

Extreme genetic structure and dynamic range evolution in a montane passerine bird: implications for tropical diversification

CARLOS DANIEL CADENA^{1,*}, JORGE L. PÉREZ-EMÁN^{2,3}, ANDRÉS M. CUERVO^{4,5}, LAURA N. CÉSPEDES¹, KEVIN L. EPPERLY⁶ and JOHN T. KLICKA⁶

¹Laboratorio de Biología Evolutiva de Vertebrados, Departamento de Ciencias Biológicas, Universidad de Los Andes, Bogotá, Colombia

²Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela, Caracas, Venezuela

³Colección Ornitológica Phelps, Caracas, Venezuela

⁴Louisiana State University Museum of Natural Science, Baton Rouge, LA, USA

⁵Instituto de Investigación en Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia

⁶Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, WA, USA

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Phylogeographical analyses provide information about the role of evolutionary processes underlying species diversity of the Neotropical montains. We used mitochondrial DNA sequence data for the *Henicorhina leucophrys* complex (Aves: Troglodytidae) from Mexico to Bolivia to describe population structure, infer relationships and divergence times among populations, examine lineage accumulation through time, assess cryptic diversity and explore the origin of elevational and latitudinal replacements of species. The complex comprises numerous lineages, reaching > 12% sequence divergence; most distinct populations are separated by geographical barriers, but several co-occur with elevational segregation or replace each other along the Andes. Some close relatives are separated by vast distances, with more distant relatives occupying intervening areas. The complex is likely to have originated in Mexico and expanded over South America while diversifying constantly into lineages persisting for millions of years. We do not propose taxonomic changes, but *H. leucophrys* is an outlier among birds in lineage diversity, with > 35 presumptive species identified by coalescent methods. Our work illustrates how diversity accumulates via divergence and persistence in allopatry, achievement of secondary sympatry, and coexistence mediated by ecological divergence. Phases of expansion and contraction of ranges and localized extinctions are likely to account for phylogeographical patterns preceding speciation and the accumulation of diversity.

ADDITIONAL KEYWORDS: Andes – elevational replacement – range expansion – speciation.

INTRODUCTION

Mountains in the tropics contribute disproportionately to regional species richness, given their area in comparison to lowlands, and are often considered global hotspots of biological diversity and endemism (Stattersfield *et al.*, 1997; Orme *et al.*, 2005; Fjeldså *et al.*, 2012). Tropical mountains exhibit particularly high beta diversity (i.e. species turnover in space) because: (1) species assemblages shift along

elevational gradients; and (2) related species occupy similar elevations in different mountains or in sectors of a mountain separated by geographical barriers. Although knowledge of the biodiversity of mountains has advanced conceptually (Graham *et al.*, 2014; Bertuzzo *et al.*, 2016; Badgley *et al.*, 2017) and empirically (e.g. Patterson *et al.*, 1998; Jankowski *et al.*, 2009; McCain, 2009; Price *et al.*, 2014; Peters *et al.*, 2016; Quintero & Jetz, 2018), accounting for species richness in montane systems remains difficult. In particular, although climate and available energy have an imprint globally on the distribution of life

*Corresponding author. E-mail: ccadena@uniandes.edu.co

(Francis & Currie, 2003; Hawkins *et al.*, 2003), they cannot predict the agglomeration of range-restricted species in tropical mountains (Jetz & Rahbek, 2002; Rahbek & Graves, 2001; Rahbek *et al.*, 2007; but see Ruggiero & Hawkins, 2008). Given that the high species richness and uniqueness of mountains in the tropics might instead reflect high speciation rates or low extinction rates (Jetz *et al.*, 2004; Badgley *et al.*, 2017), considering evolutionary processes is crucial to a better understanding of montane diversity (Graham *et al.*, 2014; Laiolo *et al.*, 2018; Quintero & Jetz, 2018).

Dozens of studies have used phylogenetic and population genetic perspectives to probe into evolutionary processes underlying patterns of avian diversity in the Neotropical mountains. Birds have diversified rapidly in the Andes, with pivotal roles of features of the landscape (e.g. low-lying valleys, high-elevation passes) and of climatic changes as drivers of divergence (Pérez-Emán, 2005; Weir, 2006; Cadena *et al.*, 2007; Ribas *et al.*, 2007; Sedano & Burns, 2010; Chaves *et al.*, 2011; Gutiérrez-Pinto *et al.*, 2012; Valderrama *et al.*, 2014; Benham *et al.*, 2015; Sánchez-González *et al.*, 2015; Winger & Bates, 2015; Prieto-Torres *et al.*, 2018). Allopatric differentiation of lineages separated by barriers to dispersal is predominant (Hazzi *et al.*, 2018), whereas evidence for speciation in parapatry along mountain slopes remains elusive (Patton & Smith, 1992; García-Moreno & Fjeldså, 2000; Cadena *et al.*, 2012; Caro *et al.*, 2013). Thus, the replacement of closely related species along elevational gradients, a salient geographical pattern in tropical avifaunas (Terborgh, 1971, 1977), appears to result largely from populations coming into secondary contact after allopatric divergence (Diamond, 1973; Cadena, 2007; Freeman, 2015). However, with hundreds of bird species living in the Neotropical mountains, much remains to be learned about the histories of individual clades and about how such histories collectively resulted in the patterns of diversity we observe today.

Before conducting analyses seeking to characterize and account for patterns of diversity, one must have proper knowledge of what species exist and where they occur (Fine, 2015). Traditionally, the species-level taxonomy of birds was considered well known (Scheffers *et al.*, 2012), with suggestions that the inventory of species was essentially complete by the mid-20th century (Mayr, 1946). This, however, proved incorrect; multiple avian species have been discovered and described over recent decades, and analyses of novel data sets (notably, of vocal and genetic variation) have revealed that species-level diversity was seriously underestimated (Fjeldså, 2013). The extent to which avian taxonomy will require revision depends on how one delimits

species (Gill, 2014; Tobias *et al.*, 2010; Toews, 2015; Barrowclough *et al.*, 2016; Remsen, 2016), but clearly there are more species of birds than traditionally thought, particularly in the tropics. Although problems with species delimitation are unlikely to affect assessments of patterns in local (alpha) diversity of birds, inadequate knowledge of species limits may seriously influence perceptions of patterns in species turnover in space, hence regional and global patterns of diversity (beta and gamma diversity). Alternative approaches for species delimitation may also influence inferences about biogeographical history (Smith *et al.*, 2018).

Birds in which species diversity is likely to be greater than traditionally thought are those in which plumages vary subtly (in which case, one would expect species recognition to be based more on vocal cues) and in which ecologically relevant traits (body size, habitat and dispersal ability) may be conducive to population isolation (Burney & Brumfield, 2009; Salisbury *et al.*, 2012; Smith *et al.*, 2014; Harvey *et al.*, 2017a). Here, we analyse the phylogeography of the grey-breasted wood-wren complex (*Henicorhina leucophrys*, Troglodytidae), a group of small, drably coloured and highly vocal songbirds of the forest interior, with poor dispersal abilities. Given that the complex is broadly distributed, from Mexico to Bolivia, and restricted to montane forest habitats, it is an appropriate system in which to ask questions relevant to understanding the role of evolutionary processes in establishing patterns of diversity in Neotropical mountains. We used extensive geographical sampling to reconstruct the phylogenetic relationships of populations in the complex and to characterize patterns of genetic variation, with the goals of: (1) gaining insight about the tempo and mode of evolutionary differentiation and the role of colonization of new regions in diversification; (2) understanding the role of geographical isolation in the differentiation of lineages; and (3) exploring the origin of elevational replacements leading to changes in species assemblages with elevation. We also examined the extent to which current taxonomy adequately reflects true diversity and reflected on the influence of cryptic differentiation for inferences about diversification processes and patterns of diversity in the tropics.

MATERIAL AND METHODS

STUDY SYSTEM

Henicorhina wood-wrens (Troglodytidae) range widely in the Neotropical region. Traditionally, taxonomists recognized two widespread species, the white-breasted wood-wren (*Henicorhina leucosticta*) and

the grey-breasted wood-wren (*H. leucophrys*). Two species with restricted ranges, the bar-winged wood-wren (*Henicorhina leucoptera*) from southern Ecuador and northern Peru (Fitzpatrick *et al.*, 1977) and the Munchique wood-wren (*Henicorhina negreti*) from western Colombia (Salaman *et al.*, 2003), were described later. More recently, another narrow endemic formerly considered a subspecies of *H. leucophrys*, the hermit wood-wren (*Henicorhina anachoreta*) from northern Colombia, was elevated to species status (Cadena *et al.*, 2016). Preliminary data on the phylogenetics and population structure of wood-wrens based on mitochondrial DNA (mtDNA) sequences suggest that both *H. leucosticta* and *H. leucophrys* are paraphyletic (*H. leucoptera* is nested within *H. leucosticta*, and *H. anachoreta* is nested within *H. leucophrys*), and both comprise multiple distinct lineages (Dingle *et al.*, 2006; Becker *et al.*, 2007; Caro *et al.*, 2013; Smith *et al.*, 2014; Aguilar *et al.*, 2016). However, no comprehensive analysis of genetic variation across the range of either widespread species has been conducted.

Wood-wrens segregate ecologically by elevation. Overall, *H. leucosticta* is a lowland species replaced in montane areas by *H. leucophrys*; their replacement is sharp and likely to be mediated by interspecific competition (Jankowski *et al.*, 2010). In the isolated Cordillera del Cóndor of southern Ecuador and northern Peru, *H. leucophrys* also replaces *H. leucosticta* in montane areas but is absent from higher elevations where *H. leucoptera* occurs (i.e. the three species turn over along the elevation gradient; Fitzpatrick *et al.*, 1977; Dingle *et al.*, 2006). Likewise, in part of the western slope of the Colombian Andes, *H. negreti* replaces *H. leucophrys* (subspecies *brunneiceps*) at higher elevations and is, in turn, replaced by nominate *H. leucophrys* east of the ridgeline on the eastern slope of the cordillera (Salaman *et al.*, 2003). In addition, two populations of *H. leucophrys* differing in mtDNA sequences, morphology and songs are parapatrically distributed along an elevational gradient in Ecuador (Dingle *et al.*, 2008, 2010), but nuclear gene flow indicates that they are conspecific (Halfwerk *et al.*, 2016). A similar scenario, with populations differing genetically, morphologically and vocally and turning over along an elevational gradient, exists in the Sierra Nevada de Santa Marta, northern Colombia (Caro *et al.*, 2013; Burbidge *et al.*, 2015); because there is little to no hybridization, these populations are now treated as separate species, with *H. anachoreta* sharply replacing *H. leucophrys* at higher elevations (Cadena *et al.*, 2016).

SAMPLING

We focused on the *H. leucophrys* complex, i.e. the clade defined by the most recent common ancestor

of populations referable to *H. leucophrys* in current taxonomy and *H. anachoreta* (Dingle *et al.*, 2006; Caro *et al.*, 2013). Although *H. negreti* was not sampled in previous molecular analyses, we consider it to be part of the complex based on phenotypic traits (Salaman *et al.*, 2003) and our data (see Results below). The *H. leucophrys* complex is widespread in Neotropical mountains, ranging from Mexico to Bolivia (Fig. 1); as currently circumscribed, it consists of 19 taxa, including *H. anachoreta*, *H. negreti* and 17 subspecies of *H. leucophrys* (Kroodsma & Brewer, 2005). For phylogeographical analyses, we sought to sample as thoroughly as possible across geography and taxonomy. Combining sequences generated for this study and published sequences available in GenBank (total of 288 individuals), we managed to cover nearly all the distributional range of the complex and all named taxa, with multiple individuals and localities per taxon whenever possible (Fig. 1; Supporting Information, Table S1). Sampling in Middle America covered all the major montane areas where members of the complex occur; within South America, sampling was especially thorough in Venezuela, Colombia and Ecuador, and less so in the southern part of the range (i.e. Peru and Bolivia). As outgroups for phylogenetic analyses, we used specimens of *H. leucosticta* and *H. leucoptera* and specimens of species of *Microcerculus*, *Campylorhynchus*, *Cistothorus*, *Troglodytes*, *Cantorchilus* and *Cyphorhinus* (for an overview of relationships among wren genera, see Barker, 2017), for a grand total of 300 individuals considered in analyses.

DNA EXTRACTION AND SEQUENCING

We extracted DNA using DNeasy tissue extraction kits (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. We then amplified an 842 bp region of the mtDNA gene ATP synthase 6 and 8 using primers described by Joseph *et al.* (2004). We chose to sequence this region because it has been used in earlier studies of *Henicorhina* (Dingle *et al.*, 2006, 2008; Caro *et al.*, 2013), which enabled us to include published sequences in analyses. Fragments were amplified via polymerase chain reaction (PCR) in 12.5 µL reactions, with denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, 54 °C for 45 s and 72 °C for 2 min, followed by 10 min elongation at 72 °C and 4 °C soak. The PCR products were sequenced at the Barrick Museum of Natural History (University of Nevada, Las Vegas, NV, USA), Universidad de los Andes (Bogotá, Colombia), or the High-Throughput Genomics Unit at the University of Washington (Seattle, WA, USA). Chromatograms were aligned using Sequencher v.4.9 (GeneCodes Corporation, Ann Arbor, MI, USA).

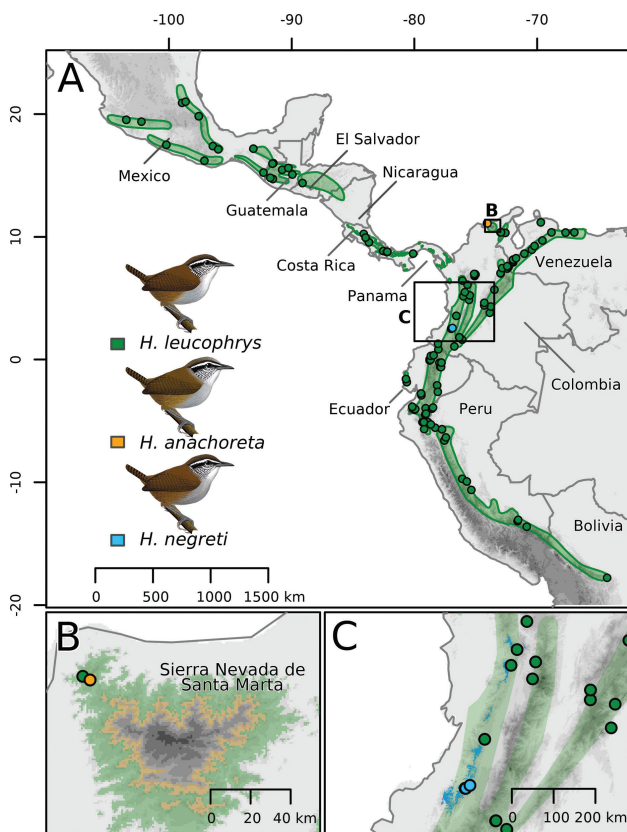


Figure 1. Geographical distribution of wood-wrens in the *Henicorhina leucophrys* complex in the Neotropical montane region and localities where specimens were sampled for our phylogeographical analyses. The complex currently comprises three species: the widely distributed *H. leucophrys*, ranging from Mexico to Bolivia, and two narrow endemics from Colombia (*Henicorhina anachoreta* in the Sierra Nevada de Santa Marta and *Henicorhina negreti* on the western slope of the Cordillera Occidental). Distribution maps were obtained from BirdLife International (*H. leucophrys*) and Velásquez-Tibatá *et al.* (2013) (*H. negreti*) or generated for the present study based on information on elevational range (*H. anachoreta*; Cadena *et al.*, 2016). Bird illustrations are by Ayerbe-Quiñones (2018), reproduced with permission from the author.

ANALYSES

Gene trees

Before phylogenetic analyses, we determined the best-fit model of evolution to be GTR+ γ with jModeltest v.2.1.7 (Posada, 2008). We then used Bayesian (BEAST v.1.8.4; Drummond *et al.*, 2012) and maximum-likelihood (ML; RAxML v.8.2.4; Stamatakis, 2006) methods to estimate phylogenetic trees. For the Bayesian analyses, we ran 50 million generations, sampling trees and parameters every 5000 generations. A relaxed uncorrelated lognormal

clock, with a rate of 2.5% (Smith & Klicka, 2010) and a birth–death speciation tree prior (Ritchie *et al.*, 2017), were applied. We confirmed likelihood stationarity and adequate effective sample sizes > 200 for all estimated parameters using Tracer v.1.6.0 (<http://tree.bio.ed.ac.uk/software/tracer>). The parameter values of the samples from the posterior distribution on the maximum clade credibility tree were summarized after discarding the first 5 million generations (10%) as burn-in using TreeAnnotator v.1.8.4 (Drummond *et al.*, 2012). Maximum-likelihood analyses were conducted using a GTR+ γ model and run for 1000 non-parametric rapid bootstrap replicates to provide an assessment of nodal support. Both Bayesian and maximum-likelihood analyses were first done with a data set containing all ATPase sequences ($N = 300$), and then repeated with a reduced data set containing only individuals having unique haplotypes ($N = 184$). To visualize and annotate trees and to produce figures, we used the R packages GGTREE (Yu *et al.*, 2017) and phytools (Revell, 2012), and QGIS v.2.18.20 with the Qgis2threejs plugin (<http://qgis.osgeo.org>).

Species delimitation

Given the uncertainty about species diversity in the *H. leucophrys* complex, we adopted two coalescent approaches using mtDNA data to identify distinct lineages that may be considered presumptive species worthy of additional study with other sources of information (Carstens *et al.*, 2013; Sukumaran & Knowles, 2017): (1) multi-rate Poisson tree processes (mPTP v.0.2.0; Kapli *et al.*, 2017); and (2) a Bayesian implementation of the general mixed Yule coalescent model (bGMYC v.1.0.2; Reid & Carstens, 2012). The trees generated by BEAST using the unique haplotype data set were used as the input for these analyses after removal of all non-*Henicorhina* taxa (i.e. outgroups).

For the mPTP analysis, we ran five replicate Markov chain Monte Carlo (MCMC) chains of 10 000 000 generations, sampling every 10 000, with a burn-in of 1 000 000 (10%), using the maximum clade credibility tree obtained as described above. The minimum branch length was calculated using the minbr_auto function before the analysis. We ran bGMYC with 100 of the 10 000 gene trees estimated in the BEAST analysis, after removing 50% as burn-in; this approach accounts for error in gene-tree estimation by integrating over uncertainty in tree topology and branch lengths. For each tree, we ran an MCMC chain of 100 000 steps, with 20 000 steps of burn-in and a thinning interval of 100 steps. We set the threshold parameter prior to range between two and 178 (i.e. the number of tips in the tree). We defined the burn-in after exploring a preliminary run with a single tree and then assessed convergence and appropriate mixing of the final run

by inspecting trace plots. We focused our results and discussion on lineages (i.e. presumptive species) defined using a threshold of 0.9 on the probability of membership of individuals, which allowed us to compare our results with similar work on other birds (Smith *et al.*, 2014, 2017; Harvey *et al.*, 2017b). However, we also considered more conservative (0.95) and less conservative (0.5; Gehara *et al.*, 2017) thresholds.

Diversification through time

To describe patterns of lineage accumulation over time, we constructed lineage-through-time (LTT) plots and estimated the γ statistic (Pybus *et al.*, 2000). We accounted for phylogenetic uncertainty by performing these analyses on a sample of credible trees in the posterior distribution obtained from the BEAST analysis. We used the 100 trees constructed using only unique haplotypes as the input for the bGMYC analyses and trimmed them to include tips corresponding to the presumptive species recognized under the 0.9 threshold. Then we used functions implemented in the R packages *ape* (Paradis *et al.*, 2004) and *paleotree* (Bapst, 2012) to build an LTT plot with a 95% confidence interval and to calculate the γ statistic for each tree.

RESULTS

We found substantial genetic differentiation among populations in the *H. leucophrys* complex. In total, we recovered 172 haplotypes among the 288 individuals analysed. Several haplotypes were highly divergent from each other, with uncorrected genetic distances between them reaching > 12% (i.e. between individuals from Sierra Madre del Sur of Mexico and from the east slope of the Cordillera Occidental of Colombia). Genetic variation was highly structured spatially but was not readily accounted for by geographical distance among populations. We did not conduct formal analyses of isolation by distance, but genetic distances among isolated populations from adjacent mountains were often much greater than genetic distances observed over larger distances in more continuous ranges. For instance, mean genetic distances among the five montane areas of Venezuela that we sampled was 7.3% (range 6.1–8.3%), whereas genetic distances within montane regions extending over comparable distances were much lower, e.g. reaching only 3.5% in the Sierra Madre Oriental of Mexico or 1.7% in the Talamanc-Chiriquí mountains of Costa Rica and Panama.

Maximum-likelihood and Bayesian phylogenetic analyses recovered similar overall patterns (Fig. 2; Supporting Information, Figs S1, S2). The deepest split in gene trees separates clades corresponding

to Mexican populations from the Sierra Madre del Sur and the western reaches of the Trans-Mexican Volcanic Belt from a large and strongly supported clade including the remainder of populations in the complex (Fig. 2). Within the latter clade, the earliest diverging group occurs in eastern Mexico and consists of three subclades, each corresponding to a unique region within the Sierra Madre Oriental. Sister to this group is a clade divided into two main groups (albeit without strong support, i.e. 0.91 posterior probability, 68% ML bootstrap in analyses using only unique haplotypes): one includes samples from lower Central America (Costa Rica and Panama, subspecies *H. l. collina*), whereas the other includes all South American populations of *H. leucophrys*, the Colombian endemic species *H. anachoreta* and *H. negreti*, and a clade formed by samples of *H. leucophrys* from Chiapas (Mexico), Guatemala and El Salvador (subspecies *H. l. castanea* and *H. l. composita*). The latter clade, of somewhat uncertain affinities within an otherwise South American group (it was recovered as sister to *H. negreti* with 0.93 posterior probability and 56% ML bootstrap in analyses using only unique haplotypes), is the only exception to the pattern in which Mexican and Central American populations are the earliest diverging lineages in the complex.

Basal relationships among major South American lineages were unresolved or poorly supported, yet some patterns in the region are noteworthy. Whereas populations from some isolated montane systems (e.g. the Serranía de Perijá, or the Venezuelan Cordillera de la Costa and Sierra de San Luis) formed distinct clades, this was not the case for the main cordilleras of the northern Andes, resulting in complicated patterns of area relationships (Fig. 2). For example, several populations from the western slope of the Andes, from Colombia through Ecuador and into northwestern Peru, formed a large clade (clade I in Fig. 2), but this clade was not exclusive because it also included some, but not all, populations from the northern sector of the Cordillera Central of Colombia and did not cluster all populations from the western Andes; *H. negreti* and lineages of *H. leucophrys* from the northern and southern sectors of the Cordillera Occidental of Colombia and from western Ecuador occupied different positions in the tree (Fig. 2). Likewise, birds from the Cordillera Oriental of the Colombian Andes formed multiple distinct clades that seemed to be distantly related to each other, and populations from the Central and Southern Andes (i.e. from Bolivia and Peru south of the Marañón Valley or North Peruvian Low) formed at least two highly divergent clades with differing affinities. Given weak support for deep branches in South America, we do not elaborate further on relationships among major biogeographical areas, but we do emphasize the complexity of phylogeographical

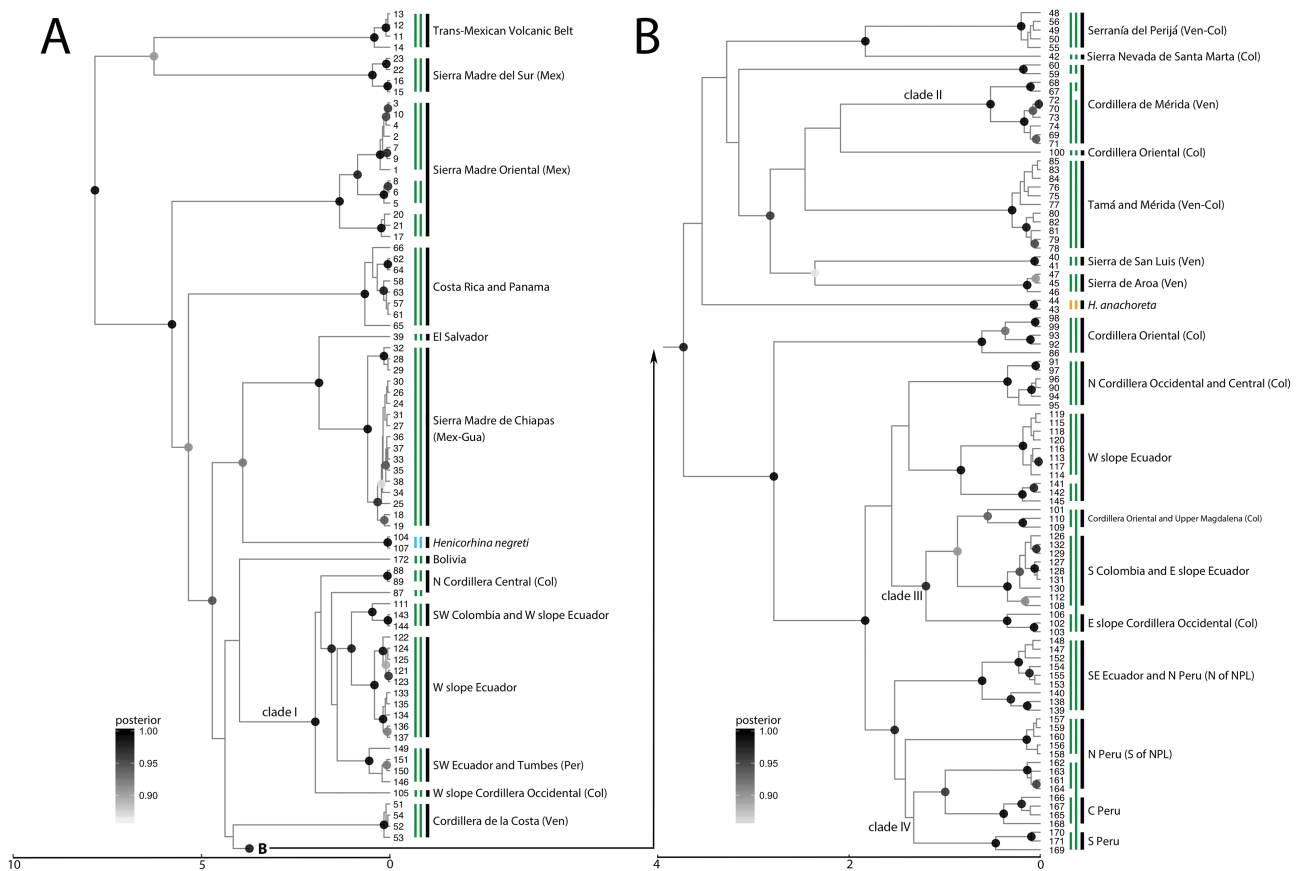


Figure 2. Phylogenetic relationships and divergence times among unique mitochondrial DNA haplotypes in the *Henicorhina leucophrys* complex inferred using BEAST suggest the following: (1) paraphyly of *H. leucophrys* with respect to *Henicorhina anachoreta* and *Henicorhina negreti*; (2) a northern origin for the complex, with subsequent colonization of South America; and (3) marked population genetic structure, partly attributable to geographical isolation mediated by physical barriers. Colour shading on nodes corresponds to posterior probabilities ≥ 0.85 . Black vertical lines indicate geographical regions; note that all deep branches correspond to clades from mountain regions in Mexico and Central America and that South American populations are also strongly structured. Green vertical lines signal presumptive species identified using multi-rate Poisson tree processes (mPTP; left) and the Bayesian general mixed Yule coalescent model (bGMYC; right); results of these analyses were similar, with minor discrepancies in clades II, III and IV. Haplotypes are numbered (based on their localities from North to South) at the tips of the tree; information on specimens having each haplotype is provided in the [Supporting Information \(Table S1\)](#). The lower branch in the tree in the panel on the left (A) is expanded in the panel to the right (B). The scale bar on the bottom indicates time before present (Mya). Abbreviation: NPL, North Peruvian Low.

pattern and the strong genetic structure existing over relatively fine spatial scales throughout the continent.

Part of the complexity in phylogeographical pattern related to occurrence of phylogenetically distant groups in the same regions can be understood by examining elevational distributions. Lineages known to replace each other along elevational gradients in northern South America are not sister to each other (Fig. 3). This was true of taxa occurring in the Ecuadorean Andes (*H. l. hilaris* and nominate *H. l. leucophrys*), in the Santa Marta mountains (*H. l. bangsi* and *H. anachoreta*) and on the western slope of the Colombian Andes (*H. l. brunneiceps* and *H. negreti*). Our data also revealed a previously unknown case

of cryptic replacement of mtDNA lineages along an elevational gradient in the Venezuelan Andes (Fig. 3). The lineage occurring in the Tamá massif near the Colombia–Venezuela border crosses the Táchira depression to the northeast into the Cordillera de Mérida, where we found it from ~1520 to ~1920 m. Only a small distance upslope in this range, a different lineage occupied elevations from ~2100 to ~2750 m. Divergence in mtDNA sequences between lineages replacing each other with elevation was substantial, in all cases $> 5\%$ uncorrected *p*-distances (Fig. 3).

In addition to examples of elevational parapatry, our analyses revealed cases where lineages may meet

in contact zones along latitudinal or east–west axes. For instance, three distinct lineages replace each other geographically in the northern sector of the Cordillera Central of the Colombian Andes (Fig. 2). Two of these lineages, not sister to each other and differing in ~3.5% uncorrected *p*-distance, occur in the west and the east of the northern tip of the cordillera in Antioquia (haplotypes 87–89 in Fig. 2). South of this region, a third lineage (haplotype 91), differing in ~7% distance from the other two, is found ranging north presumably to the southern extreme of the Aburrá Valley in the outskirts of Medellín, also in Antioquia. Some individuals occupying the northern

extreme of the Cordillera Central are most closely allied to geographically distant populations from the western slope of the Andes (i.e. subspecies *H. l. hilaris* and *H. l. brunneiceps*; clade I in Fig. 2), which tend to occur at lower elevations and are replaced upslope by nominate *H. leucophrys* in Ecuador or *H. negreti* in southwest Colombia. Several other examples of distinct lineages occurring at different latitudes within mountain systems exist in the Cordillera Oriental of Colombia and along the Andes of Ecuador (Fig. 2).

Coalescent approaches to delimit species produced consistent results. Both mPTP and bGMYC (the latter with a 0.90 probability threshold to define group

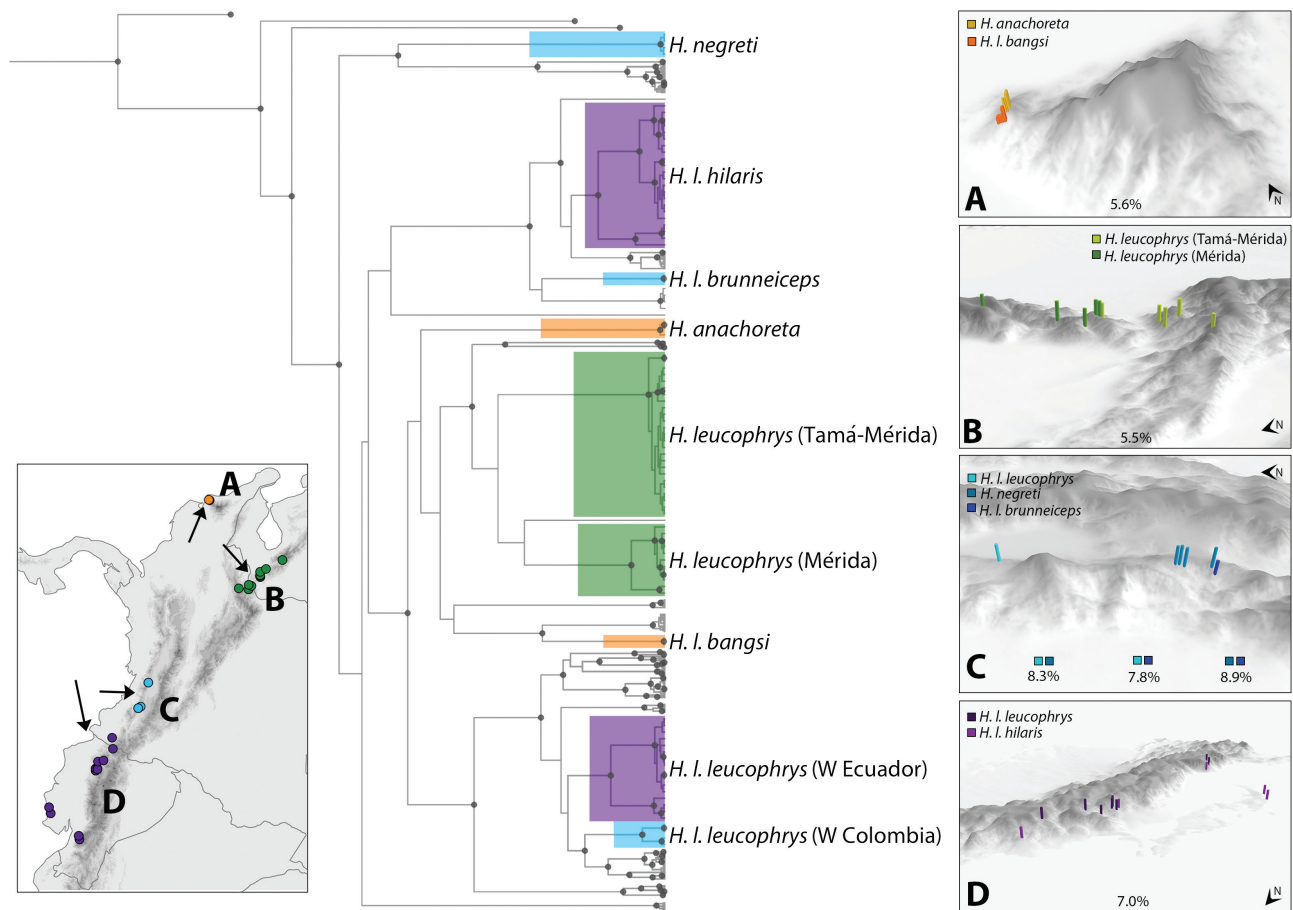


Figure 3. Elevational replacements involving distinct lineages of the *Henicorhina leucophrys* complex in montane South America. Lineages replacing each other with elevation in a region share colours on the map, the phylogeny (modified from Supporting Information, Fig. S1) and the close-up view of mountain slopes, where different shades are used for each lineage (arrows on the map show the approximate direction from which mountains are seen in A–D; arrows inside panels point to the north). Nodes indicated with grey dots on the tree have ≥ 0.85 posterior probability support; clades corresponding exclusively to Central American and Mexican populations are collapsed. In at least three regions (A, C, D), elevational replacements do not involve sister taxa, with the only possible exception being the novel case of cryptic replacement of lineages in the Cordillera de Mérida, Venezuela (B), where affinities of lineages to each other and to those from other regions are not strongly supported. Lineages involved in elevational replacements are deeply divergent from each other (panels show mean uncorrected *p*-distances in ATPase genes), occur in close proximity, and correspond to different presumptive species identified by coalescent analyses (see main text).

membership) recovered *H. anachoreta* and *H. negreti* as distinct species, and both methods identified many more additional lineages in the *H. leucophrys* complex that might prove to be distinct species (Figs 2, 4; Supporting Information, Fig. S3). Although methods did not agree exactly in how they assigned individuals to presumptive species, congruence was remarkable, with mPTP recognizing 35 and bGMYC 38 presumptive species (Fig. 2). Differences between methods were restricted to three groups, as shown in Figure 2. In clade II, formed by individuals from Cordillera de Mérida, mPTP recognized two presumptive species, whereas bGMYC recognized one. In clade III, formed by individuals from the Cordillera Oriental, Upper Magdalena, east slope of Ecuador and the east slope of the Cordillera Occidental of Colombia, bGMYC recognized three presumptive species, whereas mPTP recognized only one. Lastly, in clade IV, formed by individuals from Peru, bGMYC recognized three presumptive species, but mPTP recognized only one. Application of a more stringent probability threshold (0.95) to delimit species in bGMYC analyses also recovered 38 presumptive species, and application of a less conservative threshold (0.5) resulted in 46 presumptive species. In general, presumptive species appear to have restricted ranges (Fig. 4; Supporting Information, Fig. S4); in some cases, particular mountain systems harbour a single presumptive species (e.g. Sierra Madre del Sur and Trans-Mexican Volcanic Belt in Mexico, Cordillera de la Costa and Sierra de San Luis in Venezuela), but more than one presumptive species may also occur within a region (e.g. Sierra Madre Oriental of Mexico, Sierra Nevada de Santa Marta in Colombia), and a few of them have ranges encompassing various montane areas (e.g. across cordilleras of Costa Rica and Panama). The diversity of presumptive species is especially remarkable in northern South America, with seven or eight identified in Venezuela, 13–15 in Colombia and seven in Ecuador (Figs 2, 4). Given that our sampling was sparser in Peru and Bolivia, our figures for these countries are likely to be underestimates of presumptive species richness (Fig. 4).

Our estimates of divergence times obtained from the BEAST analysis of unique haplotypes indicated that the *H. leucophrys* complex diverged from its sister group (i.e. the clade formed by *H. leucosticta* and *H. leucoptera*) ~12.4 Mya [95% highest posterior density (HPD), 10.2–14.8 Mya], with the crown age of extant populations dating to 7.9 Mya (95% HPD, 6.4–9.4 Mya). Over this period, the complex diversified into multiple lineages; we found that it consists of roughly five lineages of > 5 Myr of age and of 19–20 lineages of > 2 Myr of age (Fig. 2; Supporting Information, Fig. S5). The estimated age of the node including all South American populations and populations from Chiapas,

Guatemala and El Salvador is 4.7 Mya (95% HPD, 4.0–5.5 Mya), whereas that of the node including all South American populations excluding *H. negreti* is 4.4 Mya (95% HPD, 3.8–5.0 Mya).

Analyses of lineage accumulation over time based on presumptive species identified by bGMYC suggested that rates of diversification in the *H. leucophrys* complex have been nearly constant and exponential through much of the history of the complex (Fig. 5). An apparent downturn in the rate of lineage accumulation over the last ~0.8 Myr, resulting in negative values of the γ statistic ($\gamma = -3.92$ to -1.79 across the 100 trees), is likely to reflect the fact that our species delimitation analyses recognized no species younger than this age.

DISCUSSION

PHYLOGEOGRAPHY: BRIDGES, BARRIERS AND THE DISTRIBUTION OF GENETIC VARIATION

As evidenced by the branching pattern in the gene tree, with deep splits involving populations from the north, the *H. leucophrys* complex is likely to have originated in the Mexican highlands ~14.8–10.2 Mya, from where it expanded south through Central America, then colonizing South America. Other birds ranging broadly in montane forests also originated in the northern Neotropics, including single species and clades that diversified in Central and South America (Pérez-Emán, 2005; Cadena *et al.*, 2007; Weir *et al.*, 2008; Sánchez-González *et al.*, 2015). Molecular-based estimates of when birds colonized South America from the north vary (Bacon *et al.*, 2015; Barker *et al.*, 2015); our results indicate that colonization by the *H. leucophrys* complex might have occurred slightly earlier (~5.5–4.0 Mya) than a pulse of avian interchange via the Isthmus of Panama 4–2 Mya (Smith & Klicka, 2010). Our estimates of the age of the *H. leucophrys* complex and of the timing of events such as its colonization of South America are old relative to what one would expect given estimates of divergence times among wren genera (Barker, 2017). However, such estimates were derived assuming that *Certhia* and *Troglodytes* diverged ~16 Mya (Moyle *et al.*, 2016), whereas analyses integrating more extensive fossil evidence suggest that this divergence occurred much earlier, ~27 Mya (Claramunt & Cracraft, 2015). The time frame for wren diversification implied by the latter study is more congruent with our estimated ages for nodes in *Henicorhina* and with previous work in other wrens based on mtDNA data (Barker, 2007).

Poorly supported relationships among South American clades, associated with short internodes subtending long branches, are common to *H. leucophrys* and other birds colonizing South America from the north (e.g. Pérez-Emán, 2005; Cadena *et al.*, 2007).

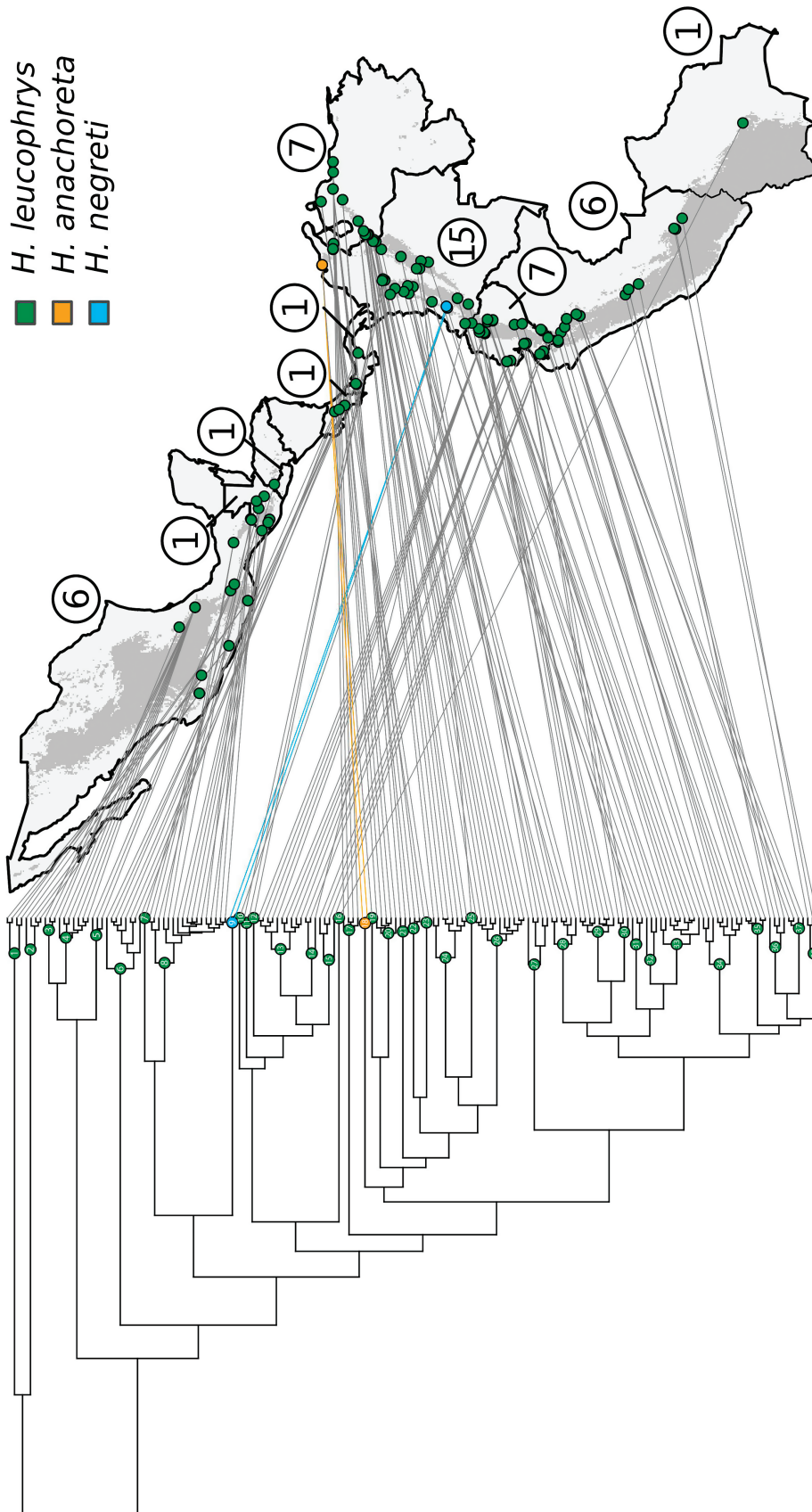


Figure 4. Geographical locations where we sampled 38 presumptive species in the *Henicorhina leucophrys* complex identified by coalescent analyses of mitochondrial DNA sequences. Dots and numbers on the tree (modified from Fig. 2) correspond to presumptive species statistically inferred by the Bayesian general mixed Yule coalescent model (bGMYC), with the threshold probability used to define group membership set at 0.90. Colours correspond to species epithets according to the current three-species taxonomy. Encircled numbers on the map indicate the number of presumptive species occurring in each country. Similar patterns were observed using the multi-rate Poisson tree processes (mPTP) method. For close-up views of geographical locations where each presumptive species was sampled, see the [Supporting Information \(Fig. S4\)](#).

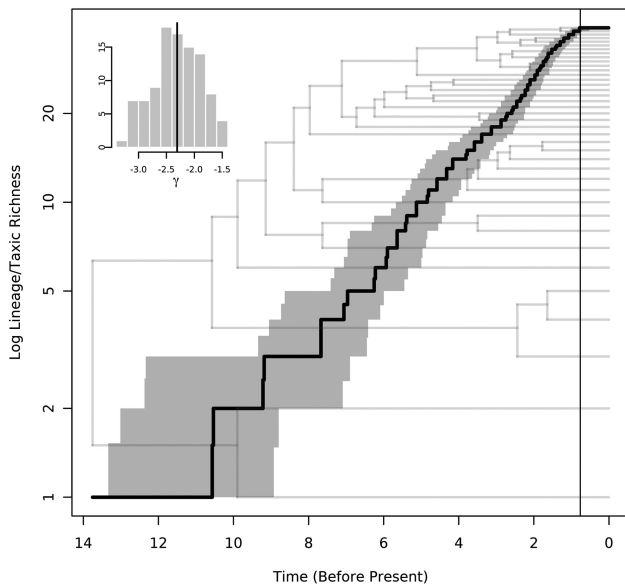


Figure 5. Lineage-through-time plot showing accumulation of lineages (i.e. presumptive species) over time in the *Henicorhina leucophrys* complex. Black lines are estimates based on the maximum clade credibility tree, and grey indicates the 95% credibility interval across 100 trees. Lineage accumulation over much of the history of the clade appears to have been roughly constant. The downturn near the present and the associated negative γ statistic reflects the fact that this analysis used results of the Bayesian general mixed Yule coalescent model (bGMYC) as input, with no presumptive species younger than ~ 0.8 Myr considered (cut-off shown with a vertical line). The pattern might also reflect, in part, incomplete sampling of young lineages, particularly within South America. The histogram in the upper left shows the distribution of the gamma statistic across trees in the posterior distribution.

This indicates range expansions and ensuing rapid diversification of lineages in geographical isolation, a pattern also documented in montane clades with South American (Chaves *et al.*, 2011) or uncertain geographical origins (Gutiérrez-Pinto *et al.*, 2012). Rapid range expansions occurring in concert across birds might indicate that geological changes, such as closure of the Isthmus of Panama and uplift of mountains, increased connectivity among formerly isolated regions, enabling subsequent diversification of various taxa; climatic changes driving population isolation are likely to have facilitated such diversification (Barrantes, 2009; Ramírez-Barahona & Eguiarte, 2013).

Genetic divergence associated with landscape features isolating montane habitats is another pattern shared by the *H. leucophrys* complex and co-distributed clades (Weir, 2009). Such features include lowland areas in Central America (Cadena

et al., 2007; Barber & Klicka, 2010), inter-Andean valleys, such as the Magdalena and Marañón (Gutiérrez-Pinto *et al.*, 2012; Benham *et al.*, 2015), and alpine areas separating slopes of cordilleras (Parra *et al.*, 2009; Valderrama *et al.*, 2014). In many Neotropical montane birds, genetic structure across geographical barriers coincides with differences in plumage (Cadena *et al.*, 2011; Winger & Bates, 2015; Winger, 2017). Phenotypic differences among distinct lineages of *H. leucophrys*, however, appear either subtle or non-existent (Kroodsma & Brewer, 2005). Reduced gene flow across barriers might have influenced vocal differentiation of wood-wrens more strongly, but confirmation of this awaits studies documenting repertoires of individuals in addition to variation within and among populations. Such data are relevant given the uncertainty about species limits in the complex, because vocalizations are likely to play a crucial role in species recognition (Caro *et al.*, 2013; but see Halfwerk *et al.*, 2016).

EXTREME POPULATION STRUCTURE, CRYPTIC DIVERGENCE AND PATTERNS IN TROPICAL DIVERSITY

We uncovered genetic structure in the *H. leucophrys* complex across well-known geographical barriers (Hazzi *et al.*, 2018), but also over fine scales in ways not associated with divergence in other tropical montane birds. For example, in the Cordillera Occidental and Cordillera Central of Colombia, where other birds show little to no population structure (Cadena *et al.*, 2007; Gutiérrez-Pinto *et al.*, 2012; Isler *et al.*, 2012; Valderrama *et al.*, 2014), we found six mtDNA lineages of ≥ 1 Myr of age. These lineages and others have restricted ranges, with some of their boundaries reflecting topographic or climatic breaks (Graham *et al.*, 2010; Supporting Information, Fig. S4). Traits affecting dispersal abilities and dependence on closed understorey habitats mediate divergence across putative barriers and thus diversification in topographically complex landscapes (Burney & Brumfield, 2009; Smith *et al.*, 2014). Given that wood-wrens are small bodied, have small and rounded wings and live in dark forest understorey, they are likely to disperse little (Moore *et al.*, 2008), and this might account, in part, for their exceptionally strong population structure (Claramunt *et al.*, 2012; Salisbury *et al.*, 2012; but see Smith *et al.*, 2017). Deep phylogeographical structure also exists in other small-bodied wrens (i.e. other *Henicorhina*, *Cistothorus* and *Troglodytes*; Dingle *et al.*, 2006; Campagna *et al.*, 2012; Galen & Witt, 2014; Robbins & Nyári, 2014), suggesting that their biology predisposes populations to become isolated and to diverge.

Although our study used a single molecular marker, some of the lineages we recovered coexist as distinct

phenotypic entities exhibiting behavioural barriers to hybridization (Salaman *et al.*, 2003; Caro *et al.*, 2013; Burbidge *et al.*, 2015), implying that several species are involved. Genetic distances (i.e. divergence times) are not appropriate surrogates for reproductive isolation (Roux *et al.*, 2016), but we note that wood-wren lineages demonstrating barriers to gene flow in sympatry (i.e. *H. anachoreta* and *H. l. bangsi*; *H. negreti* and *H. l. brunneiceps*; *H. negreti* and nominate *H. leucophrys*) last shared ancestors more recently than many other lineages in the complex. It is also remarkable that the phylogeography of *H. leucophrys* resembles that of *Atlapetes* brushfinches (Emberizidae), which also have a northern origin and montane distribution through the Neotropics, and which diverged into numerous lineages upon colonizing South America (Sánchez-González *et al.*, 2015; J. L. Pérez-Emán, unpublished data). In contrast to *H. leucophrys*, *Atlapetes* diversified extensively in plumage, and this arguably influences taxonomy, with researchers recognizing 28 species in the group (Remsen *et al.*, 2018). Just as lineages of the *H. leucophrys* complex replace each other in space, species of *Atlapetes* are, for the most part, allopatric

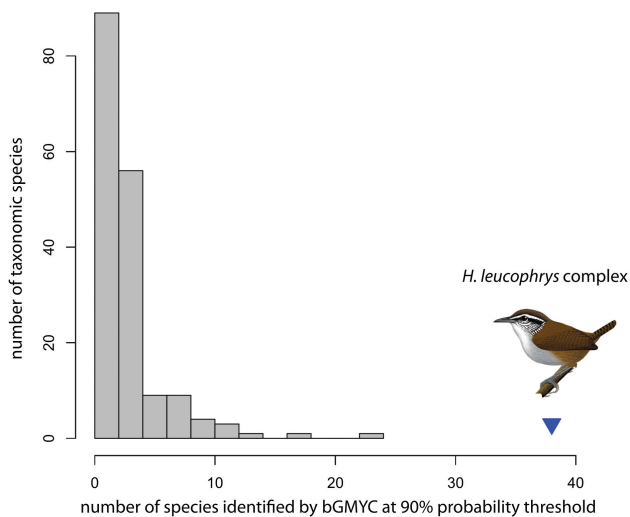


Figure 6. Relative to the frequency distribution of the number of presumptive species identified by coalescent analyses of mitochondrial DNA sequence data within 173 taxonomic species of New World birds (data from Harvey *et al.*, 2017b; Smith *et al.*, 2017), our result for the *Henicorhina leucophrys* complex is a distant outlier. Even if many lineages cannot be shown to be isolated reproductively from others, the data reveal a remarkable and previously undocumented degree of population genetic structuring despite our sparse sampling in the Peruvian and Bolivian Andes, where one would expect more lineages to exist. Bird illustration is by Ayerbe-Quiñones (2018), reproduced with permission from the author.

or replace each other sharply along elevational or latitudinal axes in the Andes (Remsen & Graves, 1995), with their ranges often matching those of lineages of *H. leucophrys* uncovered by our study. This comparison serves to illustrate what might be a more general situation, in which clades with roughly similar ages and genetic structure might be split to different degrees by taxonomists because of differences among clades in the traits birds use for signalling and in the lability of such traits. In other words, birds such as wood-wrens might be under-split owing to their conserved plumage and because the role of vocalizations in species recognition remains understudied (see also D’Horta *et al.*, 2013).

Our coalescent analyses suggested that taxonomy underestimates species diversity in the *H. leucophrys* complex. We consistently identified > 35 presumptive species across methods, a remarkable figure relative to similar studies. In 27 clades of lowland birds, ranging from Central America across the Andes through much of Amazonia and even into the Atlantic Forest (Smith *et al.*, 2014), the mean number of species identified using bGMYC with a threshold of 0.95 was 5.3 (range 1–18); figures were slightly higher in understory birds (11 clades; mean = 6.6, range 3–11 presumptive species) but still much lower than our estimate of 38 presumptive species for the *H. leucophrys* complex. Moreover, in 173 taxonomic species of birds from the New World subject to phylogeographical analyses using mtDNA data, the largest number of presumptive species identified by bGMYC with a threshold of 0.90 was 23 (Harvey *et al.*, 2017b; Smith *et al.*, 2017), highlighting the *H. leucophrys* complex as a distinct outlier (Fig. 6). We do not argue that all lineages we uncovered are species given existing evidence, but several are candidates for studies examining other molecular markers, morphology, voices and behaviour (Caro *et al.*, 2013; Burbidge *et al.*, 2015; Halfwerk *et al.*, 2016). The allopatric ranges of most wood-wren lineages preclude tests of intrinsic barriers to gene flow, but some of them might well be reproductively isolated (Pulido-Santacruz *et al.*, 2018).

Given that most wood-wren lineages are not sympatric, our finding that the *H. leucophrys* complex might comprise multiple species has no influence on local estimates of diversity. However, species richness and endemism at regional scales might need revision. For example, current taxonomy recognizes only one species (and only five subspecies) in the complex in Venezuela (Kroodsma & Brewer, 2005), yet we uncovered seven or eight presumptive species, each endemic to a particular montane system, in the country. If presumptive species are elevated to species status and similar patterns exist in other taxa not yet studied, then geographical variation in population structure might alter knowledge of spatial patterns

of diversity (e.g. differences in species richness among cordilleras and slopes of cordilleras of Colombia; Kattan *et al.*, 2004; Fig. 4; Supporting Information, Fig. S4). Alternative classifications also affect inferences about beta diversity; under current taxonomy, there is no species turnover along thousands of kilometres and across multiple geographical barriers from Mexico to Bolivia except for the local replacements involving *H. anachoreta* and *H. negreti*. At the other extreme, if distinct lineages of *H. leucophrys* are species, then spatial turnover would be substantial even over relatively short distances (e.g. in Colombia), probably exacerbating differences among regions in beta diversity (Gaston *et al.*, 2007; Fjelds  *et al.*, 2012) and with potential conservation implications (Socolar *et al.*, 2016). The recognition of distinct lineages as species would also alter the perceived role of features of montane landscapes in setting range limits and explaining spatial turnover in assemblages (Graham *et al.*, 2010).

In addition to affecting perceptions of patterns of diversity, our results have implications for thinking about historical processes underlying such patterns. The accumulation of biological diversity via diversification within a region such as the montane Neotropics requires that: (1) populations become isolated to initiate divergence; (2) budding population isolates persist in time; (3) populations expand their ranges and come into secondary sympatry; and (4) newly sympatric populations are differentiated enough that they may coexist without coalescing owing to hybridization or without excluding each other via competition (Mayr, 1942; Ricklefs & Bermingham, 2007). In the following sections, we discuss our results in the context of these steps.

LINEAGE SPLITTING, PERSISTENCE AND THE ORIGIN OF DIVERSITY

A leading explanation for high tropical diversity involves latitudinal differences in net diversification rates (Fischer, 1960; Schluter & Pennell, 2017). In particular, rapid diversification (Madri n n *et al.*, 2013) might explain the high species richness and concentration of narrow-ranged species of birds in tropical mountains, which cannot be accounted for by area or contemporary climate (Jetz *et al.*, 2004; Fjelds  *et al.*, 2012). However, evidence that diversification rates vary with latitude remains mixed in birds (Ricklefs, 2006; Martin & Tewksbury, 2008; Jetz *et al.*, 2012; Belmaker & Jetz, 2015) and other taxa (e.g. Pyron & Wiens, 2013; Pyron, 2014; Rolland *et al.*, 2014; Schluter, 2016; Rabosky *et al.*, 2018). Furthermore, differences in the rate at which species originate might not be as important as the rate at which they go extinct in establishing spatial patterns in avian

diversity (Hawkins *et al.*, 2006; Weir & Schluter, 2007; Pulido-Santacruz & Weir, 2016). Accordingly, the high diversity and endemism of tropical montane areas might reflect low extinction rates of species (Fjelds  *et al.*, 2012), and increasing diversification rates with elevation (Quintero & Jetz, 2018) might result from both high speciation and low extinction (Fjelds  & Irestedt, 2009). A complementary explanation for diversity in tropical mountains less commonly addressed in the literature is high persistence of budding populations, an important control of rates of speciation (Mayr, 1963; Dynesius & Jansson, 2014; Rabosky, 2016).

We found that the *H. leucophrys* complex radiated rapidly into multiple lineages, several of which have persisted for periods exceeding millions of years. Also, LTT plots suggested nearly constant rates of exponential accumulation of lineages over nearly 8 Myr, with an apparent slowdown in diversification towards the present. Although LTT plots with such a shape and their associated negative γ statistic are often considered evidence of ecological limits to diversification (Rabosky & Hurlbert, 2015), we interpret the pattern more as an artefact of our methods resulting from: (1) using species based on a model specifying a divergence threshold separating population-level processes (gene coalescence) from diversification dynamics (speciation and extinction) as units for analysis (Reid & Carstens, 2012); and (2) potential limitations in geographical sampling, leading to failure to identify additional independent lineages of young age. Given that the bGMYC analysis we used to delimit presumptive species established an age cut-off of ~0.8 Mya defining the units included in the LTT analysis, we solely conclude that diversification was nearly constant through much of the history of the *H. leucophrys* complex. To the extent that similar diversification dynamics might characterize the evolutionary history of other Neotropical montane birds, high rates of lineage splitting (Harvey *et al.*, 2017b) and high persistence of such lineages over time (Smith *et al.*, 2017) have probably contributed to diversification, probably accounting for the high diversity of tropical montane systems and, more broadly, have contributed to large-scale biodiversity patterns, such as latitudinal gradients in species richness.

RANGE DYNAMICS, SECONDARY SYMPATRY AND THE REGIONAL BUILD-UP OF DIVERSITY

Our data revealed that mtDNA lineages in the *H. leucophrys* complex, which are likely to have diverged in geographical isolation, have come into secondary sympatry. This is most evident where divergent mtDNA lineages not sister to each other

segregate with elevation. In addition to previously documented cases of elevational replacements of lineages involving distinct taxa (i.e. different species or subspecies in the Sierra Nevada de Santa Marta, in western Colombia and in western Ecuador), we discovered a novel elevational replacement of distinct lineages in the Mérida Cordillera of Venezuela, where no phenotypic differences had been noted. Likewise, previous work in other wood-wrens revealed that although *H. leucoptera* is nested within *H. leucosticta*, the lineage of *H. leucosticta* replaced by *H. leucoptera* at higher elevations in the Cordillera del Cóndor east of the Andes is distantly related to it, whereas its closest relative seemingly occurs in the Chocó region west of the Andes (Dingle *et al.*, 2006). The finding that elevational replacements consistently involve fairly distant relatives as opposed to sister lineages fits the hypothesis that evolutionary divergence in tropical montane birds occurs largely in allopatry and not in parapatry along mountain slopes (Patton & Smith, 1992; García-Moreno & Fjeldså, 2000; Caro *et al.*, 2013).

In addition to documenting elevational replacements, we found evidence of regional co-occurrence of lineages replacing each other with latitude (e.g. along the cordilleras of Colombia and Ecuador). More fine-scaled sampling is required to determine whether geographical gaps separate the ranges of such lineages or if they come into close contact. Part of the observed genetic differentiation along the latitudinal axis might reflect the propensity of the linear distributions of tropical montane birds to become fragmented (Graves, 1988). However, some lineages replacing each other with latitude in a region are not sisters and may even be distantly related, which suggests range expansions and secondary contact rather than primary divergence along cordilleras.

Other intriguing phylogeographical patterns aside from secondary contact of lineages in elevational or latitudinal parapatry speak to the dynamism of geographical ranges over broad scales. For example, populations of *H. leucophris* from southern Mexico (Chiapas), Guatemala and El Salvador are not closely related to other Middle American populations; within a large, otherwise South American clade, these specimens appeared closest to *H. negreti*, a species endemic to western Colombia whose northernmost records are ~1700 km south of montane El Salvador. Likewise, the only sequence analysed from Bolivia is a long branch seemingly more closely allied to lineages from northern South America than to geographically much closer lineages. Given that closest relatives may occur in distant areas, spatial patterns of genetic variation are not easily accounted for by geography (e.g. by isolation by distance; Seeholzer & Brumfield, 2018). Given that such patterns are unlikely to be evidence of long-distance dispersal and are not unique

to wood-wrens in the region (Cadena *et al.*, 2007), considering the dynamics of expansion and contraction of geographical ranges involving localized extinctions is crucial to understand the biogeographical and demographic processes underlying the distribution of genetic and species diversity in Neotropical birds.

Shifting climatic conditions affecting habitat connectivity drive changes in species ranges, thereby influencing phylogeographical patterns and the build-up of montane diversity (Ramírez-Barahona & Eguiarte, 2013; Flantua & Hooghiemstra, 2018). Species ranges may also experience phases of expansion and contraction linked to shifts in ecological specialization and interactions with natural enemies (Ricklefs & Bermingham, 2002; i.e. the taxon cycle; Wilson, 1959). Although taxon cycles are more evident in insular settings with discrete populations and areas (e.g. Ricklefs & Bermingham, 1999; Jönsson *et al.*, 2014), they may also take place on continents (Graves, 1982). Lineages experiencing the taxon cycle might account for what one might call continental great speciators, such as *Henicorhina* wood-wrens, which occur widely in space (revealing an ability to expand their ranges), yet split into isolated populations at a fast rate owing to cessation of gene flow (cf. Diamond *et al.*, 1976). Wood-wrens disperse little at present because of their morphology and ecology, which arguably explain their remarkable patterns of genetic structure, reflecting long-term population isolation. However, our findings that wood-wrens dispersed throughout much of the montane Neotropics from a northern area of origin and that several lineages achieved secondary sympatry indicate that episodes of range expansion interspersed with periods of divergence occurred at various moments, possibly in synchrony with morphological or behavioural changes influencing their abilities to disperse (Pigot & Tobias, 2015; Hosner *et al.*, 2017). Furthermore, gaps separating the ranges of closely related lineages of wood-wrens arguably reflect extinctions of intervening populations of formerly widespread lineages, which left vacant spaces that could, in turn, become occupied by other expanding lineages.

Phylogeographers will often not detect range dynamics embodied in the taxon cycle because incomplete reproductive isolation between young lineages can result in homogenization of gene pools upon secondary contact (Kearns *et al.*, 2018). Furthermore, niche similarities between incipient species achieving contact may preclude long-term sympatry owing to interspecific competition (Pigot & Tobias, 2013). Irrespective of whether the patterns we observed resulted from the taxon cycle, we identified aspects making the *H. leucophris* complex well suited for further work on the origins of tropical diversity and its accumulation over time and space. Our results and

other work on the complex reveal that the completion of reproductive isolation between lineages meeting in secondary sympatry seemingly exhibits a continuum ranging from neutral divergence with no obvious phenotypic differences (forms in southwestern montane Venezuela) to phenotypic and behavioural divergence with persistent interbreeding (*H. l. hilaris* and *H. l. leucophrys* in Ecuador) to completed speciation with little to no hybridization (*H. l. anachoreta* and *H. negreti* vs. various forms of *H. leucophrys* in Colombia; Salaman *et al.*, 2003; Dingle *et al.*, 2008, 2010; Caro *et al.*, 2013; Burbidge *et al.*, 2015; Halfwerk *et al.*, 2016). Furthermore, divergence in elevational ranges occurring during periods of isolation (Cadena, 2007; Tobias *et al.*, 2014) or arising in secondary sympatry (Diamond, 1973; Freeman, 2015) has enabled coexistence of lineages at the landscape scale in various regions. Given that range boundaries may be maintained (and possibly reinforced) evolutionarily by phenotypic and behavioural barriers to interbreeding and ecologically by competition (Jankowski *et al.*, 2010), our study has uniquely captured wood-wren populations in the act of building up diversity via divergence and persistence in allopatry, achievement of secondary sympatry, and coexistence mediated by ecological and evolutionary divergence. Comparative work on the structure and dynamics of contact zones between lineages should provide rich insights into the origin and maintenance of high diversity in tropical mountains.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Phylogenetic relationships among individuals in the *Henicorhina leucophrys* complex inferred using Bayesian analysis of sequences of the ATPase 6/8 mitochondrial genes. The phylogeny is the maximum clade credibility tree obtained in BEAST. Nodal support (i.e. posterior probabilities ≥ 0.85) is shown using a grey scale. Nodes with a coloured outline (green, *H. leucophrys*; blue, *Henicorhina negreti*; orange, *Henicorhina anachoreta*) were also recovered with strong support ($\geq 80\%$ bootstrap) in maximum-likelihood analysis ([Supporting Information, Fig. S2](#)).

Figure S2. Phylogenetic relationships among individuals in the *Henicorhina leucophrys* complex inferred using maximum-likelihood analysis of sequences of the ATPase 6/8 mitochondrial genes. The phylogeny is the maximum-likelihood tree obtained in RAxML. Nodal support (i.e. bootstrap values $\geq 80\%$) is shown using a grey scale. Nodes with a coloured outline (green, *H. leucophrys*; blue, *Henicorhina negreti*; orange, *Henicorhina anachoreta*) were also recovered with strong support (≥ 0.90 posterior probability) in Bayesian analysis ([Supporting Information, Fig. S1](#)).

Figure S3. Results of species delimitation analysis in the *Henicorhina leucophrys* complex using the Bayesian general mixed Yule coalescent model (bGMYC). The phylogeny showing relationships among haplotypes is the

maximum clade credibility obtained using BEAST, and the table to the right is a sequence-by-sequence matrix, in which cells are colour coded to indicate the posterior probability that each pair of sequences is conspecific.

Figure S4. Close-up views of geographical locations where presumptive species in the *Henicorhina leucophrys* complex identified by coalescent analyses of mitochondrial DNA sequences were sampled. Maps showing known locations of each presumptive species are ordered roughly from North to South and are numbered according to numbers on nodes in the tree in [Figure 4](#); points on maps are coloured based on the current three-species taxonomy recognizing *H. leucophrys*, *Henicorhina negreti* and *Henicorhina anachoreta*. Some presumptive species have relatively large ranges (e.g. no. 6 across Costa Rica and Panama), whereas others appear to be much more restricted, in some cases found at single localities so far (e.g. no. 11 in the northern extreme of the Cordillera Central in Antioquia, Colombia). Asterisks next to numbers denote presumptive species represented by only one sequence in our sampling.

Figure S5. Number of mitochondrial DNA lineages in the *Henicorhina leucophrys* complex of various ages (from 5 to 1 Mya). Vertical black lines (when shown) correspond to the median number of lineages dating to at least each of the four ages (i.e. splitting from their common ancestor with other lineages before each age) observed in a sample of 100 trees in the posterior distribution obtained using BEAST; vertical green lines correspond to the number of lineages dating to at least each of the four ages in the consensus tree shown in [Figure 2](#). When the median number of lineages equals that in the consensus tree, only the latter is shown. Grey bars are the frequency distributions of the number of lineages per age across all trees.

Table S1. Information on specimens considered in phylogeographical analyses, including museum catalogue or collector numbers, locality data and GenBank accession numbers when available. GenBank accession numbers starting with MK correspond to specimens sequenced for the present study. For each specimen, we also indicate the name used to refer to it in the [Supporting Information \(Figs S1, S2\)](#), the ATPase 6/8 haplotype as shown in [Figure 2](#), and the identity of the presumptive species identified using bGMYC to which it belongs using the threshold of 0.9 ([Fig. 4](#); [Supporting Information, Fig. S4](#)). Specimens belonging to the *Henicorhina leucophrys* complex are ordered roughly from North to South.