Climate explains recent population divergence, introgression and persistence in tropical mountains: phylogenomic evidence from Atlantic Forest warbling finches

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Abstract

Taxa with disjunct distributions are common in montane biotas and offer excellent opportunities to investigate historical processes underlying genetic and phenotypic divergence. In this context, subgenomic datasets offer novel opportunities to explore historical demography in detail, which is key to better understand the origins and maintenance of diversity in montane regions. Here we used a large ultraconserved elements dataset to get insights into the main biogeographic processes driving the evolution of the Montane Atlantic Forest biota. Specifically, we studied two species of warbling finches disjunctly distributed across a region of complex geological and environmental history. We found that a scenario of three genetically differentiated populations is best supported by genomic clustering methods. Also, demographic simulations support simultaneous isolation of these populations at ~10 kya, relatively stable population sizes over recent time, and recent gene flow. Our results suggest a dual role of climate: population divergence, mediated by isolation in mountain tops during warm periods, as well as population maintenance - allowing persistence mediated by shifts in elevation distribution during periods of climate change, with episodic bouts contact and gene flow. Additional support for the role of climate comes from evidence of their contact in a recent past. We propose that two major gaps, which we call São Paulo and Caparaó subtropical gaps, have been historically important in the divergence of cold adapted organisms in the Atlantic Forest, and could be associated to cryptic diversity. Finally, our results suggest that shallow divergence and past gene flow may be common in montane organisms, but complex demographic histories may be detectable only when using subgenomic or genomic datasets.

Introduction

Montane organisms often exhibit intriguing patterns of disjunct distributions, which are frequently associated to phenotypic divergence. Among the many mechanisms that have been proposed to explain such patterns, the ones with the strongest support are the historical fragmentation of formerly continuous habitats and dispersal (Mayr & Diamond 1976; Patton & Smith 1992; Knowles & Massatti 2017) associated with rugged topography and climate fluctuations. That is, changes in climate over time, which are well known to promote shifts in the elevational distributions of suitable habitats and associated organisms (Hooghiemstra & Van der Hammen 2004, Moritz et al. 2008, Chen et al. 2009), could lead to cyclical isolation and connection of population through temporal bridges (Brown 1971, McCormack et al. 2009). During the late Pleistocene glacial periods, warmer periods often lead to isolation in high-elevation habitat pockets, whereas colder periods often lead to range expansion into lower elevations and previously unsuitable regions. Under this scenario, warmer periods (such as the current interglacial) would create opportunities for population divergence by genetic drift and natural selection, while colder periods might promote secondary contact and genetic homogenization of previously isolated populations. This interplay between selection and drift with topography and a dynamic climate could lead not only to genetic divergence, but to the evolution of reproductive isolation, thus rendering mountains as potential hotspots of population divergence and speciation (Fjeldsa 1994, McCormack et al. 2009). Alternatively, isolation could be initially mediated by topographic changes. For example, the splitting of formerly continuous highlands into multiple isolated mountain ranges could be the result of geological changes, and organisms adapted to higher habitats would then become separated by ecologically unsuitable valleys. Under such scenario, population divergence should be temporally associated to geotectonic changes (Badgley 2010).

These biogeographic processes likely have played an important role in the building up of the enormous diversity found in mountain regions (Graham et al. 2014, Antonelli 2015; Knowles & Massatti 2016). However, their exact contribution is not well understood. In particular, the evolutionary processes underlying the high levels of biological diversity in the South American Montane Atlantic Forest are yet to be elucidated. The Montane Atlantic Forest (hereafter MAF) is a cradle of biodiversity (Stotz et al. 1996) and has great potential to provide a wealthy natural laboratory for evolutionary research since its high levels of endemism likely encompass a plethora of diversification mechanisms. In particular, MAF organisms showing phenotypic breaks that coincide with major highlands are especially interesting models for

montane phylogeography, as such pattern presumably reflects genome-wide divergence resulting from an interplay between climate, topography and the evolutionary outcomes of drift, selection and gene flow. Of these, vagile organisms such as birds may be particularly useful models to understand large-scale historical events, as their fidelity to specific habitats and high dispersal ability may promote genetic differentiation and homogenization, except in face of strong events of habitat isolation.

Much of what is known about the Atlantic Forest comes from studies of either lowland species or species with broad elevational distributions (e.g. Cabanne et al. 2007, Carnaval et al. 2009, Maldonado-Coelho 2012, Amaral et al. 2013, Carnaval et al. 2014), and less than a handful of phylogeographic studies have investigated cold-adapted species whose distributions include mostly montane habitats (e.g. Amaro et al. 2013, Batalha-Filho et al. 2012, Peres et al. 2015, Frikowski et al. 2016, Franoso et al. 2016, Pie et al. 2018). Conclusions from the few studies on MAF-inhabiting species are often discordant; consequently, there is still little consensus about what are the major drivers of diversification in the MAF. A common finding by many of them is the lack of both population structure and strong demographic fluctuations, which could speak against Pleistocene climatic fluctuations as major drivers of population divergence and diversification in MAF organisms. Importantly, all but one (Pie et al. 2018) of the studies performed so far were based on five or less loci, raising the possibility that shallow population structure—which is expected under recent climate fluctuations—may have been overlooked (Amaral et al. 2018). Furthermore, none of these studies have performed demographic analyses explicitly testing for historical gene flow among currently isolated and genetically structured populations—a prediction of montane climate-driven dynamics.

Here we use the largest population-level sub-genomic dataset of any MAF organism and coalescent-based methods to ask (1) how does genetic variation relates to distribution breaks in the MAF, and (2) what are the influences of climate and topographic evolution (i.e. mountain building) on the current patterns of genetic variation in endemic Atlantic Forest montane birds. To address these questions we use two common cold-adapted species of Atlantic Forest warbling finches as models: the Gray-Throated Warbling Finch (*Microspingus cabanisi*) and the Buff-Throated Warbling Finch (*Microspingus lateralis*). Their distributions and phenotypic breaks are congruent with major isolated highlands in S and SE Brazil (Assis et al. 2007), and a detailed phylogeographic analysis of these species represents a fundamental step towards a better understanding of population history in MAF and biotic diversification in tropical montane systems as a whole.

Methods

Study system: biological models and geological and environmental history of the MAF

The two warbling finches studied here are sister-species (Amaral *et al.* 2015), once considered conspecific (Assis *et al.* 2007), and whose combined distributions cover most of the MAF. The northern species, *M. lateralis* inhabits highland areas above 900 m of two major isolated mountain ranges in SE Brazil, the Mantiqueira and the northern portion of the Serra do Mar. This species also occurs in the Caparaó highlands, an isolated northernmost massif of the Mantiqueira mountain range (Fig.1). The southern species, *M. cabanisi*, occurs in the southern portion of Serra do Mar and also in the Serra Geral mountain range mostly above 900 m, but also in lower elevation areas towards its southern limit, which includes areas in Argentina and Paraguay (Assis *et al.* 2007). These two species are currently separated by more than 100 km of lower elevation forest habitats (Fig. 1). They differ phenotypically in both plumage and vocalizations. Plumage differences are mostly in ventral color (yellow in *M. lateralis* and gray in *M. cabanisi*) and amount of white in tail feathers (more extensive in *M. cabanisi*), whereas the songs and the calls of these species differ in syntax and harmonic structure, respectively (Assis *et al.* 2007).

The tectonic processes that originated the eastern Brazilian highlands as well as their ages are still a matter of debate. In one hand, the major uplift events were suggested to have started during the lower Cretaceous (ca. 120 mya) as a consequence of the Gondwanaland breakup (Melo *et al.* 1985, Franco-Magalhães *et al.* 2014). On the other hand, the uplift is proposed to have resulted from the three main pulses of Andean orogeny, with the early ages of the mountain ranges (i.e. Serra do Mar) estimated to be ca. 90 mya (Meisling *et al.* 2001, Karl *et al.* 2013). Regardless, a range of evidence indicates subsequent multi-episodic uplift events during the upper Cretaceous, the Tertiary and the Quaternary (Freitas 1951, Melo *et al.* 1985, Almeida & Carneiro 1998, Modenesi-Gauttieri *et al.* 2002, Tello Saens *et al.* 2003, Hackspacher *et al.* 2004, Franco-Magalhães *et al.* 2014), with the uplift of the Serra do Mar mountain range thought to have extended until recent times (i.e. Holocene, Cogné *et al.* 2012).

Available evidence from fossil pollen records in southern and southeastern Brazil highland sampling sites (> 900 m a.s.l.) at Mantiqueira, Serra do Mar and Serra Geral mountain ranges suggest broadly concordant cold and dry conditions during the last glacial maximum, with forests completely absent or likely present only along ravines and/or at lower altitudes (Behling 1997, 2007, Behling & Pillar 2007, Behling *et al.* 2007, Oliveira *et al.* 2008, Ledru *et al.* 2009, Behling & Safford 2010, Oliveira *et al.* 2012). The onset of a moister climate after this

period favored the return of montane forest species (Behling 1997, Behling & Pillar 2007, Behling et al. 2007, Ledru et al. 2009, Behling and Safford 2010, Oliveira et al. 2012). Importantly, the highland vegetation seems to have had a complex dynamics during the last 130,000 yr BP (i.e. late Pleistocene and Holocene), with multiple shifts in community composition and elevational distribution of montane forest plant species (Ledru et al. 2009, Behling & Safford 2010, Oliveira et al. 2012).

Sampling and laboratory methods

We sampled 90 individuals (50 of *M. cabanisi* and 40 of *M. lateralis*) in nine localities—10 per locality, spread throughout the species' known distributions (Fig. 1, Supplementary Information Table S1). Specimens were attracted by playback and collected as described in Amaral *et al.* 2012 (see Acknowledgments for permit number and Table S1 for specimen and tissue collection holdings).

We extracted total DNA from pectoral muscle using the Qiagen DNeasy kit (Valencia, CA) according to the manufacturer's protocol, including a RNase treatment. We obtained genomic data using sequence capture and Illumina sequencing of ultraconserved elements (UCEs) using standard protocols (Faircloth *et al.* 2012) with a few modifications: enrichment was done using 650 probes, targeting 634 loci covering all *Gallus gallus* chromosomes; use of 100 bp paired-end Illumina HiSeq 2000 sequencing run, and use of 16 cycles in both pre- and post-capture PCR reactions. Sequencing was performed in two lanes. Library preparation was performed by RAPiD Genomics (Gainesville, FL, USA).

Sequence quality control, mtDNA, UCE assembly and SNP calling

We sorted raw sequences by individual tags using Illumina's Casava software. Initial quality control was performed using FastQC 0.10.1 (Andrews 2014). Adapters, barcodes and low quality regions were trimmed using Illumiprocessor 2.0.7 (Faircloth 2014), which processes Illumina sequences using Trimmomatic 0.32.1 (Bolger *et al.* 2014). Assembly, removal of non-UCE loci and final loci alignment was performed with Phyluce 1.4 (Faircloth 2014). The largest contig of each locus was used as a reference for mapping individual reads using BWA-mem (Li 2013) and SNP calling, which was performed using GATK (McKenna *et al.* 2010). We kept only >Q30 SNPs for downstream analyses. We sampled one random SNP per locus from the collection of all zero-missing biallelic SNPs recovered for the locus. We removed Z-linked SNPs using a local BLAST search based on the zebra-finch Z chromosome (Emsembl taeGut3.2.4) to avoid any bias due to the idiosyncratic evolution of sex

chromosomes and different ploidy (unfortunately the limited number of Z-linked SNPs recovered precluded us from running independent analyses on this dataset). Since mtDNA is a common subproduct of sequence capture experiments (see Amaral *et al.* 2015), we also isolated complete cytochrome b sequences from assemblies that were generated by Phyluce using a local BLAST search based on the complete mitogenome of *Microspingus lateralis* (Genbank NC_028039.1) to obtain a Median Joining Network using POPART v 1.7 (Leigh & Bryant 2015).

Population structure

We explored population structure first with sparse non-negative matrix factorization (sNMF, Frichot *et al.* 2014, implemented in the R package LEA, Frichot & Olivier 2015) as well as with the multivariate Discriminant Analysis of Principal Components (Jombart *et al.* 2010). The sNMF runs were performed for a range of K values from 1 to 9 (following the number of localities), with 500 runs per K. We used the minimum cross-entropy method to identify the best fitting K. Runs with the lowest values of minimum entropy were selected. Since sNMF's regularization parameter alpha may affect the inferences (Frichot *et al.* 2014), we evaluated the results under different values of alpha (1, 10, 100 and 1,000). To further assess population structure we ran a DAPC analysis using a k-clustering algorithm to determine the *a priori* grouping of individuals based on the Bayesian Information Criterion (BIC). The first set of principal components accounting for 80% of the variance was included in the analyses.

Inference of historical processes

Based on the results from the previous analyses (Fig. 2), we tested 12 alternative models to assess the most likely diversification scenario for *Microspingus*. The models tested explored the relationship of the three genetic clusters inferred by sNMF, the presence of gene flow and instantaneous population size change after divergence (Fig. 3). To compare simulated data under each specific demographic model with the empirical data we implemented a coalescent model-based approach using Fastsimcoal2 (FSC; Excoffier *et al.* 2013). Since FSC summarizes the complexity of the data by using the site frequency spectrum (SFS) as summary statistics, we first estimated the empirical multi-SFS (single SFS with all populations included) in *PopGen* 1.7 (Gutenkunst *et al.* 2009). For each demographic model we ran 50 independent replicates, retaining the parameters that maximized the composite likelihood across all iterations. Parameter optimization was performed through 50 cycles of the Brent algorithm and

the composite likelihood calculated using 100,000 simulations per replicate. The runs with the highest likelihood of each model were used in model selection using Akaike Information Criterion (AIC; Akaike 1973), $AIC=2k-2\ln(L)$, where k is the number of parameters estimated in the model and L the composite likelihood value. The model with best fit had its confidence intervals estimated with 50 parametric bootstrap runs, by simulating multi-SFSs under the maximum likelihood estimates and re-estimating parameters for each of these simulated data sets. For all simulations, we used a rate of 2.5×10^{-9} substitution per site per generation (average of all avian species analysed in Nadachowska-Brzyska et al. 2015) and assumed a generation time of 2.33 years (Maldonado-Coelho 2012).

Results

Sequencing results

We obtained 634 UCE loci with median length of 633 bp (range: 216-811 bp) and 8,465 SNPs passed our quality filtering. Each UCE locus had a median of 13 SNPs (range: 1-35). The final dataset containing 1 randomly sampled SNP per variable locus had 588 SNPs.

Population structure

sNMF runs indicated three groups based on the best cross-entropy values across alpha values, with the only exception of alpha of 1,000 showing a smaller cross-entropy value for a single group (Supporting Information Figure S1). Plots based on alpha of 1, 10 and 100 for $K=3$ support the Caparaó population of *M. lateralis*, the remaining *M. lateralis* populations, and *M. cabanisi* as three distinct clusters with somewhat varying levels of ancestry coefficients (Fig. 2). DAPC based on k-clustering algorithm suggests two as the best number of groups, although BIC values are very similar among $K=1$, $K=2$ and $K=3$ (246.7343, 246.5362 and 247.0419, respectively, Supporting Information Figure S2). These two clusters correspond to: 1) the Caparaó population of *M. lateralis* + some *M. cabanisi* individuals and 2) remaining *M. lateralis* individuals + remaining *M. cabanisi* individuals (Supporting Information Figure S2). If the three sNMF populations are considered in the DAPC analysis, the groupings are similar: one of them included only *M. lateralis* Caparaó individuals and the other two mostly matched *M. cabanisi* and *M. lateralis* excluding Caparaó (Fig. 2 and Supporting Information Fig. S1). Accordingly, the mtDNA haplotype network showed no haplotypes shared among populations, although some haplotypes of *M. lateralis* were more closely related to those of *M. cabanisi* than to conspecific haplotypes, while Caparaó haplotypes formed a cluster (Fig. 2).

Demographic relationships and population history

The best demographic model according to AIC assumed a polytomy of the three populations, presence of asymmetric gene flow and stable population sizes after the divergence (Fig. 3; Tables 1 and 2). The second and third best-fit models included alternative branching (instead of simultaneous divergence) with gene flow, but neither supported population size changes (Table 1). Parameter estimations based on the best-fit model supported current effective population sizes (N_e) that were positively related with the modern range size of the three lineages. The geographically most restricted population (i.e. Caparaó) had the smallest N_e , with ~4,000 individuals, followed by the populations with intermediate (rest of *M. lateralis*) and largest (*M. cabanisi*) geographic ranges, with respectively ~11,000 and ~19,500 individuals (Table 2). The divergence time estimate supports a very recent (Late Pleistocene) event for the simultaneous separation of the three populations at approximately 12,400 years ago (5,325 generations, Table 2).

The estimated gene flow between *M. cabanisi* and *M. lateralis* (excluding Caparaó) was greater (3.15 and 2.65 individuals per generation from *M. lateralis* to *M. cabanisi* and from *M. cabanisi* to *M. lateralis*, respectively) than between *M. lateralis* and the Caparaó population of this species (0.324 and 1.147 individuals per generation from *M. lateralis* to Caparaó and from Caparaó to *M. lateralis*, respectively), potentially reflecting the more isolated and restricted distribution of the Caparaó population (Table 2).

Discussion

The dual role of AF mountains: population divergence and persistence

Mountains may function both as generators and maintainers of diversity (Fjeldsa et al. 2012), and here we show Atlantic Forest organisms that support this idea. First, the ancient AF highlands may generate diversity by fostering population isolation and divergence, as indicated by our population structure estimates with three distinct groups associated with three isolated mountain ranges. While geotectonic changes could explain phylogeographic breaks in the AF (e.g. Thomé et al. 2010, Amaro et al. 2012, Amaral et al. 2013), the shallow divergences found here cannot be reconciled with the major uplift events of eastern Brazil highlands, which very likely took place long before the estimated recent divergence times (< 20 k years). In addition, support for a model of synchronous isolation among the three populations is especially compelling in terms of large scale effects of climate across the entire range of

subtropical AF. Interestingly, the strong population structure found here contradicts previous studies on other co-distributed montane organisms, which did not recover recent (< 100 k years) phylogeographic breaks (Amaro et al. 2012, Batalha-Filho et al. 2012, Peres et al. 2015). These findings suggest that although warbling-finches are forest-associated species that often use forest edges, such ecological flexibility was presumably not sufficient to preclude isolation and divergence. In contrast, the White-browed Warbler (*Myiothlypis leucoblephara*), for example, which is sympatric - and often syntopic - to *M. cabanisi* and *M. lateralis*, did not show detectable population structure (Batalha-Filho et al. 2012). These phylogeographic differences may be due to the interaction of traits and historical processes (see Zamudio et al. 2016 for a review), differences in altitudinal distribution, lack of power of Sanger datasets to detect shallow divergences (Amaral et al. 2018) or a combination of those factors, and warrants additional studies on both novel and previously studied organisms using subgenomic or genomic datasets.

Second, it has been long suggested that the MAF (Brown & Ab'Saber, 1979; Brown, 1987) and other Neotropical mountains (Fjelds et al. 1999; Garca-Moreno & Fjelds, 2000, Mastretta-Yanes et al. 2018) are safe harbors (i.e. refuges) for montane and non-montane organisms during historical periods of harsh climates. Mountains may buffer population size changes of montane forest organisms during historical periods of unsuitable climate by holding moisture in the leeward slopes (Brown & Ab'Saber 1979) and by allowing shifts in elevational distributions, a process that may be even seen in ecological time (Moritz et al. 2008). In line with this observation, our estimates of historical demography echo the lack of strong population size fluctuations found in other co-distributed organisms (Amaro et al. 2012, Batalha-Filho et al. 2012, Peres et al. 2015, Pie et al. 2018), what underscores the notion that montane habitats are important refuges, hampering extinction of isolated population during periods of climate change.

While our results illustrate how montane habitats may act both as drivers and keepers of diversity, partial concordance with previous studies, specially in terms of population structure, is intriguing. New comparative phylogenomic studies will help understand the generality of the patterns found here, and contribute to tease apart the effects of history, ecology and issues related to number of loci. We hypothesize that population structure among highland species in the AF will be more common than previously thought, and in many cases may be detectable only using sufficiently sized nuclear datasets (e.g. Pie et al. 2018; present study). Fine-scale inferences of population structure will be essential not only to better describe patterns and infer processes involved in Neotropical montane diversification, but may also reveal important

hotspots of divergence and cryptic diversity of conservation importance, which may have been overlooked due to recent divergences (as in the Araripe Highlands, Amaral et al. 2018).

Historical climate changes explain diversification in MAF warbling-finches

A main question in the MAF system is whether one can disentangle the relative influence of topography and climate when explaining diversification patterns. For example, under a strict geomorphological model of diversification, genetic divergences among warbling finch populations should be coincident with the known major pulses of uplift of eastern Brazilian highlands during the last ca.100 mya (e.g. Melo et al. 1985, Meisling et al. 2001, Franco-Magalhães et al. 2014). However, we argue that one cannot rule out biogeographic scenarios in which historical climate changes taking place during or subsequent to the major uplift events had a main role on biotic diversification. In fact, the interplay between topography and climate implies that the expected model of diversification for warbling finches and other MAF organisms would be one of isolation and divergence on distinct highlands during the warm-interglacial periods, when populations retracted upwards over the mountains. In addition, any event of genetic admixture (i.e. introgression) between resulting lineages ensued when populations expanded downwards, during cold-glacial periods. The results of our coalescent analyses and the current altitudinal distribution of warbling finches confirm key components of this model. Specifically, the recent estimates of genetic divergence and past admixture (i.e. for the late Pleistocene) as well as their occurrence on highlands during the present-day warm-interglacial period undoubtedly implicate a strong influence of historical climate changes on range shifts and associated population evolutionary dynamics across a longstanding rugged landscape. One especially intriguing result is the lack of recent variation in effective size despite signs of introgression. We hypothesize that secondary contact may not necessarily involve large population size fluctuation, as shifts in elevational distribution could occur without significant changes in effective size. In addition, small elevational shifts (e.g. only 100 m) could readily connect the two species (Fig. 1). Alternatively, we can also speculate that past gene flow may have erased signs of population expansion and contraction, or limited expansion associated with long-distance dispersal could explain introgression without large N_e variation.

Elevational distribution and its role in population isolation

Population structure was detected in the northern species (*M. lateralis*) but was absent in the southern species (*M. cabanisi*), and it is possible that the phylogeographic structure of

Microspingus warblers may reflect historical differences in forest dynamics between southeastern and southern AF. One interesting biological difference between *M. cabanisi* and *M. lateralis* is their distinct elevational range: populations of *M. lateralis* are found only above 900 m, while in the southernmost part of its range, *M. cabanisi* reaches sea-level. This pattern may be a consequence of either thermal niche divergence (Janzen 1967), biological interactions (i.e. competition) or simply a latitudinal compensation of elevational distribution (Barry 1992). In any case, differences in altitudinal ranges across a latitudinal gradient may lead to greater opportunities for isolation in the populations in the northern extreme edges of SE/S subtropical habitats than those in the southern edges. Additional multi-taxon studies are warranted to test this hypothesis, as it could explain distinct levels of endemism in different portions of the MAF.

Caparaó highlands as a hotspot of divergence

The Caparaó is the highest mountain massif in the AF, reaching ca. 2900 m asl. Despite its small area compared to the neighbouring highlands (Fig. 1), this mountain system not only harbors a strongly differentiated population of *Microspingus* warbling-finches, but also endemic taxa (i.e. *Caparaonia* lizards, Rodrigues et al. 2009) whose divergences from taxa in other larger AF highlands are possibly much older than our divergence estimates. This suggests that the Caparaó highlands possibly represent a microrefugium (*sensu* Rull 2009) with a long-term history of connection and isolation with the neighboring highlands. This makes the Caparaó highlands an overlooked hotspot of population divergence and cryptic diversity, similarly to the Chapada Diamantina highlands (Amaral et al. 2013) and the Araripe plateau (Amaral et al. 2018). The presence of both recent and old endemic lineages in the Caparaó mountains may be related to higher population persistence in contrast to lower neighboring mountains. Interestingly, although the Caparaó population of *M. lateralis* diverged from the remaining *M. lateralis* populations, genetic differentiation was not detected between the other two currently isolated (by the warm lowlands of the Paraíba do Sul river valley) Serra do Mar and Serra da Mantiqueira populations. This could be related to a greater geographic proximity between the latter, thus allowing genetic homogenization. Given that population size changes may be buffered by shifts in elevational ranges and that extreme interglacial-warm periods could crash mountain-top populations (Moritz et al. 2008), it is possible that higher mountain ranges are related to higher persistence, higher levels of endemism, wider temporal ranges of population divergence compared to lower neighboring mountain ranges. Populations of many species that occur in Mantiqueira and Serra do Mar mountain systems can also be found in

Caparaó and offer exciting future opportunities to identify cryptic diversity.

The São Paulo and Caparaó subtropical gaps

Low passes and inter-mountain valleys often constitute formidable barriers to dispersal in montane tropical organisms (e.g. Africa - Bowie et al. 2006; Andes - Cadena et al. 2007, Winger and Bates 2015). However, such ecological barriers to genetic exchange remain largely overlooked in the MAF biota. In our study, more than 100 kilometers of lowlands that reach elevations down to 250 m a.s.l. in the Ribeira de Iguape River Valley (Karl et al. 2013) separate *M. lateralis* from *M. cabanisi*. This coincides with range and phenotypic discontinuities in other cold-adapted avian species complexes, including passerines (e.g. *Hemitriccus* flycatchers, *C. thoracica* warbling-finch) and non-passerines (*Stephanoxis* hummingbirds, Cavarzere et al. 2014), as well as with a narrow zone of secondary contact between cold-adapted lineages of *Bombus* bees (Françoso et al. 2016). Lower elevation forests also seem to isolate the highland biota of Caparaó from the ones of southern mountain ranges. We suggest that these lower elevation forests represent the ecological barrier underlying the differentiation in these and possibly other MAF organisms and could be named “São Paulo subtropical gap” and “Caparaó subtropical gap”. These ecological gaps highlights the influence of historical climate changes and associated shifts in the geographic distribution of MAF organisms, and coincides with the genetic differentiation observed in our study system. Additional studies will show if the historical processes affecting montane organisms in this region are shared, thus generating population divergence in other co-distributed organisms.

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Figures and tables

Table 1. Composite likelihood (Max ln(L)), number of parameter (param), Akaike information criterion (AIC) and relative contribution (Weights) for each of the demographic models tested. The name of the models represent the topology, in newick format and the demographic syndromes tested (see Figure 3). mc - *Microspingus cabanisi*; ml - *Microspingus lateralis*; cp - Caparaó population of *Microspingus lateralis*. In bold: best model.

Model	Max ln(L)	param	AIC	Weights
(cp(mc,ml)_no_gene_flow	-984,043	7	4545,69	7,59E-14
(mc(ml,cp)_no_gene_flow	-992,599	7	4585,09	2,11E-22
(ml(mc,cp)_no_gene_flow	-995,667	7	4599,22	1,80E-25
polytomy_no_gene_flow	-995,458	5	4594,25	2,16E-24
(cp(mc,ml)_gene_flow	-969,639	13	4491,35	4,77E-02
(ml(mc,cp)_gene_flow	-969,7	13	4491,63	4,15E-02
(mc(ml,cp)_gene_flow	-969,165	13	4489,17	1,42E-01
polytomy_gene_flow	-969,301	11	4485,80	7,68E-01
(cp(mc,ml)_bottleneck_gene_flow	-969,658	19	4503,44	1,13E-04
(mc(ml,cp)_bottleneck_gene_flow	-971,675	19	4512,73	1,09E-06
(ml(mc,cp)_bottleneck_gene_flow	-968,982	19	4500,33	5,37E-04
polytomy_bottleneck_gene_flow	-970,391	17	4502,82	1,55E-04

Model	NMm c/ml	TDIV 1	TDIV 2	SCcp	SCm l	SCm c	TSC cp	TSC ml	TSC mc
(cp(mc,ml)_no_gene_flow	n/a	3159	8984	n/a	n/a	n/a	n/a	n/a	n/a
(mc(ml,cp)_no_gene_flow	n/a	4926	10049	n/a	n/a	n/a	n/a	n/a	n/a
(ml(mc,cp)_no_gene_flow	n/a	5082	5415	n/a	n/a	n/a	n/a	n/a	n/a
polytomy_no_gene_flow	n/a	n/a	4380	n/a	n/a	n/a	n/a	n/a	n/a
(cp(mc,ml)_gene_flow	3,20	9637	15832	n/a	n/a	n/a	n/a	n/a	n/a
(ml(mc,cp)_gene_flow	2,03	10448	16403	n/a	n/a	n/a	n/a	n/a	n/a
(mc(ml,cp)_gene_flow	1,97	17042	23414	n/a	n/a	n/a	n/a	n/a	n/a
polytomy_gene_flow	2,65	n/a	8742	n/a	n/a	n/a	n/a	n/a	n/a
(cp(mc,ml)_bottleneck_gene_flow	0,15	21967	35353	8,31	7,03	7,26	6068	7529	7524
(mc(ml,cp)_bottleneck_gene_flow	1,42	15331	16823	2,05	6,87	3,41	3499	3883	4519
(ml(mc,cp)_bottleneck_gene_flow	3,47	14064	15042	6,22	3,86	5,28	2583	2972	4031
polytomy_bottleneck_gene_flow	1,55	n/a	12407	38,15	5,53	6	1777	1602	1881
polytomy_gene_flow lo	0,68	n/a	10140	n/a	n/a	n/a	n/a	n/a	n/a
polytomy_gene_flow up	4,25	n/a	47404	n/a	n/a	n/a	n/a	n/a	n/a

683

684 mc - *Microspingus cabanisi*; ml - *Microspingus lateralis*; cp - Caparaó population of
685 *Microspingus lateralis*. Ne - effective population size; a1 - ancestral population after the first
686 divergence event backwards in time; a2 - ancestral population; NMx/y - number of migrants
687 per generation going from population x to y backwards in time; TDIV1 - first divergence event
688 backwards in time; TDIV2 second divergence event backwards in time; SC - proportion of the

ancestral effective population size in relation to the present, before instantaneous population
size change; TSC - Time of the instantaneous population size changes.

Figure 1. Localities sampled. Blue and yellow circles represent *Microspingus lateralis* while red circles represent *Microspingus cabanisi*. Major highlands include Caparaó (1), Northern Serra do Mar (2), Mantiqueira (3 and 4), Southern Serra do Mar (5) and Serra Geral (6 and 7). Two lower elevation regions that mark phenotypic and genotypic shifts, named here Caparaó and São Paulo subtropical gaps, are indicated by pink dashed lines. See Supplementary Information Table S1 for details on samples and coordinates.

Figure 2. Plots depicting population structure according to (a) the best fitting sNMF model $K=3$ based on different values of the regularization parameter α (1, 10, 100) and (b) the three clusters inferred with DAPC and (c) cytochrome-b median-joining network. Black dots in the haplotype network indicate inferred haplotypes.

Figure 3. Demographic models simulated in Fastsimcoal2 for (a) three populations and (b) alternative topologies tested under these demographic scenarios, in a total of 12 simulated models. Na – ancestral effective population size; θ' – effective population size before population size change; Tdiv – divergence time; Mc – *Microspingus cabanisi*; MI – *Microspingus lateralis*; Cp - Caparaó population of *Microspingus lateralis*

Supplementary Information

Table S1. Samples used in the present study.

Field number	Species	Locality number	Municipality	Latitude	Longitude	MZUSP number	LGEMA number
FRA46	<i>Poospiza lateralis</i>	1	Dores do Rio Preto	-20,5125	-41,8075	pending	pending
FRA49	<i>Poospiza lateralis</i>	1	Caparaó	-20,4097	-41,8376	pending	pending
FRA50	<i>Poospiza lateralis</i>	1	Caparaó	-20,4097	-41,8376	pending	pending
FRA52	<i>Poospiza lateralis</i>	1	Alto Jequitibá	-20,4074	-41,8382	pending	pending
FRA54	<i>Poospiza lateralis</i>	1	Caparaó	-20,4097	-41,8376	pending	pending
FRA55	<i>Poospiza lateralis</i>	1	Caparaó	-20,4748	-41,8291	pending	pending
FRA56	<i>Poospiza lateralis</i>	1	Espera Feliz	-20,4861	-41,8211	pending	pending
FRA57	<i>Poospiza lateralis</i>	1	Caparaó	-20,4792	-41,8297	pending	pending
FRA58	<i>Poospiza lateralis</i>	1	Caparaó	-20,4806	-41,8298	pending	pending
FRA59	<i>Poospiza lateralis</i>	1	Caparaó	-20,4722	-41,8267	pending	pending
MA02	<i>Poospiza lateralis</i>	2	Delfim Moreira	-22,5442	-45,2108	pending	pending
MA03	<i>Poospiza lateralis</i>	2	Delfim Moreira	-22,5444	-45,2073	pending	pending

	<i>Poospiza</i>						
MA04	<i>lateralis</i>	2	Delfim Moreira	-22,5426	-45,1897	pending	pending
	<i>Poospiza</i>						
MA05	<i>lateralis</i>	2	Delfim Moreira	-22,5429	-45,1837	pending	pending
	<i>Poospiza</i>						
FRA05	<i>lateralis</i>	2	Delfim Moreira	-22,5446	-45,2158	pending	pending
	<i>Poospiza</i>						
FRA06	<i>lateralis</i>	2	Piquete	-22,5160	-45,1521	pending	pending
	<i>Poospiza</i>						
FRA07	<i>lateralis</i>	2	Piquete	-22,5696	-45,2276	pending	pending
	<i>Poospiza</i>						
FRA08	<i>lateralis</i>	2	Piquete	-22,5159	-45,1510	pending	pending
	<i>Poospiza</i>						
FRA09	<i>lateralis</i>	2	Piquete	-22,5693	-45,2291	pending	pending
	<i>Poospiza</i>						
FRA11	<i>lateralis</i>	2	Piquete	-22,5160	-45,1507	pending	pending
	<i>Poospiza</i>						
FRA13	<i>lateralis</i>	3	Cunha	-23,1438	-44,8761	pending	pending
	<i>Poospiza</i>						
FRA14	<i>lateralis</i>	3	Paraty (PN Serra da Bocaina)	-23,1722	-44,8339	pending	pending
	<i>Poospiza</i>						
FRA16	<i>lateralis</i>	3	Paraty (PN Serra da Bocaina)	-23,1673	-44,8396	pending	pending
	<i>Poospiza</i>						
FRA17	<i>lateralis</i>	3	Paraty (PN Serra da Bocaina)	-23,1734	-44,8366	pending	pending
	<i>Poospiza</i>						
FRA19	<i>lateralis</i>	3	Cunha	-23,1516	-44,8353	pending	pending
	<i>Poospiza</i>						
FRA20	<i>lateralis</i>	3	Paraty (PN Serra da Bocaina)	-23,1516	-44,8215	pending	pending
	<i>Poospiza</i>						
FRA21	<i>lateralis</i>	3	Cunha	-23,1526	-44,8417	pending	pending

	<i>Poospiza</i>						
FRA22	<i>lateralis</i>	3	Cunha	-23,1491	-44,9328	pending	pending
	<i>Poospiza</i>						
FRA26	<i>lateralis</i>	3	Cunha	-23,2053	-44,9966	pending	pending
	<i>Poospiza</i>						
FRA27	<i>lateralis</i>	3	Cunha	-23,1386	-44,9064	pending	pending
	<i>Poospiza</i>						
MV01	<i>lateralis</i>	4	Camanducaia	-22,8378	-46,0551	pending	pending
	<i>Poospiza</i>						
MV02	<i>lateralis</i>	4	Camanducaia	-22,8584	-46,0664	pending	pending
	<i>Poospiza</i>						
MV03	<i>lateralis</i>	4	Camanducaia	-22,8419	-46,0606	pending	pending
	<i>Poospiza</i>						
MV04	<i>lateralis</i>	4	Camanducaia	-22,8419	-46,0606	pending	pending
	<i>Poospiza</i>						
MV05	<i>lateralis</i>	4	Camanducaia	-22,8036	-46,0473	pending	pending
	<i>Poospiza</i>						
MV07	<i>lateralis</i>	4	Camanducaia	-22,8214	-46,0438	pending	pending
	<i>Poospiza</i>						
FRA01	<i>lateralis</i>	4	Camanducaia	-22,8499	-46,0642	pending	pending
	<i>Poospiza</i>						
FRA02	<i>lateralis</i>	4	Camanducaia	-22,8037	-46,0472	pending	pending
	<i>Poospiza</i>						
FRA03	<i>lateralis</i>	4	Camanducaia	-22,8112	-46,0425	pending	pending
	<i>Poospiza</i>						
FRA04	<i>lateralis</i>	4	Camanducaia	-22,8500	-46,0642	pending	pending
	<i>Poospiza</i>						
FRA28	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
FRA29	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending

	<i>Poospiza</i>						
FRA32	<i>cabanisi</i>	5	Apiáí	-24,4762	-48,8972	pending	pending
	<i>Poospiza</i>						
FRA33	<i>cabanisi</i>	5	Apiáí	-24,4762	-48,8972	pending	pending
	<i>Poospiza</i>						
FRA34	<i>cabanisi</i>	5	Apiáí	-24,4762	-48,8972	pending	pending
	<i>Poospiza</i>						
FRA39	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
FRA40	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
FRA41	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
FRA42	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
FRA43	<i>cabanisi</i>	5	Apiáí	-24,4284	-48,6742	pending	pending
	<i>Poospiza</i>						
SC01	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1419	-49,6353	pending	pending
	<i>Poospiza</i>						
SC02	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1419	-49,6353	pending	pending
	<i>Poospiza</i>						
SC03	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1419	-49,6353	pending	pending
	<i>Poospiza</i>						
SC05	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1449	-49,6324	pending	pending
	<i>Poospiza</i>						
SC08	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending
	<i>Poospiza</i>						
SC09	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending
	<i>Poospiza</i>						
SC10	<i>cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending

SC11	<i>Poospiza cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending
SC14	<i>Poospiza cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending
SC15	<i>Poospiza cabanisi</i>	6	Urubici (PN São Joaquim)	-28,1116	-49,4968	pending	pending
SC17	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC18	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC19	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC20	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC21	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC22	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC23	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Aparados da Serra)	-29,1746	-50,1148	pending	pending
SC24	<i>Poospiza cabanisi</i>	7	Cambará do Sul	-29,1149	-50,1064	pending	pending
SC25	<i>Poospiza cabanisi</i>	7	Cambará do Sul (PN Serra Geral)	-29,1447	-50,0804	pending	pending
SC26	<i>Poospiza cabanisi</i>	7	Cambará do Sul	-29,0568	-50,1187	pending	pending
FRA74	<i>Poospiza cabanisi</i>	8	Itaara	-29,6130	-53,7529	pending	pending
FRA75	<i>Poospiza cabanisi</i>	8	Santa Maria	-29,5974	-53,7006	pending	pending

	<i>Poospiza</i>						
FRA76	<i>cabanisi</i>	8	Santa Maria	-29,5974	-53,7006	pending	pending
	<i>Poospiza</i>						
FRA77	<i>cabanisi</i>	8	Itaara	-29,5896	-53,7934	pending	pending
	<i>Poospiza</i>						
FRA78	<i>cabanisi</i>	8	Itaara	-29,5920	-53,7521	pending	pending
	<i>Poospiza</i>						
FRA80	<i>cabanisi</i>	8	Itaara	-29,5892	-53,7933	pending	pending
	<i>Poospiza</i>						
FRA81	<i>cabanisi</i>	8	Itaara	-29,5929	-53,7598	pending	pending
	<i>Poospiza</i>						
FRA82	<i>cabanisi</i>	8	Itaara	-29,5929	-53,7598	pending	pending
	<i>Poospiza</i>						
FRA84	<i>cabanisi</i>	8	Itaara	-29,5937	-53,7343	pending	pending
	<i>Poospiza</i>						
FRA85	<i>cabanisi</i>	8	Itaara	-29,5819	-53,8062	pending	pending
	<i>Poospiza</i>						
FRA61	<i>cabanisi</i>	9	Pelotas	-31,6277	-52,5227	pending	pending
	<i>Poospiza</i>						
FRA62	<i>cabanisi</i>	9	Pelotas	-31,6088	-52,5188	pending	pending
	<i>Poospiza</i>						
FRA64	<i>cabanisi</i>	9	Pelotas	-31,6317	-52,5416	pending	pending
	<i>Poospiza</i>						
FRA65	<i>cabanisi</i>	9	Pelotas	-31,6071	-52,5486	pending	pending
	<i>Poospiza</i>						
FRA66	<i>cabanisi</i>	9	Pelotas	-31,6071	-52,5486	pending	pending
	<i>Poospiza</i>						
FRA67	<i>cabanisi</i>	9	Pelotas	-31,5265	-52,5505	pending	pending
	<i>Poospiza</i>						
FRA68	<i>cabanisi</i>	9	Pelotas	-31,5265	-52,5505	pending	pending

	<i>Poospiza</i>							
FRA69	<i>cabanisi</i>	9	Pelotas	-31,5178	-52,5042	pending	pending	
	<i>Poospiza</i>							
FRA71	<i>cabanisi</i>	9	Canguçu	-31,4565	-52,6348	pending	pending	
	<i>Poospiza</i>							
FRA72	<i>cabanisi</i>	9	Canguçu	-31,4579	-52,6150	pending	pending	

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Figure S1. Cross-entropy sNMF runs for alpha values of 1, 10, 100 and 1,000.

Figure S2. BIC values of (a) K-clustering method and (b) DAPC plot for two populations. Colors in the DAPC plot follow the ones assumed for Figures 1 and 2.

Data Accessibility

Dryad XXXX and SRA XXXX

Raw Illumina files, Set of SNPs, Joint SFS

Author contributions

FRA, DFA and GT designed the study. FRA, JACM and GT performed field work. FRA, DFAS and GT performed data analysis. FRA, KCMP, CYM and MJH contributed funds for lab or field work. All authors participated in the discussion of the results. FRA, MMC and GT wrote the manuscript with input from the other authors. All authors approved the final version of the manuscript.