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ORIGINAL ARTICLE

Biogeography of the Andean metaltail hummingbirds: contrasting evolutionary histories of tree line and habitat-generalist clades

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ABSTRACT

Aim To assess geographical and temporal patterns of diversification in *Metallura* hummingbirds, particularly with respect to topographical barriers and climatic variation between different populations.

Location Tropical Andes.

Methods We estimated a multilocus phylogeny for all nine species of *Metallura* and evaluated phylogeographical patterns within *Metallura tyrianthina* using mitochondrial DNA sequences from across its range ($n = 192$). We tested mechanisms of diversification using climatic classification of sampling sites, coalescent-based dating, lineages-through-time plots and dispersal–vicariance analysis.

Results *Metallura* consists of two main clades: (1) the tree line specialists in the *M. aeneocauda* superspecies; and (2) a habitat-generalist clade that includes *M. tyrianthina* and *M. iracunda. Metallura phoebe* was recovered as sister to the tree line clade in some analyses. In both clades, there was marked genetic structure across topographical barriers and almost no structure between climatically distinct regions in the absence of barriers. The tree line clade exhibited deeper divergences in the Central Andes than elsewhere, and a south-to-north history of diversification, whereas the habitat-generalist clade showed deeper divergences in the Northern Andes and a history of southward expansion. A pure-birth model explained the steady net rate of diversification of *Metallura* hummingbirds through the Pliocene and the Pleistocene.

Main conclusions Isolation across topographical barriers best explained the genetic structure in *M. tyrianthina*. The two *Metallura* clades expanded from opposite ends of the Andes, leading to asynchronous divergence across common topographical barriers. Cycles of expansion followed by isolation may explain the preponderance of idiosyncratic area relationships that are typical of Andean clades. Geoclimatic dynamism during the Pleistocene caused *Metallura* and other Andean bird clades to undergo radical range shifts, including dispersal across topographical barriers, that became key to their diversification.

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Keywords

Allopatric speciation, Andes, ecological speciation, elevational replacement, glaciation, hummingbirds, *Metallura*, orogeny, phylogeography, tree line.

INTRODUCTION

The tropical Andes harbour one of the most diverse biological assemblages on the planet (Myers *et al.*, 2000). The mechanisms underlying the historical build-up of this biota are thought to include physical isolation through complex topography (Cracraft, 1985; Weir, 2009; Valderrama *et al.*, 2014), variable ecological pressures (Chapman, 1926; Graham *et al.*, 2004; Elias *et al.*, 2009; Caro *et al.*, 2013), a dynamic Neogene uplift history (Ribas *et al.*, 2007; Picard *et al.*, 2008;

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Antonelli *et al.*, 2009; Chaves *et al.*, 2011) and Pleistocene climatic oscillations (Vuilleumier, 1969; García-Moreno *et al.*, 1999a,b; Weir, 2006; Koscinski *et al.*, 2008). Andean endemic radiations might contain phylogenetic and population-genetic signatures of these different historical mechanisms of diversification that could be evaluated using spatially explicit, time-calibrated phylogenies with comprehensive sampling (Chaves *et al.*, 2011; Smith *et al.*, 2014).

Topographical complexity and ecoclimatic variation are prominent features of the Andes, but their impacts on divergence are rarely evaluated simultaneously (but see Graham *et al.*, 2004; Caro *et al.*, 2013). Multiple or recurring barriers are often implicated in animal diversification (Mayr, 1947; Price, 2008): for tropical montane animals, topographical barriers created by elevational shifts (e.g. deep valleys or high ridges) create intervening regions of unsuitable habitat, fragmenting the species' range. These barriers are pervasively associated with genetic and phenotypic variation and provide support for an allopatric model of Andean speciation (Cracraft, 1985; Graves, 1988; Weir, 2009). Alternatively, the high levels of beta diversity found along environmental gradients in the Andes has led some authors to propose that speciation occurs via local adaptation to differential ecological pressures, regardless of gene flow (Chapman, 1926; Endler, 1977; Milá *et al.*, 2009). This parapatric speciation model has received mixed support in the Andes, however, with sister species apparently replacing one another along environmental gradients in frogs (Graham *et al.*, 2004) and butterflies (Elias *et al.*, 2009). In Andean mammals and birds, elevational replacement seems to occur only after allopatric divergence and range expansion into secondary contact (Patton & Smith, 1992; Dingle *et al.*, 2006; Ribas *et al.*, 2007; Caro *et al.*, 2013), but genetic structure between environmentally distinct regions within a species' realized niche has not yet been assessed for the vast majority of species.

The dynamic history of orogeny and glaciation in the Andes must have had a profound impact on high-elevation species (Rull, 2011). Andean montane habitats are likely to have originated when the central Andean plateau attained about half its current elevation, *c.* 10 Ma, followed by a period of rapid uplift in the late Miocene, *c.* 10–6 Ma (Garzione *et al.*, 2008; Hoorn *et al.*, 2010). The northern Andes were uplifted later, but exhibit a more complex history with independent late-Miocene tectonic events forming the Central and Western cordilleras of Colombia, the Sierra Nevada de Santa Marta, the Serranía de Perijá and the Mérida Andes (Montes *et al.*, 2010; Kalm & Mahaney, 2011). The final uplift of the Eastern Cordillera of Colombia *c.* 5–3 Ma completed the formation of the Andean chain (Gregory-Wodzicki, 2000). This uplift sequence created an expanding corridor of montane habitat from south to north and a topographically complex landscape conducive to vicariance.

Glacial cycles commenced *c.* 2.5 Ma and intensified after *c.* 0.9 Ma (Clark *et al.*, 1999). These glacial cycles correspond to major shifts in habitat, with the tree line pushed down-slope as much as 1200 m (Hooghiemstra *et al.*, 2006; Cárdenas *et al.*, 2011). Repeated habitat migration is likely to have allowed range expansion of montane taxa during glacial periods when cold-adapted vegetation types descended and spanned river valleys, followed by range contraction and isolation during interglacials (Hooghiemstra & Van der Hammen, 2004; Hooghiemstra *et al.*, 2006). The major periods of orogeny substantially pre-date the glacial cycles, making it possible to test their relative influences on diversification (Weir, 2006; Ribas *et al.*, 2007; Chaves *et al.*, 2011).

The replacement of related lineages along elevational gradients could result from repeated cycles of range fragmentation and expansion during uplift or glaciation (Terborgh, 1971). Taxa at higher elevations are thought to undergo faster diversification because of the reduced habitat continuity (Graves, 1985, 1988). Previous phylogenetic comparisons of elevational-replacement clades in the hummingbird genus *Metallura* were consistent with this elevation effect on diversification (García-Moreno *et al.*, 1999a). The finding of lower diversity in the lower-elevation lineage may, however, have resulted from recent range expansion into the study region, rather than a lower rate of diversification (Kessler, 2002). Detailed analyses of the historical biogeography of elevational-replacement lineages could resolve whether different elevational effects or colonization histories underlie the observed patterns of diversity. Similar areas of origin and histories of expansion through the Andes would indicate that elevational replacement evolved early and differences in diversity may be primarily due to elevation. In contrast, different areas of origin would suggest that secondary contact and elevational replacement are relatively recent.

Metallura is an Andean-endemic hummingbird genus that is ideal for testing ecological and geographical mechanisms of diversification in the Andes. Previous work has identified two main groups: (1) the six members of the *Metallura aeneocauda* superspecies, which replace one another latitudinally from Bolivia to Colombia in humid tree line habitat (*c.* 2900–3800 m elevation; Fig. 1a); and (2) the seven subspecies within *Metallura tyrianthina* (Fig. 1b), which occupy a latitudinally, elevationally (1900–4200 m) and ecologically broad range (Graves, 1980; Heindl & Schuchmann, 1998; García-Moreno *et al.*, 1999a; nomenclature follows Schuchmann, 1999). Where these two groups co-occur, *M. tyrianthina* is found at lower elevations than members of the *M. aeneocauda* superspecies (Fjeldså & Krabbe, 1990). The evolutionary relationships of the west Peruvian *Metallura phoebe* and the Serranía de Perijá endemic *Metallura iracunda* remain poorly understood.

In the present study, we estimate a multilocus, time-calibrated phylogeny for all nine species of *Metallura*, with comprehensive geographical sampling of all seven *M. tyrianthina* subspecies, to assess the mode and tempo of diversification in this Andean radiation. We addressed three biogeographical hypotheses: (1) that geographical patterns of neutral genetic diversity in *M. tyrianthina* were structured primarily by topographical barriers rather than climatic variation; (2) that the contemporary *Metallura* diversity arose through

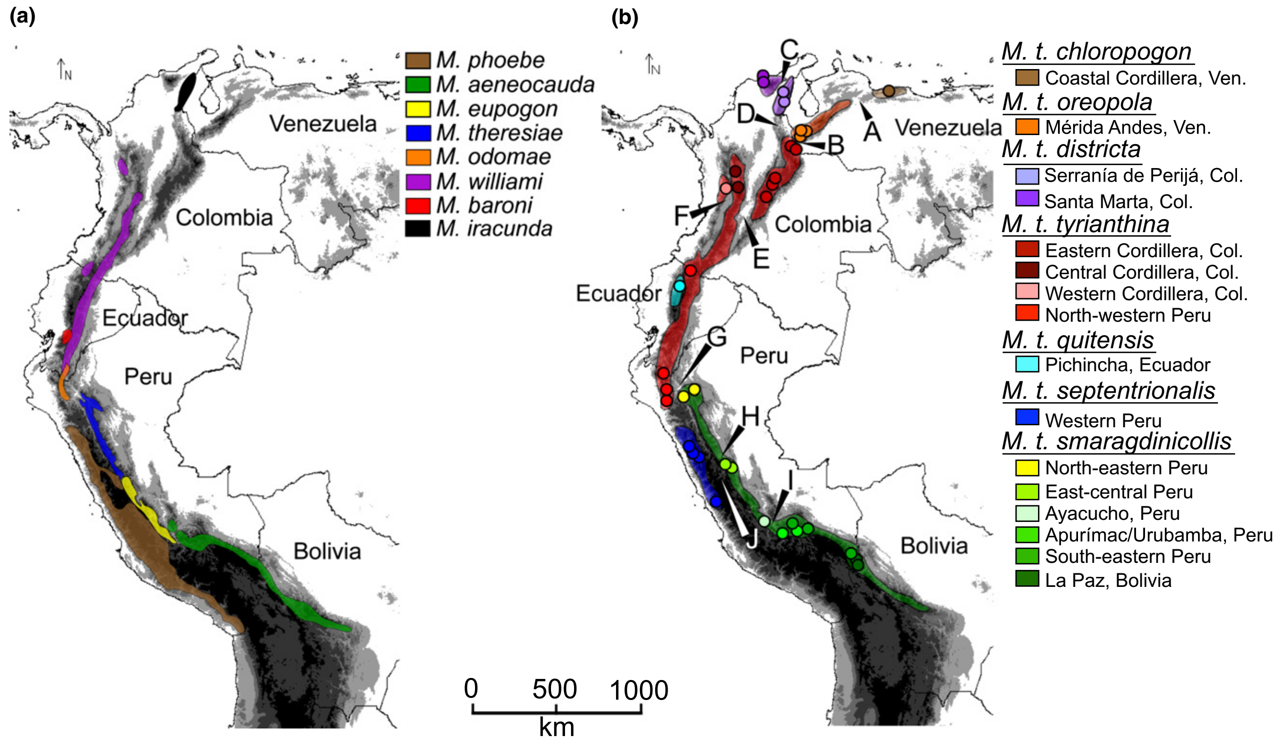

Figure 1 The geographical distributions of all species of *Metallura* and subspecies of *Metallura tyrianthina* in the tropical Andes: (a) tree line specialists of the *M. aeneocauda* superspecies, *M. phoebe* and *M. iracunda*; (b) the more generalist, forest-dwelling subspecies of *M. tyrianthina*. Circles represent the sampling localities of all *M. tyrianthina* tissues, and colour-coding of circles corresponds to geographically cohesive populations, which largely matches the *ND2* phylogeny (Col, Colombia; Ven, Venezuela). Capital letters refer to the major topographical barriers evaluated in this study, including: A, Turbio–Yaracuy Depression; B, Táchira Depression; C, Cesar Depression; D, Motilones Low Pass; E, Magdalena Valley; F, Cauca Valley; G, Marañón Valley; H, Huallaga Bend; I, Apurímac Valley; J, high Andean ridge of Peru.

distribution shifts during Pleistocene glacial cycles rather than through vicariance during earlier orogeny; and (3) that elevational replacement evolved recently following range expansions from distinct areas of origin.

MATERIALS AND METHODS

Samples and sequencing

We sequenced the mitochondrial gene NADH dehydrogenase subunit 2 (*ND2*) from muscle tissue of 192 *M. tyrianthina* individuals and sequenced or obtained sequence data from GenBank for 1–5 individuals of every other *Metallura* species (see Appendix S1 in Supporting Information). We also sequenced nuclear intron 1 of the adenylate kinase gene (*AK1*), intron 7 of the β-fibrinogen gene (*Bfib7*), and the Z-linked muscle/skeletal receptor tyrosine kinase (*MUSK*), for 22 *M. tyrianthina* individuals representing distinct *ND2* genetic clusters and 1–3 individuals of every other *Metallura* species (33 ingroup individuals). *ND2*, *AK1* and *BFib7* sequences were obtained from GenBank for five outgroup species (*Lophornis chalybeus*, *Aglaiocercus kingi*, *Oreotrochilus estella*, *Lesbia nuna* and *Chalcostigma herrani*) and we sequenced *MUSK* for these outgroup taxa (Appendix S1). See Appendix S2 for details of sequencing.

Phylogenetic analyses

We estimated phylogenies using Bayesian and maximum-likelihood (ML) methods based on *ND2* for all 216 samples, and five datasets with a subset of 38 individuals: 1, *AK1* only; 2, *BFib7* only; 3, *MUSK* only; 4, concatenated nuclear genes; and 5, concatenated nuclear and mitochondrial genes. The Akaike information criterion (AIC) was used in jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) to select the following substitution models: *ND2*, GTR+I+Γ; *AK1*, HKY+I; *Bfib7*, HKY+I+Γ; *MUSK*, HKY+Γ. The *ND2* dataset was partitioned by codon position (see McGuire *et al.*, 2007). We ran Bayesian analyses in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller *et al.*, 2010). Four Markov chain Monte Carlo (MCMC) runs of four chains were run for 40 million generations with a chain heating parameter of 0.2 (0.175 for *ND2*), trees were sampled every 1000 generations, and the first 10% of trees discarded as burn-in. Convergence was assessed using AWTY (Nylander *et al.*, 2008a). Three datasets (*Bfib7*, *MUSK* and concatenated nuclear) did not converge after 40 million generations and were rerun for 60 million generations with chain temperature reduced to 0.15 in order to increase the rates of acceptance and swapping between chains.

ML analyses were performed using RAxML 7.2.8 (Stamatakis, 2006) using a GTRGAMMA model for each locus. The data were partitioned by codon position for *ND2* and by locus for concatenated datasets. We conducted 100 RAxML searches for each dataset (200 searches for the *ND2* gene tree) to identify the best topology, and nodal support was estimated through 1000 iterations of the rapid bootstrapping algorithm.

To further evaluate the structure in *M. tyrianthina*, we identified haplotypes in DnaSP 5 (Librado & Rozas, 2009) and visualized these as a network using a median-joining method in network 4.6.11 (Bandelt *et al.*, 1999).

Phylogeographical structure

Genetic structure was evaluated across putative topographical barriers spanned by *Metallura* (Fig. 1b) that correspond to borders between areas of endemism in the Andes that are frequently implicated in other studies (Cracraft, 1985; Chaves & Smith, 2011; Gutiérrez-Pinto *et al.*, 2012; Valderrama *et al.*, 2014). To determine the role of climatic differences in structuring populations of *M. tyrianthina*, we performed principal components analysis on 19 bioclimatic variables (Table 1; WorldClim; Hijmans *et al.*, 2005) at all 41 localities. We used the first four principal components (accounting for 99.8% of the variance) to identify climatically distinct regions using a clustering analysis in the R package mclust (Fraley *et al.*, 2014). mclust compares models of varying cluster number and shape, and selects the best model according to the Bayesian information criterion (BIC). Although topographical barriers in the Andes represent inherently abrupt climatic shifts, we distinguish between these barriers and variation among climatic clusters, which can exist in the absence of elevational discontinuities and are within the realized niche of *M. tyrianthina*. We compared the genetic structure of *M. tyrianthina* across topographical barriers and between the identified climatic clusters with corrected pairwise distances (Nei, 1987), Φ_{ST} values (Excoffier *et al.*, 1992) and AMOVA analyses (Excoffier *et al.*, 1992), all calculated in Arlequin 3.5 (Excoffier *et al.*, 2005).

Divergence times

Reported substitution rates for *ND2* vary in the range 2–12.3% Myr^{-1} (Arbogast *et al.*, 2006; Fuchs *et al.*, 2011; Johnson & Weckstein, 2011; Lerner *et al.*, 2011; Patel *et al.*, 2011), with little consensus. We therefore employed a fossil and geological calibration for hummingbirds to avoid compounding errors from previous studies. First, fossil swifts, *Scaniacypselus* spp., are known from the middle Eocene (38.0–48.6 Ma) of Europe (Harrison, 1984) and their position within Apodidae is well supported (Mayr, 2003). The sister relationship between Apodidae and Hemiprocnidae to the exclusion of Trochilidae is also supported by multiple datasets (e.g. Mayr, 2002; Hackett *et al.*, 2008). Second, we calibrated the node between two coquette hummingbirds, the endemic Juan Fernández firecrown (*Sephanoides fernandensis*) and its sister species, *Sephanoides sephaniodes*, using K–Ar dates for the emergence of Robinson Crusoe Island *c.* 4–5 Ma (Stuessy *et al.*, 1984). GenBank sequences of Apodi-

Table 1 Loadings from the first four principal components from an analysis of 19 bioclimatic variables for each of the 41 *Metallura tyrianthina* sampling localities in the tropical Andes. Blank spaces signify loadings < 0.1.

Variable	PC1	PC2	PC3	PC4
Annual mean temperature				
Mean diurnal range				
Isothermality				
Temperature seasonality		−0.116	−0.346	
Maximum temperature of the warmest month				
Minimum temperature of the coldest month				
Annual temperature range				
Mean temperature of the wettest quarter				
Mean temperature of the driest quarter				
Mean temperature of the warmest quarter				
Mean temperature of the coldest quarter				
Annual precipitation	0.894		0.233	0.266
Precipitation in the wettest month	0.126	−0.195	−0.167	−0.105
Precipitation in the driest month		0.114		
Precipitation seasonality			−0.254	−0.129
Precipitation in the wettest quarter	0.304	−0.470	−0.535	−0.156
Precipitation in the driest quarter	0.127	0.369	0.206	
Precipitation in the warmest quarter	0.181		0.280	−0.930
Precipitation in the coldest quarter	0.205	0.753	−0.567	
Proportion of variance	0.932	0.052	0.005	0.004
Cumulative variance	0.937	0.989	0.994	0.998

dae (*Streptoprocne zonaris*), Hemiprocnidae (*Hemiprocne mystacea*) and both *Sephanoides* species (Appendix S1) were obtained to apply these calibrations to an *ND2* dataset of 35 ingroup individuals in BEAST 1.7.0 (Drummond *et al.*, 2012). We also constructed a four-locus species tree with *BEAST (Heled & Drummond, 2010), because multiple independent loci should yield superior estimates of species divergence times (Edwards, 2009). Independent evolutionary units were specified a priori for *BEAST analyses if populations met at least one of two criteria: (1) phenotypically distinct populations (i.e. subspecies); and (2) geographically contiguous clades supported by the *ND2* phylogeny (Fig. 2). To improve coalescent estimation of the species tree, we analysed a dataset of 70 individuals, including 1–5 individuals from each of the 23 ingroup species (Heled & Drummond, 2010). Input files were created using BEAUti 1.7.0 (Drummond *et al.*, 2012), where we calibrated the node between Apodidae and Hemiprocnidae using an exponential prior with a hard minimum divergence date of 38.0 Ma and a 97.5% highest posterior density (HPD) upper bound of 93.3 Ma. A normal prior was applied to the node between the two *Sephanoides* species (mean, 4.2 Ma; SD, 0.5 Myr; 95% HPD, 3.5–5 Ma). An uncorrelated relaxed lognormal clock model (Drummond *et al.*, 2006) was selected for *ND2* and a strict clock was selected for nuclear loci based on preliminary runs. For BEAST and *BEAST, we performed eight MCMC runs (300 million generations; 30,000 steps; 33% of trees discarded as burn-in).

We constructed a lineages-through-time (LTT) plot using the time-calibrated species tree to evaluate deviations from a constant rate of diversification and to assess the extent to which lineage accumulation can be associated

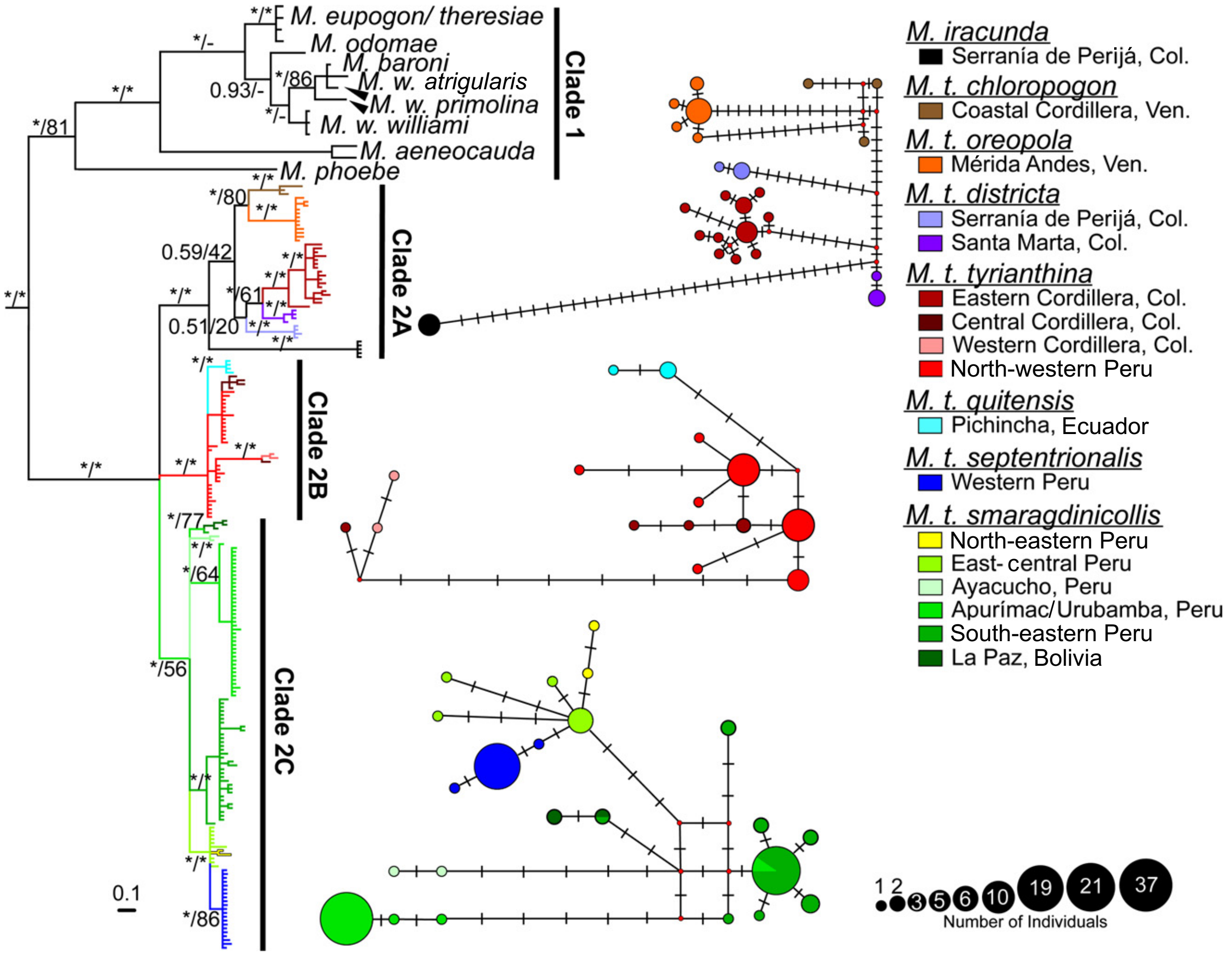

Figure 2 50% majority-rule Bayesian phylogeny of the *ND2* dataset in *Metallura* from the tropical Andes. Branch annotations refer to the posterior probabilities followed by bootstrap values from the maximum-likelihood phylogeny (* = posterior probabilities ≥ 0.95 and bootstrap > 90%). Clade 1, *M. aeneocauda* superspecies and *M. phoebe*; clade 2, *M. iracunda* and *M. tyrianthina*. Haplotype networks are shown to the right of each of the three main *M. tyrianthina* clades. Dashes represent mutations and red dots at junctions are inferred ancestral haplotypes. The size of each circle in the network represents the number of individuals sharing that haplotype (see scale at bottom right). Coloured branches and haplotypes correspond to localities for each of the well-supported clades/ haplogroups (see Fig. 1b). Col., Colombia; Ven., Venezuela.

with orogeny or Pleistocene glacial cycles. We used each evolutionary unit as defined in the species tree for this analysis following d'Horta *et al.* (2013). Diversification models implementing constant or shifting rates (pure-birth, birth–death, exponential or logistic density-dependent) were evaluated using the AIC in APE 3.0.4 (Paradis *et al.*, 2004), LASER 2.3 (Rabosky, 2006) and GEIGER 1.3 (Harmon *et al.*, 2008), coupled with the 'rbdtree.n3.R' function (http://ib.berkeley.edu/courses/ib200b/labs/lab12/rbdtree.n3.R).

Historical biogeography

We estimated the history of spread through the Andes in *Metallura* using statistical dispersal–vicariance analysis (S-DIVA) implemented in RASP 2.1 (Yu *et al.*, 2010) on 80,000 post-burn-in trees from the BEAST analysis. *Metallura* populations were clustered into six geographical regions: A, Venezuelan mountains; B, Serranía de Perijá and Sierra Nevada de Santa Marta mountains; C, the Eastern Cordillera of Colombia; D, the Central and Western cordilleras of Colombia, south to northern Peru; E, the eastern slope of the Central Andes from northern Peru to Bolivia; and F, the western Peruvian Andes. These regions are divided by topographical barriers (Weir, 2009; Valderrama *et al.*, 2014), are regional areas of endemism (Cracraft, 1985), and are associated with major discontinuities in genetic and phenotypic diversity in *Metallura* (Fig. 2). Ancestral taxa were permitted to occupy two to five regions. Given the linearity of Andean bird distributions (Graves, 1988), we also constrained ancestral distributions to be contiguous with respect to regions (e.g. Venezuelan Andes, Eastern Cordillera of Colombia, Serranía de Perijá) and not disjunct (e.g. Venezuelan Andes and Peruvian Andes). Although many Andean species have disjunct distributions within the predefined regions (e.g. *M. williami* within region D), few species have disjunct distributions at the scale of our predefined regions (e.g. *Diglossa brunneiventris*), and no *Metallura* taxa exhibit this pattern.

To detect signatures of recent range expansion in *M. tyrianthina* that are consistent with dispersal patterns identified in S-DIVA analyses, we estimated population-genetic parameters and mismatch distributions in ARLEQUIN 3.5 (Excoffier *et al.*, 2005). We calculated the number of variable sites, haplotype diversity, nucleotide diversity, Tajima's *D* and Fu's F_S (1000 simulations) on *ND2* sequence data for populations with at least 10 sampled individuals (Cordillera de Mérida, Venezuela; Eastern Cordillera, Colombia; north-western Peru; Western Andes, Peru; Apurímac and Urubamba valleys, Peru; and south-eastern Peru). Mismatch distributions were used to evaluate deviations from models of sudden population expansion, via the sum of squared deviation (SS) and Harpending's raggedness index (Rogers & Harpending, 1992), with the expectation that populations of *M. tyrianthina* that had recently arrived in a region would fit a model of sudden population expansion.

RESULTS

Phylogenetic estimate

Bayesian and ML analysis of the *ND2* dataset strongly supported a monophyletic *Metallura* (Fig. 2), with a deep dichotomy between two well-supported clades: (1) *M. phoebe* as sister to all members of the *M. aeneocauda* superspecies (hereafter, clade 1: posterior probability, PP, 0.96; bootstrap, BS, 81%); and (2) *M. iracunda* nested within a geographically structured clade of all *M. tyrianthina* populations (hereafter, clade 2: PP 1.0; BS 100%). Within clade 1, *Metallura eupogon* and *Metallura theresiae* formed a clade, and *Metallura odomae* appeared as the sister to a northern clade in which *Metallura williami* was paraphyletic with respect to *Metallura baroni*. Clade 2 can be divided into three well-supported groups: 2a, a north-eastern Andean clade including *M. iracunda*, *M. t. chloropogon*, *M. t. oreopola*, *M. t. districta* and populations from the Serranía de Perijá and Eastern Cordillera of Colombia that resemble *M. t. districta* and *M. t. tyrianthina*, respectively; 2b, a central clade comprising populations of *M. t. tyrianthina* from the Central and Western cordilleras of Colombia south to the Marañón valley, plus *M. t. quitensis* from north-western Ecuador; and 2c, a clade south of the Marañón valley including *M. t. smaragdinicollis* and *M. t. septentrionalis* (PP, 0.96; BS, 56%). Relationships among clades 2a, 2b and 2c were weakly supported and, although phylogeographical structure was found within each clade, there was little resolution. Bayesian and ML analyses of individual nuclear loci were poorly resolved despite evidence for good mixing in AWTY (see Figs S1 & S2 in Appendix S3). The four-locus concatenated tree generally confirmed results from the *ND2* tree, whereas the concatenated nuclear dataset weakly placed *M. phoebe* as sister to the rest of the genus (PP 0.58; BS 77%). Analysis of the concatenated nuclear dataset also yielded high support for clade 2, but it generally failed to resolve the relationships among populations of *M. tyrianthina* (see Figs S3 & S4 in Appendix S3).

Geographical and climatic structuring of genetic diversity

Within clade 1, the highest corrected *ND2* pairwise divergence was between *M. phoebe* and the *M. aeneocauda* superspecies across the high Andean ridge of Peru (7.96%). Within the *M. aeneocauda* superspecies, divergence ranged from 0.16% to 5.42%, with the deepest divergence across the Apurímac valley. Within *M. tyrianthina*, sequence divergence across major topographical barriers ranged from 0.06% to 3.40% (Table 2). The greatest divergences were within clade 2a, ranging from 1.62% (Turbio–Yaracuy Depression) to 2.63% (Táchira Depression), and also had high Φ_{ST} values, most of which were significant. *Metallura iracunda* was 2.89% divergent from syntopic populations of *M. tyrianthina*. There was little structure within clade 2b, with low divergence (0.92%; Φ_{ST} non-signifi-

Table 2 Corrected pairwise differences across major topographical barriers in the tropical Andes for clade 1 (917 bp) and clade 2 (832 bp) of *Metallura*. Boxed cells correspond to barriers within clades 2a (solid line), 2b (dashed line) and 2c (dotted line). Φ_{ST} values are only shown for clade 2. Localities in bold represent transitions between climatically distinct regions, and localities without letters represent points of climatic transition in the absence of a topographical barrier.

	Clade 2		Clade 1
Barrier	Sequence divergence	Φ_{ST}	Sequence divergence
A. Turbio–Yaracuy Depression	1.62%	0.936^{**}	—
B. Táchira Depression	2.63%	0.912^{***}	—
C. Cesar Depression	1.85%	$0.969^{\mathrm{n.s.}}$	—
D. Motilones Low Pass	2.10%	0.869^{***}	—
Cundinamarca–Boyacá, Colombia	0.631%	$0.653^{\mathrm{n.s.}}$	
E. Magdalena Valley	3.40%	0.887^{***}	—
F. Cauca Valley	0.92%	$0.567^{\mathrm{n.s.}}$	—
G. Marañón Valley	2.15%	0.848^{***}	1.49%
H. Huallaga Valley	0.08%	0.186^{*}	0.16%
San Martín–Huánuco, Peru	0.116%	0.855^{*}	—
I. Apurímac Valley	0.39%	0.341^{***}	5.42%
J. High Andean Ridge	0.32%	0.692^{***}	7.96%
Cusco–Puno, Peru	0.177%	0.488^{***}	—

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\mathrm{n.s.}}P > 0.05$.

cant) across the Cauca Valley between the Western and Central cordilleras of Colombia. We did find evidence for structure across a low-lying region in the northern Central Cordillera of Colombia, isolating populations from the Western Cordillera to the northern Central Cordillera (near Antioquia) from populations near Caldas in the Central Cordillera (Fig. 2). Within the southern clade 2c, sequence divergence across putative barriers was 0.08–0.39% and Φ_{ST} values were low but significant (0.186–0.692).

Loadings for the first four principal components of the BioClim variables (accounting for 99.8% of the variance) were heaviest on annual precipitation (PC1), precipitation of the coldest quarter (PC2 and PC3), precipitation of the wettest quarter (PC3) and precipitation of the warmest quarter (PC4). Cluster analysis based on these components identified a diagonal, varying-volume, equal-shape model with five clusters as the best fit to the data (log-likelihood, −958.14; *n*, 41; d.f., 28; BIC, −2020.3). Certain localities within each cluster were geographically disjunct from other ecologically similar clusters. In these cases, we subdivided the cluster to create geographically and ecologically cohesive groups, resulting in eight clusters: (1) La Paz, Bolivia, to Puno, Peru; (2) south-eastern Peru from Cusco to Ayacucho; (3) Junin and Pasco, Peru; (4) central Peru, including the regions of Huánuco, Lima and Ancash; (5) northern Peru and Ecuador; (6) the Central and Western cordilleras of Colombia plus the department of Cundinamarca in the Eastern Cordillera; (7) the Eastern Cordillera of Colombia north of Cundinamarca to the Coastal Cordillera, Venezuela; and (8) the Sierra Nevada de Santa Marta and Serranía de Perijá mountains. Most transitions between climatic clusters corresponded to topographical barriers, but there were three exceptions (Table 2). These included a region in the Eastern Cordillera of the Colombian Andes between Cundinamarca and Boyacá (0.631% divergent; Φ_{ST} $0.653^{\mathrm{n.s.}}$); in north-eastern Peru between San Martín and Huánuco (0.116% divergent; Φ_{ST} 0.855, $P < 0.05$); and in south-eastern Peru between Cusco and Puno (0.177% divergent; Φ_{ST}: 0.488, $P < 0.001$).

AMOVA analyses indicated that 92.4% (d.f., 16; SS, 1311.8; variance, 7.45) of the genetic variation in *M. tyrianthina* exists among groups isolated by topographical barriers, whereas only 1.5% (d.f., 4; SS, 5.7; variance, 0.12) is within groups. In contrast, 60.9% (d.f., 7; SS, 1156.4; variance, 6.63) of the genetic variation was distributed among climatic clusters, and 33.5% was within clusters (d.f., 24; SS, 500.9; variance, 3.65).

Historical biogeography

Divergence times

Substitution rates were faster for the *ND2* locus estimated in BEAST (1.94% Myr^{-1}) than for the multilocus dataset in *BEAST (1.46% Myr^{-1}). Nuclear loci evolved at rates similar to those estimated by Lerner *et al.* (2011) for Hawaiian honeycreepers (0.18–0.22% Myr^{-1}; Appendix S3: Table S4). BEAST (see Fig. S5 in Appendix S3) and *BEAST (Fig. 3a) topologies were largely consistent with other phylogenetic analyses (see above). Some key differences included: (1) strong support for clade 2a as sister to clades 2b and 2c in the *ND2* BEAST tree; (2) weak support for *M. phoebe* as sister to clade 2 in the *BEAST tree; and (3) *M. odomae* embedded within the *M. williami* complex.

Estimated median divergence times were more recent for the species-tree analysis (Fig. 3a) than the *ND2* gene tree (Fig. S5), but with broadly overlapping 95% HPDs. In the *BEAST tree, we found that *Metallura* and *Chalcostigma* diverged *c.* 6.7 Ma, clade 1 and clade 2 diverged *c.* 4.8 Ma, *M. eupogon* /

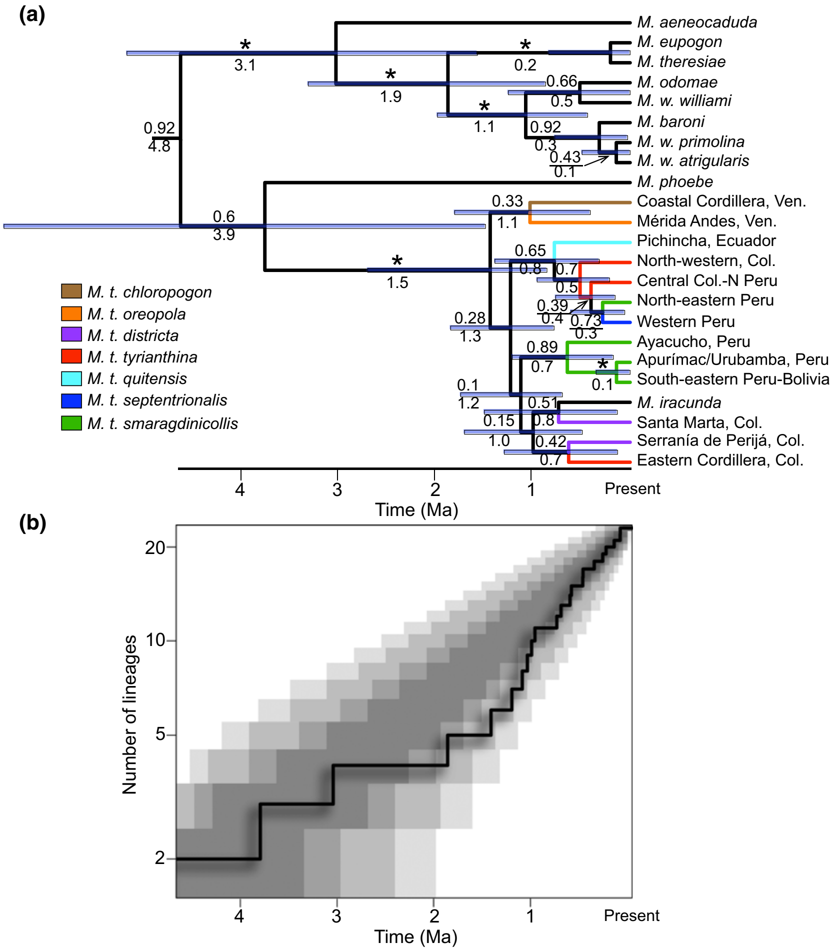

Figure 3 Phylogeny and diversification of *Metallura* hummingbirds in the tropical Andes. (a) Four-locus (*ND2*, *AK1*, *Bfib7* and *MUSK*) species tree constructed in *BEAST. Posterior probabilities are shown above and median divergence times (Ma) below each branch. Colours correspond to different subspecies of *M. tyrianthina* (outgroups not shown). Col., Colombia; Ven., Venezuela. (b) Lineages-through-time (LTT) plot (black line) illustrating the diversification of *Metallura* based on the multilocus species tree. Background grey envelope represents 0.5 (darkest) to 0.99 (lightest) confidence intervals surrounding the expected distribution of a pure-birth model. The *y*-axis is on a logarithmic scale.

M. theresiae diverged from *M. aeneocauda* across the Apurímac valley *c.* 3.1 Ma, and clade 1 was divided by the Marañón valley *c.* 1.9 Ma. All clade 1 taxa distributed north of the Apurímac valley diversified less than 1 Ma. Within clade 2, all divergence events estimated from *BEAST occurred less than 1.5 Ma.

Metallura is characterized by a steady rate of net diversification ($r = 0.68$ lineages Myr^{-1}), leading to an exponentially increasing accumulation of species. Visual examination of the LTT plot suggested an apparent acceleration in the rate of net diversification beginning *c.* 1.5 Ma (Fig. 3b), although the increase was insufficient for the data to reject a pure-birth model ($\Delta\mathrm{AIC} = 0$; $\mathrm{AIC} = -36.54$; log-likelihood = 19.27).

Dispersal–vicariance analysis

S-DIVA indicates that the ancestor of *Metallura* and *Chalcostigma* was distributed in the eastern Andes of Peru and Bolivia (Fig. 4). The common ancestor of clade 1 and clade 2 is likely to have expanded westwards from the eastern Peruvian Andes [node probability of E, $P(\mathrm{E}) = 0.7$] to occupy both slopes of the Peruvian Andes (EF) and northwards to span the Ecuadorian, Central and Western Colombian Andes (D) and the Perijá/Santa Marta region (B). A vicariance event subsequently isolated clade 1 in the Peruvian Andes. Within the Peruvian Andes, *M. phoebe* and the *M. aeneocauda* superspecies diverged across the high Andean ridge. This was followed by expansion and divergence across the Marañón valley in the *M. aeneocauda* superspecies. Within clade 2, when the number of ancestral areas was restricted to two, the inferred ancestral distribution was probably northern-restricted, BD ($P = 0.88$); with the number of ancestral areas set to three or greater, however, the ancestral distribution at this node was more likely to be BDE ($P = 0.68$) than BD ($P = 0.27$). Although it seems clear that the ancestor of clade 2 was distributed in the Northern Andes, it is uncertain whether its range extended into the eastern Peruvian Andes. Regardless, the initial split between clade 2a and clades 2b and 2c isolated clade 2a in the Perijá/Santa Marta region and the rest of ancestral *M. tyrianthina* was likely to have been distributed in D or possibly DE.

Demographic expansion

Nucleotide diversity (π) for mtDNA sequences was greatest within the Eastern Cordillera of Colombia and lowest in the Mérida Andes and western Peru (Table 3). Significant deviations for values of Tajima's D and Fu's F_S that may indicate population expansion were found in south-eastern Peru and the Mérida Andes (Fu's F_S only). Mismatch distributions

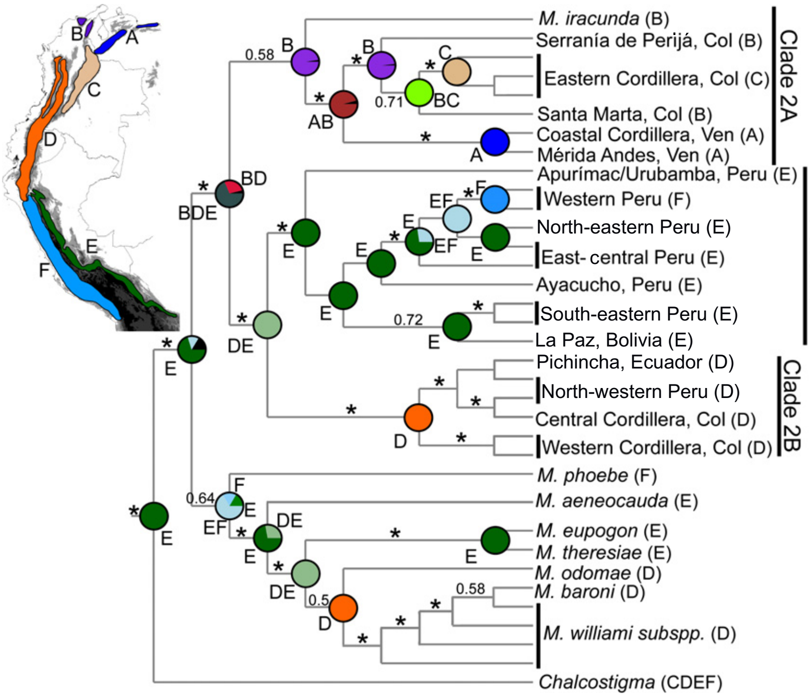

Figure 4 Results from S-DIVA analysis performed in RASP. Tree topologies were from BEAST analysis of the *Metallura ND2* dataset. Inset map exhibits the extent of the six regions used for S-DIVA analyses. Pie charts at node represent probabilities of the ancestral distributions, and letters denote which regions are coded by each colour in the pie chart. Letters in parentheses following tip labels indicate the extant distribution of each tip. Annotations on branches refer to posterior probability support for each node (* > 0.9 posterior probability). Col, Colombia; Ven, Venezuela.

Table 3 Summary statistics of genetic diversity for the six populations of *Metallura tyrianthina* with more than 10 sampled individuals. *n*, number of individuals; Var, number of variable sites; π, nucleotide diversity; *h*, haplotype diversity.

Population	*n*	Var	π	*h*	Tajima's *D*	Fu's F_S
Mérida Andes	12	4	0.000771 ± 0.000672	0.6667 ± 0.1409	−1.385	−2.471*
Eastern Cordillera	17	21	0.004118 ± 0.002401	0.8824 ± 0.0595	−1.231	−2.055
Lambayeque/Cajamarca	29	9	0.002374 ± 0.001498	0.7759 ± 0.0472	−0.069	−0.149
Ancash/Lima	21	1	0.000093 ± 0.000188	0.1857 ± 0.1102	−1.164	−0.919
Urubamba/Apurímac	41	12	0.001721 ± 0.001154	0.8341 ± 0.0536	−1.295	1.082
South-eastern Peru	30	12	0.001202 ± 0.000902	0.7471 ± 0.0828	−2.079*	−4.869*

*$P < 0.05$.

revealed trends of demographic expansion in the Peruvian and Mérida Andes populations (Fig. 5). Although a model of demographic expansion was only rejected for the population from the Eastern Cordillera of Colombia, the fit between observed and simulated mismatch distributions under population expansion was closest in the three southernmost populations (Table 4).

DISCUSSION

Phylogeny

All analyses recovered a monophyletic *Metallura* sister to *Chalcostigma*, consistent with previous studies (García-Moreno *et al.*, 1999a; McGuire *et al.*, 2007; Kirchman *et al.*, 2010). Although the position of *M. phoebe* varied, it is clear that *M. phoebe* split very soon after the initial divergence between clades 1 and 2, where the presence of successive short internodes is likely to have fostered discordant gene topologies. It is unlikely that the unresolved position of *M. phoebe* has affected other analyses, because BEAST, *BEAST and S-DIVA all account for phylogenetic uncertainty (Nylander *et al.*, 2008b; Heled & Drummond, 2010). *Metallura iracunda* was embedded within a highly structured *M. tyrianthina* in all analyses. Because *M. iracunda* overlaps extensively with a population of *M. tyrianthina* in the Serranía de Perijá, their history is likely to have involved the rapid evolution (in < 1.5 Myr) of reproductive isolation and secondary sympatry. This is analogous to the rapid origin of elevational replacements between the two *Henicorhina* wood-wrens of the Santa Marta region (Caro *et al.*, 2013). The phylogenetic pattern and sympatry of the Serranía de Perijá *Metallura* species indicates that multiple biological species are likely to exist within the polytypic and paraphyletic *M. tyrianthina.* Likewise, our sampling within *M. williami* indicated that this species is not monophyletic with respect to either *M. baroni* or *M. odomae* (Fig. 3a).

Geographical and ecological structuring of genetic diversity

Consistent with the patterns shown in other Andean lineages (e.g. Chaves & Smith, 2011; Gutiérrez-Pinto *et al.*, 2012; Val-

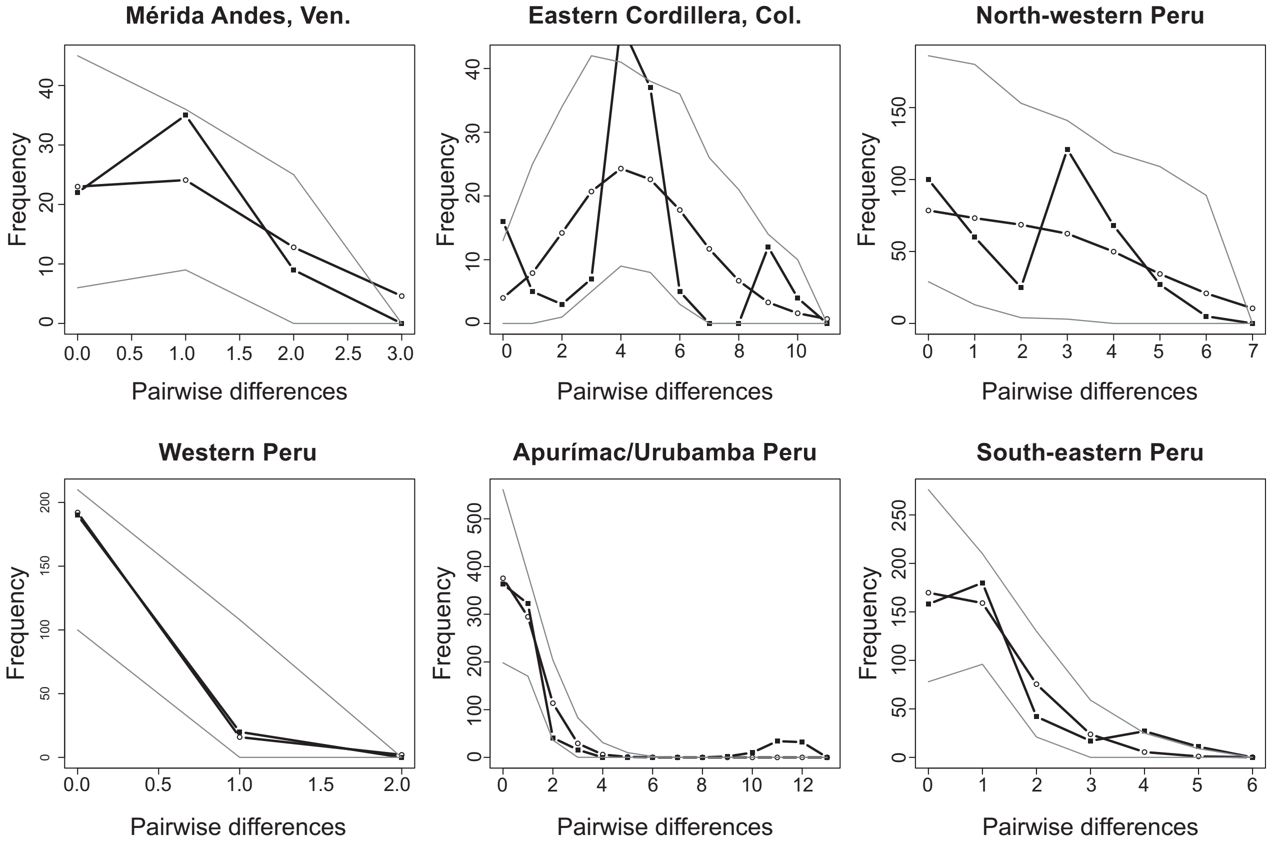

Figure 5 Mismatch distribution for each of the six *Metallura tyrianthina* populations that include 10 or more sampled individuals. These populations belong to the following *ND2* clades: Mérida Andes and Eastern Cordillera (clade 2a); north-western Peru (clade 2b); Western Peru, Apurímac / Urubamba, Peru, south-eastern Peru (clade 2c). For each population, the data simulated according to a sudden demographic expansion model are plotted (open circles) with the observed mismatch distribution (closed squares). A model of sudden demographic expansion can only be rejected for the population from the Eastern Cordillera of the Colombian Andes (Table 4).

Table 4 Comparisons of mismatch distributions (Fig. 5) to a null model of sudden demographic expansion across six populations of *Metallura tyrianthina* in the tropical Andes. Estimates of time since expansion (τ) and population size before (θ_0) and after (θ_1) expansion are also included. Populations are listed from north to south. The only significant deviation from the null model is marked in bold.

Population	SSD	*P*	Raggedness index	*P*	τ	θ_0	θ_1
Mérida Andes	0.0298	0.169	0.2126	0.184	1	0	99999
Eastern Cordillera	**0.0887**	**0.012**	**0.1684**	**0.007**	**4.7**	**0**	**45.078**
Lambayeque/Cajamarca	0.0402	0.198	0.1034	0.248	3.8	0.0018	4.502
Ancash/Lima	0.00005	0.246	0.6644	0.84	3	0	0.11
Urubamba/Apurímac	0.0128	0.151	0.1237	0.247	0.7	0	99999
South-eastern Peru	0.0119	0.211	0.109	0.267	0.9	0	99999

derrama *et al.*, 2014), deep river valleys and the high Andean ridge of Peru played prominent roles in structuring the genetic diversity in *Metallura*. The patterns of divergence across barriers differed strikingly between the two *Metallura* clades, however, with clade 1 exhibiting a clear pattern of decreasing depth of divergence from south to north and clade 2 showing an opposite, north-to-south pattern (Table 2).

Local adaptation to climatically distinct regions appears to have played a lesser or negligible role in initial diversification. Although AMOVA found that 60.9% of genetic variation occurs among climatically distinct regions, topographical barriers also separated these regions in many cases. In the three cases where the transition between climatically distinct regions was independent of a topographical barrier, divergence was relatively shallow (Table 2). Divergence was no higher across topographical barriers that were associated with climatic transitions, as might be expected if climatic differences had facilitated geographical isolation. Despite climatic differences not appearing to enhance geographical population structure at neutral loci, future studies should evaluate divergence in functional traits that are more directly affected by ecological pressures (Chaves *et al.*, 2007; Milá *et al.*, 2009; Cadena *et al.*, 2011; DuBay & Witt, 2014).

Timing of diversification

The earliest divergences within *Metallura* were contemporaneous with Miocene–Pliocene uplift in the Andes, but the majority (81%) of divergence events occurred much later, during the mid–late Pleistocene (< 1.5 Ma; Fig. 3a), consistent with the visible uptick in lineage accumulation *c.* 1.5 Ma (Fig. 3b). In contrast, statistical comparisons of diversification models indicated a steady rate of lineage accumulation (Fig. 3b). These inconsistent results may be due to the limited power of LTT model-selection methods for detecting changes in diversification rate (McGuire *et al.*, 2014). Regardless of whether or not diversification accelerated during the Pleistocene, the extant *Metallura* diversity primarily arose during the Pleistocene.

Contrasting biogeography of the tree line and temperate zone in *Metallura*

The temporal and spatial patterns of genetic and phylogenetic structure suggest contrasting biogeographical histories for the two major *Metallura* clades. The ancestor of the *Metallura* lineage is likely to have expanded to occupy the Andes from Bolivia to Venezuela. Following expansion, it diverged along the latitudinal axis of the Andes, *c.* 4.8 Ma, to form a central Andean clade (clade 1) and a northern Andean clade (clade 2). The final uplift of the Peruvian Andes (Garzione *et al.*, 2008) is likely to have isolated *M. phoebe* from other *Metallura* (*c.* 3.9 Ma). This initial northward expansion and differentiation in *Metallura* follows the uplift history of the Andes (Hoorn *et al.*, 2010) and mirrors patterns found previously in Andean nematodes (Picard *et al.*, 2008) and speckled hummingbirds (Chaves *et al.*, 2011).

The tree line specialists in clade 1 – the *M. aeneocauda* complex – exhibited a south-to-north pattern of expansion and differentiation during the last 3.1 Myr, largely after Andean uplift. Based on the close correspondence of clade distribution limits with topographical barriers, we surmise that speciation was facilitated by the dramatic erosion of river valleys and associated isostatic rebound that continued after the uplift of the Central Andean Plateau was largely complete (Garzione *et al.*, 2008).

The ancestor of the *M. tyrianthina*–*M. iracunda* group (clade 2) was probably restricted to northern Andean latitudes before expanding southwards (Fig. 4). This result was sensitive to constraints on ancestral distribution size (number of areas), and the possibility of a more widespread ancestral distribution for clade 2 cannot be completely rejected. It should be noted, however, that a tendency towards widespread ancestral distributions is a bias inherent in S-DIVA analyses, which apply a greater cost to dispersal than to vicariance (Ronquist, 1997). Despite the inconclusiveness of the S-DIVA analyses regarding the origin of clade 2, patterns of Φ_{ST} (Table 2), nucleotide diversity (Table 3) and mismatch distributions (Fig. 5) corroborate a deeper history of diversification in the northern Andes followed by more recent colonization of the Peruvian Andes.

García-Moreno *et al.* (1999a) inferred more extensive and rapid differentiation in the *M. aeneocauda* superspecies than in *M. tyrianthina* as a result of its narrower, tree line niche, which is more susceptible to fragmentation than the varied forest habitats occupied by *M. tyrianthina* across its broader elevational range. Our phylogeographical results based on broader geographical and population-genetic sampling of *M. tyrianthina* demonstrate much greater differentiation within *M. tyrianthina* in the northern part of its range than in the southern part of the range, approaching the level of divergence found in the *M. aeneocauda* superspecies (Fig. 2). Thus, the lower diversity found in *M. tyrianthina* in the central Andes by García-Moreno *et al.* (1999a) cannot be attributed to elevational or habitat effects, but instead reflects a recent history of southward expansion from its northern centre of origin, after which it came into contact with several deeply divergent clades of the *M. aeneocauda* superspecies. The two major clades appear to have expanded in opposite directions from their origins in the northern and central Andes, respectively, indicating that the elevational replacement pattern evolved recently.

CONCLUSIONS

Topographical barriers, rather than climatic variation, were the primary landscape feature that structured diversity in *Metallura*. Recently evolved lineages in both major clades of *Metallura* appear to have undergone range expansions, albeit in different directions. This history of range expansion within the genus allowed for divergence across common topographical barriers spanned by both *Metallura* clades. It is likely that, without repeated expansion across these barriers, diversification within *Metallura* would have decelerated towards the present (Pigot *et al.*, 2010); instead, lineage accumulation in *Metallura* has been steady or accelerating, with most divergence events occurring in the past *c.* 1.5 Myr. Cycles of allopatric divergence and dispersal across topographical barriers caused diversification to proceed differently in the two major subclades of *Metallura*, as they appear to have done in numerous other Andean taxa (Weir, 2009), culminating in elevational parapatry only in cases when divergence was sufficient for reproductive isolation and differentiation of the elevational niche (Patton & Smith, 1992; d'Horta *et al.*, 2013). Molecular patterns of diversification within *Metallura* thus reflect a dynamic history of latitudinal range expansions and allopatric divergence across topographical barriers that were already at least partly in place, rather than fragmentation of a static distribution. Similar mechanisms also appear to be a key driver of lowland Neotropical diversification (Smith *et al.*, 2014).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Taxon sampling and GenBank sequences.
Appendix S2 Sequencing protocols and characteristics.
Appendix S3 Supplementary results.

BIOSKETCH

Phred M. Benham received his Master of Science degree from the University of New Mexico's Department of Biology in 2012 and is currently a doctoral student in the Department of Animal Biology, University of Illinois, Urbana–Champaign. During his training, Phred has made substantial contributions to avian collections, including the LSU Museum of Natural Science, Museum of Southwestern Biology and CORBIDI. His primary research interests involve the biogeography, speciation and evolution of New World birds.

Author contributions: P.M.B. led this study, which comprises part of his master's thesis, completed under the guidance of C.C.W.; P.M.B. and A.M.C. collected samples and conducted analyses; J.A.M. contributed sequence data; P.M.B. and C.C.W. wrote the manuscript with input from all authors.

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