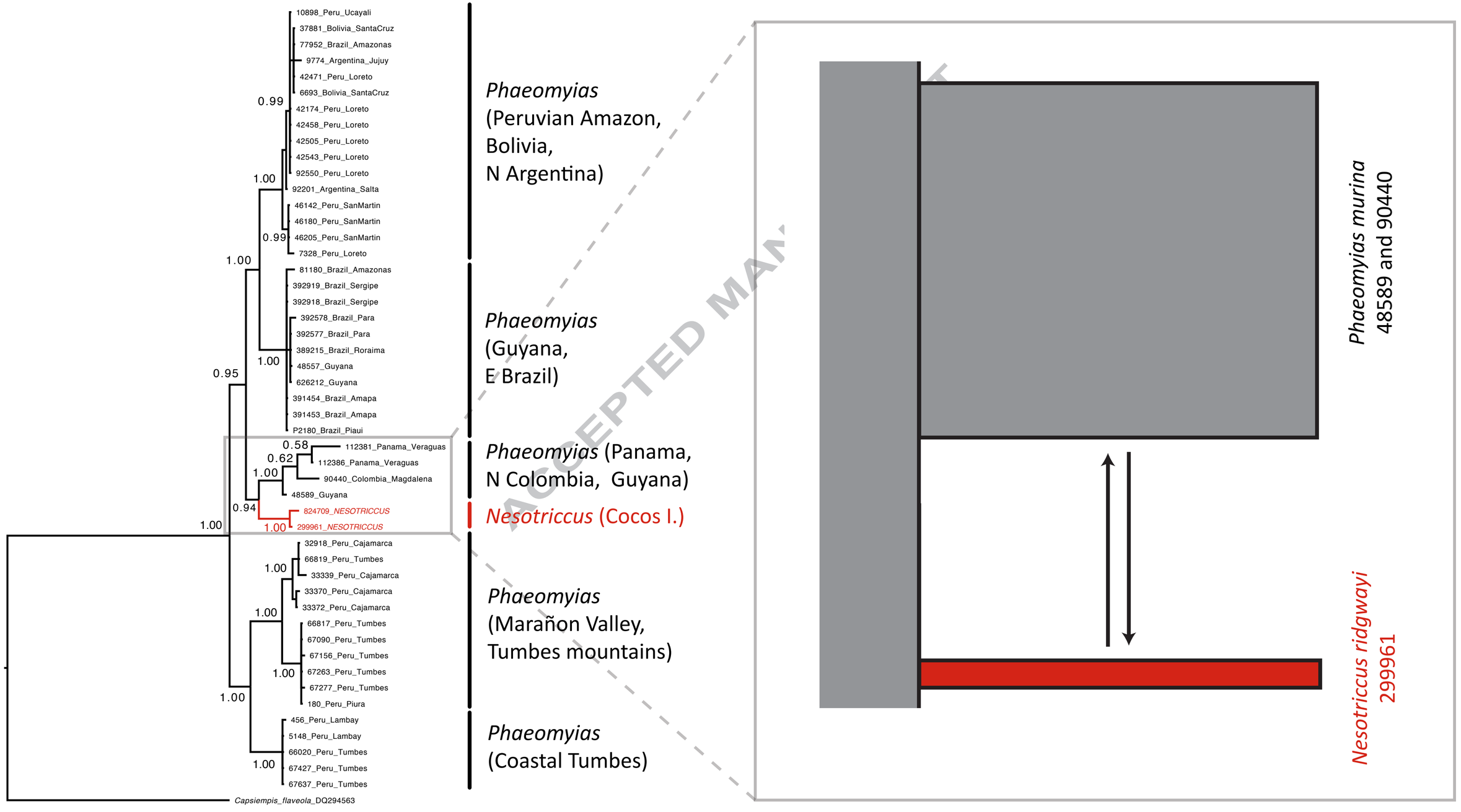
Table S2. Internal primers used for toe pads.

Table S3. Jukes-Cantor corrected genetic distances at ND2 between populations.

Table 1

	Effective Population Size (Number of Individuals)			Migration Rate (Indiv./Yr.)	
	Nesotriccus	***Phaeomyias***	**Ancestral**	***Nesotriccus* → *Phaeomyias***	***Phaeomyias* → *Nesotriccus***
Migration	249,000 (44,000-534,000)	3,056,000 (508,000-6,541,000)	183,532,000 (171,781,000-19,531,000)	1.17-6 (5.83-12-7.49-6)	9.52-8 (5.00-13-6.11-7)
No migration	249,000 (30,000-518,000)	3,060,000 (359,000-6,431,000)	191,156,000 (179,076,000-203,487,000)	NA	NA
Guyana removed	322,000 (4000-876,000)	47,376,000 (6,061,000-98,624,000)	38,674,000 (35,240,000-42,156,000)	5.68-5 (2.18-10-3.52-4)	3.85-7 (1.50-13-3.14-6)

Graphical Abstract



- Cocos Flycatcher is nested within populations of Mouse-colored Tyrannulet
- Cocos Flycatcher underwent a population bottleneck
- Mouse-colored Tyrannulet contains high levels of phylogeographic diversity
- Mouse-colored Tyrannulet may represent four or more species