

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

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35 **Abstract**

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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.

## 57 **Introduction**

58  
59 Montane organisms often exhibit intriguing patterns of disjunct distributions, which are  
60 frequently associated to phenotypic divergence. Among the many mechanisms that have been  
61 proposed to explain such patterns, the ones with the strongest support are the historical  
62 fragmentation of formerly continuous habitats and dispersal (Mayr & Diamond 1976; Patton &  
63 Smith 1992; Knowles & Massatti 2017) associated with rugged topography and climate  
64 fluctuations. That is, changes in climate over time, which are well known to promote shifts in  
65 the elevational distributions of suitable habitats and associated organisms (Hooghiemstra &  
66 Van der Hammen 2004, Moritz et al. 2008, Chen et al. 2009), could lead to cyclical isolation  
67 and connection of population through temporal bridges (Brown 1971, McCormack et al. 2009).  
68 During the late Pleistocene glacial periods, warmer periods often lead to isolation in  
69 high-elevation habitat pockets, whereas colder periods often lead to range expansion into lower  
70 elevations and previously unsuitable regions. Under this scenario, warmer periods (such as the  
71 current interglacial) would create opportunities for population divergence by genetic drift and  
72 natural selection, while colder periods might promote secondary contact and genetic  
73 homogenization of previously isolated populations. This interplay between selection and drift  
74 with topography and a dynamic climate could lead not only to genetic divergence, but to the  
75 evolution of reproductive isolation, thus rendering mountains as potential hotspots of  
76 population divergence and speciation (Fjeldsa 1994, McCormack et al. 2009). Alternatively,  
77 isolation could be initially mediated by topographic changes. For example, the splitting of  
78 formerly continuous highlands into multiple isolated mountain ranges could be the result of  
79 geological changes, and organisms adapted to higher habitats would then become separated by  
80 ecologically unsuitable valleys. Under such scenario, population divergence should be  
81 temporally associated to geotectonic changes (Badgley 2010).

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

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

97       Much of what is known about the Atlantic Forest comes from studies of either lowland  
98 species or species with broad elevational distributions (e.g. Cabanne et al. 2007, Carnaval et al.  
99 2009, Maldonado-Coelho 2012, Amaral et al. 2013, Carnaval et al. 2014), and less than a  
100 handful of phylogeographic studies have investigated cold-adapted species whose distributions  
101 include mostly montane habitats (e.g. Amaro et al. 2013, Batalha-Filho et al. 2012, Peres et al.  
102 2015, Frikowski et al. 2016, Franoso et al. 2016, Pie et al. 2018). Conclusions from the few  
103 studies on MAF-inhabiting species are often discordant; consequently, there is still little  
104 consensus about what are the major drivers of diversification in the MAF. A common finding  
105 by many of them is the lack of both population structure and strong demographic fluctuations,  
106 which could speak against Pleistocene climatic fluctuations as major drivers of population  
107 divergence and diversification in MAF organisms. Importantly, all but one (Pie et al. 2018) of  
108 the studies performed so far were based on five or less loci, raising the possibility that shallow  
109 population structure—which is expected under recent climate fluctuations—may have been  
110 overlooked (Amaral et al. 2018). Furthermore, none of these studies have performed  
111 demographic analyses explicitly testing for historical gene flow among currently isolated and  
112 genetically structured populations—a prediction of montane climate-driven dynamics.

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

124 **Methods**

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

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

141

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

152

153 Available evidence from fossil pollen records in southern and southeastern Brazil  
154 highland sampling sites (> 900 m a.s.l.) at Mantiqueira, Serra do Mar and Serra Geral mountain  
155 ranges suggest broadly concordant cold and dry conditions during the last glacial maximum,  
156 with forests completely absent or likely present only along ravines and/or at lower altitudes  
157 (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

158 period favored the return of montane forest species (Behling 1997, Behling & Pillar 2007,  
159 Behling et al. 2007, Ledru et al. 2009, Behling and Safford 2010, Oliveira et al. 2012).  
160 Importantly, the highland vegetation seems to have had a complex dynamics during the last  
161 130,000 yr BP (i.e. late Pleistocene and Holocene), with multiple shifts in community  
162 composition and elevational distribution of montane forest plant species (Ledru et al. 2009,  
163 Behling & Safford 2010, Oliveira et al. 2012).

164

#### 165 *Sampling and laboratory methods*

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

171 We extracted total DNA from pectoral muscle using the Qiagen DNeasy kit (Valencia,  
172 CA) according to the manufacturer's protocol, including a RNase treatment. We obtained  
173 genomic data using sequence capture and Illumina sequencing of ultraconserved elements  
174 (UCEs) using standard protocols (Faircloth *et al.* 2012) with a few modifications: enrichment  
175 was done using 650 probes, targeting 634 loci covering all *Gallus gallus* chromosomes; use of  
176 100 bp paired-end Illumina HiSeq 2000 sequencing run, and use of 16 cycles in both pre- and  
177 post-capture PCR reactions. Sequencing was performed in two lanes. Library preparation was  
178 performed by RAPiD Genomics (Gainesville, FL, USA).

179

#### 180 *Sequence quality control, mtDNA, UCE assembly and SNP calling*

181 We sorted raw sequences by individual tags using Illumina's Casava software. Initial  
182 quality control was performed using FastQC 0.10.1 (Andrews 2014). Adapters, barcodes and  
183 low quality regions were trimmed using Illumiprocessor 2.0.7 (Faircloth 2014), which  
184 processes Illumina sequences using Trimmomatic 0.32.1 (Bolger *et al.* 2014). Assembly,  
185 removal of non-UCE loci and final loci alignment was performed with Phyluce 1.4 (Faircloth  
186 2014). The largest contig of each locus was used as a reference for mapping individual reads  
187 using BWA-mem (Li 2013) and SNP calling, which was performed using GATK (McKenna *et*  
188 *al.* 2010). We kept only >Q30 SNPs for downstream analyses. We sampled one random SNP  
189 per locus from the collection of all zero-missing biallelic SNPs recovered for the locus. We  
190 removed Z-linked SNPs using a local BLAST search based on the zebra-finch Z chromosome  
191 (Emsembl taeGut3.2.4) to avoid any bias due to the idiosyncratic evolution of sex

192 chromosomes and different ploidy (unfortunately the limited number of Z-linked SNPs  
193 recovered precluded us from running independent analyses on this dataset). Since mtDNA is a  
194 common subproduct of sequence capture experiments (see Amaral *et al.* 2015), we also  
195 isolated complete cytochrome b sequences from assemblies that were generated by Phyluce  
196 using a local BLAST search based on the complete mitogenome of *Microspingus lateralis*  
197 (Genbank NC\_028039.1) to obtain a Median Joining Network using POPART v 1.7 (Leigh &  
198 Bryant 2015).

199

#### 200 *Population structure*

201 We explored population structure first with sparse non-negative matrix factorization  
202 (sNMF, Frichot *et al.* 2014, implemented in the R package LEA, Frichot & Olivier 2015) as  
203 well as with the multivariate Discriminant Analysis of Principal Components (Jombart *et al.*  
204 2010). The sNMF runs were performed for a range of K values from 1 to 9 (following the  
205 number of localities), with 500 runs per K. We used the minimum cross-entropy method to  
206 identify the best fitting K. Runs with the lowest values of minimum entropy were selected.  
207 Since sNMF's regularization parameter alpha may affect the inferences (Frichot *et al.* 2014),  
208 we evaluated the results under different values of alpha (1, 10, 100 and 1,000). To further  
209 assess population structure we ran a DAPC analysis using a k-clustering algorithm to  
210 determine the *a priori* grouping of individuals based on the Bayesian Information Criterion  
211 (BIC). The first set of principal components accounting for 80% of the variance was included  
212 in the analyses.

213

#### 214 *Inference of historical processes*

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



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

235

## 236 **Results**

237

### 238 *Sequencing results*

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

### 242 *Population structure*

243 sNMF runs indicated three groups based on the best cross-entropy values across alpha  
244 values, with the only exception of alpha of 1,000 showing a smaller cross-entropy value for a  
245 single group (Supporting Information Figure S1). Plots based on alpha of 1, 10 and 100 for  
246  $K=3$  support the Caparaó population of *M. lateralis*, the remaining *M. lateralis* populations,  
247 and *M. cabanisi* as three distinct clusters with somewhat varying levels of ancestry coefficients  
248 (Fig. 2). DAPC based on k-clustering algorithm suggests two as the best number of groups,  
249 although BIC values are very similar among  $K=1$ ,  $K=2$  and  $K=3$  (246.7343, 246.5362 and  
250 247.0419, respectively, Supporting Information Figure S2). These two clusters correspond to:  
251 1) the Caparaó population of *M. lateralis* + some *M. cabanisi* individuals and 2) remainig *M.*  
252 *lateralis* individuals + remaining *M. cabanisi* individuals (Supporting Information Figure S2).  
253 If the three sNMF populations are considered in the DAPC analysis, the groupings are similar:  
254 one of them included only *M. lateralis* Caparaó individuals and the other two mostly matched  
255 *M. cabanisi* and *M. lateralis* excluding Caparaó (Fig. 2 and Supporting Information Fig. S1).  
256 Accordingly, the mtDNA haplotype network showed no haplotypes shared among populations,  
257 although some haplotypes of *M. lateralis* were more closely related to those of *M. cabanisi*  
258 than to conspecific haplotypes, while Caparaó haplotypes formed a cluster (Fig. 2).

259



## 260 *Demographic relationships and population history*

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

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

279

## 280 **Discussion**

281

### 282 *The dual role of AF mountains: population divergence and persistence*

283

284 Mountains may function both as generators and maintainers of diversity (Fjeldsa et al.  
285 2012), and here we show a Atlantic Forest organisms that support this idea. First, the ancient  
286 AF highlands may generate diversity by fostering population isolation and divergence, as  
287 indicated by our population structure estimates with three distinct groups associated with three  
288 isolated mountain ranges. While geotectonic changes could explain phylogeographic breaks in  
289 the AF (e.g. Thomé et al. 2010, Amaro et al. 2012, Amaral et al. 2013), the shallow divergences  
290 found here cannot be reconciled with the major uplift events of eastern Brazil highlands, which  
291 very likely took place long before the estimated recent divergence times (< 20 k years). In  
292 addition, support for a model of synchronous isolation among the three populations is  
293 especially compelling in terms of large scale effects of climate across the entire range of

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

307         Second, it has been long suggested that the MAF (Brown & Ab'Saber, 1979; Brown,  
308 1987) and other Neotropical mountains (Fjeldså et al. 1999; García-Moreno & Fjeldså, 2000,  
309 Mastretta-Yanes et al. 2018) are safe harbors (i.e. refuges) for montane and non-montane  
310 organisms during historical periods of harsh climates. Mountains may buffer population size  
311 changes of montane forest organisms during historical periods of unsuitable climate by holding  
312 moisture in the leeward slopes (Brown & Ab'Saber 1979) and by allowing shifts in elevational  
313 distributions, a process that may be even seen in ecological time (Moritz et al. 2008). In line  
314 with this observation, our estimates of historical demography echo the lack of strong  
315 population size fluctuations found in other co-distributed organisms (Amaro et al. 2012,  
316 Batalha-Filho et al. 2012, Peres et al. 2015, Pie et al. 2018), what underscores the notion that  
317 montane habitats are important refuges, hampering extinction of isolated population during  
318 periods of climate change.

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

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

330

### 331 *Historical climate changes explain diversification in MAF warbling-finches*

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

358

### 359 *Elevational distribution and its role in population isolation*

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

362 *Microspingus* warblers may reflect historical differences in forest dynamics between  
363 southeastern and southern AF. One interesting biological difference between *M. cabanisi* and  
364 *M. lateralis* is their distinct elevational range: populations of *M. lateralis* are found only above  
365 900 m, while in the southernmost part of its range, *M. cabanisi* reaches sea-level. This pattern  
366 may be a consequence of either thermal niche divergence (Janzen 1967), biological  
367 interactions (i.e. competition) or simply a latitudinal compensation of elevational distribution  
368 (Barry 1992). In any case, differences in altitudinal ranges across a latitudinal gradient may  
369 lead to greater opportunities for isolation in the populations in the northern extreme edges of  
370 SE/S subtropical habitats than those in the southern edges. Additional multi-taxon studies are  
371 warranted to test this hypothesis, as it could explain distinct levels of endemism in different  
372 portions of the MAF.

373

#### 374 *Caparaó highlands as a hotspot of divergence*

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

396 Caparaó and offer exciting future opportunities to identify cryptic diversity.

397

### 398 *The São Paulo and Caparaó subtropical gaps*

399 Low passes and inter-mountain valleys often constitute formidable barriers to dispersal  
400 in montane tropical organisms (e.g. Africa - Bowie et al. 2006; Andes - Cadena et al. 2007,  
401 Winger and Bates 2015). However, such ecological barriers to genetic exchange remain largely  
402 overlooked in the MAF biota. In our study, more than 100 kilometers of lowlands that reach  
403 elevations down to 250 m a.s.l. in the Ribeira de Iguape River Valley (Karl et al. 2013) separate  
404 *M. lateralis* from *M. cabanisi*. This coincides with range and phenotypic discontinuities in  
405 other cold-adapted avian species complexes, including passerines (e.g. *Hemitriccus* flycatchers,  
406 *C. thoracica* warbling-finch) and non-passerines (*Stephanoxis* hummingbirds, Cavarzere et al.  
407 2014), as well as with a narrow zone of secondary contact between cold-adapted lineages of  
408 *Bombus* bees (Françoso et al. 2016). Lower elevation forests also seem to isolate the highland  
409 biota of Caparaó from the ones of southern mountain ranges. We suggest that these lower  
410 elevation forests represent the ecological barrier underlying the differentiation in these and  
411 possibly other MAF organisms and could be named “São Paulo subtropical gap” and “Caparaó  
412 subtropical gap”. These ecological gaps highlights the influence of historical climate changes  
413 and associated shifts in the geographic distribution of MAF organisms, and coincides with the  
414 genetic differentiation observed in our study system. Additional studies will show if the  
415 historical processes affecting montane organisms in this region are shared, thus generating  
416 population divergence in other co-distributed organisms.

417

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419

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436

#### 437 **Literature cited**

438

439 Akaike H (1973) Information theory and an extension of the maximum likelihood principle.

440 *International symposium on information theory*, 267–281.

441 Almeida FFM, Carneiro CDR (1998) Origem e evolução da Serra do Mar. *Revista Brasileira*  
442 *de Geociências*, **28**, 135–150.

443 Amaral FR, Piacentini VQ, Brito GRR, Curcio FF (2012) A simple and effective air shotgun  
444 for collecting small birds and other vertebrates at close range. *Journal of Field*  
445 *Ornithology*, **83**, 403–406.

446 Amaral FR, Neves LG, Resende Jr MFR *et al.* (2015) Ultraconserved Elements Sequencing as  
447 a Low-Cost Source of Complete Mitochondrial Genomes and Microsatellite Markers in  
448 Non-Model Amniotes. *PLoS ONE*, **10**, e0138446.

449 Amaral FR, Albers PK, Edwards S V, Miyaki CY (2013) Multilocus tests of Pleistocene  
450 refugia and ancient divergence in a pair of Atlantic Forest antbirds (Myrmeciza).  
451 *Molecular Ecology*, **22**, 3996–4013.

452 Amaral FR, Maldonado-Coelho M, Aleixo A, Luna LW, Rêgo PS, Araripe J, Souza TO, Thom,  
453 G. 2018. Recent chapters of Neotropical history overlooked in phylogeography: Shallow  
454 divergence explains phenotype and genotype uncoupling in *Antilophia* manakins.  
455 *Molecular Ecology*.

456 Amaro RC, Rodrigues MT, Yonenaga-Yassuda Y, Carnaval AC (2012) Demographic  
457 processes in the montane Atlantic rainforest: Molecular and cytogenetic evidence from the  
458 endemic frog *Proceratophrys boiei*. *Molecular Phylogenetics and Evolution*, **62**,  
459 880–888.

460 Andrews S (2014) FastQC: A quality control tool for high throughput sequence data.

461 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.

462 Antonelli A (2015) Biodiversity: Multiple origins of mountain life. *Nature*, **524**, 300-301.

463 Assis CP, Raposo MA, Parrini R (2007) Validação de *Poospiza cabanisi* Bonaparte, 1850



- 464 (Passeriformes: Emberizidae). *Revista Brasileira de Ornitologia*, 15, 103–112.
- 465 Badgley C (2010) Tectonics, topography, and mammalian diversity. *Ecography*, 33, 220–231.
- 466 Barry, R. G. (1992). Mountain weather and climate (Third edition). Cambridge University  
467 Press.
- 468 Batalha-Filho H, Cabanne GS, Miaki CY (2012) Phylogeography of an Atlantic Forest  
469 passerine reveals demographic stability through the last glacial maximum. *Molecular*  
470 *Phylogenetics and Evolution*, 65, 892–902.
- 471 Behling H (1997) Late Quaternary vegetation, climate and fire history from the tropical  
472 mountain region of Morro de Itapeva, SE Brazil. *Palaeogeography, Palaeoclimatology,*  
473 *Palaeoecology*, 129, 407–422.
- 474 Behling H (2007) Late Quaternary vegetation, fire and climate dynamics of Serra do Araçatuba  
475 in the Atlantic coastal mountains of Paraná State, southern Brazil. *Vegetation History and*  
476 *Archaeobotany*, 16, 77–85
- 477 Behling H, Pillar V (2007) Late Quaternary vegetation, biodiversity and fire dynamics on the  
478 southern Brazilian highland and their implication for conservation and management of  
479 modern Araucaria forest and grassland ecosystems. *Philosophical Transactions of the*  
480 *Royal Society of London Series B Biological Sciences*, 362, 243–251.
- 481 Behling H, Dupont L, Safford HD, Wefer, G (2007) Late Quaternary vegetation and climate  
482 dynamics in the Serra da Bocaina, southeastern Brazil. *Quaternary International*, 161,  
483 22–31.
- 484 Behling H, Safford, HD (2010) Late-glacial and Holocene vegetation, climate and fire  
485 dynamics in the Serra dos Orgãos Mountains of Rio de Janeiro State, southeastern Brazil.  
486 *Global Change Biology*, 16, 1661–1671.
- 487 Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina  
488 sequence data. *Bioinformatics*, 30, 2114–2120.
- 489 Bowie RCK, Fjeldså J, Hackett SJ, Bates JM, Crowe TM (2006) Coalescent models reveal the  
490 relative roles of ancestral polymorphism, vicariance, and dispersal in shaping  
491 phylogeographical structure of an African montane forest robin. *Molecular Phylogenetics*  
492 *and Evolution*, 38, 171–188.
- 493 Brown JH (1971) Mammals on Mountaintops: Nonequilibrium Insular Biogeography. *The*  
494 *American Naturalist*, 105, 467.
- 495 Brown Jr., K. S., & Ab'Saber, A. N. (1979). Ice-age forest refuges and evolution in the  
496 neotropics: correlation of paleoclimatological, geomorphological and pedological data  
497 with modern biological endemism. *Paleoclimas*, 5, 1–30.

- 498 Brown KSJr (1987) Conclusions, synthesis and alternative hypotheses. In: Whitmore, T. C.  
499 and G. T. Prance (eds). *Biogeography and Quaternary History in Tropical America*.  
500 Oxford Science Publications.
- 501 Cabanne GS, Santos FS, Miyaki CY (2007) Phylogeography of *Xiphorhynchus fuscus*  
502 (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in  
503 southern Atlantic forest. *Biological Journal of the Linnean Society*, **91**, 73–84.
- 504 Cadena CD, Klicka J, Ricklefs RE (2007) Evolutionary differentiation in the Neotropical  
505 montane region: molecular phylogenetics and phylogeography of *Buarremon*  
506 brush-finches (Aves, Emberizidae). *Molecular Phylogenetics and Evolution*, **44**,  
507 993–1016.
- 508 Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability predicts  
509 genetic diversity in the Brazilian Atlantic Forest hotspot. *Science*, **323**, 785–789.
- 510 Carnaval AC, Waltari E, Rodrigues MT, Rosauer D, VanDerWal J, Damasceno R, Prates I,  
511 Strangas M, Spanos Z, Rivera D, Pie MR, Firkowski CR, Bornschein MR, Ribeiro LF,  
512 Moritz C (2014). Prediction of phylogeographic endemism in an environmentally  
513 complex biome. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141461.
- 514 Cavarzere, V., Silveira, L. F., Vasconcelos, M. F. de, Grantsau, R., & Straube, F. C. (2014).  
515 Taxonomy and biogeography of *Stephanoxis* Simon, 1897 (Aves: Trochilidae). *Papéis*  
516 *Avulsos de Zoologia*, **54**, 69-79.
- 517 Chen IC, Shiu HJ, Benedick S, Holloway JD, Chey VK, Barlow HS, Hill JK, Thomas CD  
518 (2009) Elevation increases in moth assemblages over 42 years on a tropical mountain.  
519 *Proceedings of the National Academy of Sciences of the United States of America*, **106**,  
520 1479–1483.
- 521 Cogné N, Gallagher K, Cobbold PR, Riccomini C, Gautheron C (2012) Post-breakup tectonics  
522 in southeast Brazil from thermochronological data and combined inverse-forward thermal  
523 history modeling. *Journal of Geophysical Research*, **117**, B11413.
- 524 Excoffier L, Dupanloup I, Huerta-Sánchez E, Sousa VC, Foll M (2013) Robust  
525 Demographic Inference from Genomic and SNP Data. *PLoS Genetics*, **9**.
- 526 Faircloth BC, McCormack JE, Crawford NG, Harvey MG, Brumfield RT, Glenn TC (2012)  
527 Ultraconserved elements anchor thousands of genetic markers spanning multiple  
528 evolutionary timescales. *Systematic Biology*, **61**, 717–726.
- 529 Faircloth BC (2014) Illumiprocessor: a Trimmomatic wrapper for parallel adapter and quality  
530 trimming. <https://github.com/faircloth-lab/illumiprocessor>.
- 531 Faircloth BC (2014) Phyluce: phylogenetic estimation from ultraconserved elements.

- 532 <https://github.com/faircloth-lab/phyluce>.
- 533 Fjeldså, J. (1994). Geographical patterns for relict and young species of birds in Africa and  
534 South America and implications for conservation priorities. *Biodiversity & Conservation*,  
535 **3**, 207–226.
- 536 Fjeldså J, Lambin E, Mertens B (1999). Correlation between Endemism and Local Ecoclimatic  
537 Stability Documented by Comparing Andean Bird Distributions and Remotely Sensed  
538 Land Surface Data. *Ecography*, **22**, 63–78.
- 539 Fjeldså J, Bowie RCK, Rahbek C (2012). The Role of Mountain Ranges in the Diversification  
540 of Birds. *Annual Review of Ecology, Evolution, and Systematics*. **43**, 249-265.
- 541 Franco-Magalhaes AOB, Cuglieri MAA, Hackspacher PC, Saad AR (2014) Long-term  
542 landscape evolution and post-rift reactivation in the southeastern Brazilian passive  
543 continental margin: Taubaté basin. *International Journal of Earth Science*, **103**, 441–453.
- 544 Françoso E, Zuntini AR, Carnaval AC, Arias MC (2016). Comparative phylogeography in the  
545 Atlantic forest and Brazilian savannas: pleistocene fluctuations and dispersal shape spatial  
546 patterns in two bumblebees. *BMC Evolutionary Biology*, 16(1), 267.
- 547 Freitas, RO (1951) Relevos policíclicos na tectônica do escudo Brasileiro. *Boletim Paulista*  
548 *Geociências*, **7**, 3–19.
- 549 Frichot E, Mathieu F, Trouillon T, Bouchard G, François O (2014) Fast and efficient  
550 estimation of individual ancestry coefficients. *Genetics*, **196**, 973–983.
- 551 Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological  
552 association studies. *Methods in Ecology and Evolution*, **6**, 925–929.
- 553 Firkowski CR, Bornschein MR, Ribeiro LF, Pie MR (2016). Species delimitation, phylogeny  
554 and evolutionary demography of co-distributed, montane frogs in the southern Brazilian  
555 Atlantic Forest. *Molecular Phylogenetics and Evolution*, **100**, 345–360.
- 556 Fjeldså J, Lambin E, Mertens B (1999). Correlation between Endemism and Local Ecoclimatic  
557 Stability Documented by Comparing Andean Bird Distributions and Remotely Sensed  
558 Land Surface Data. *Ecography*, **22**, 63–78.
- 559 Graham CH, Carnaval AC, Cadena CD, Zamudio KR, Roberts TE, Parra JL, McCain CM,  
560 Bowie RC, Moritz C, Baines SB, Schneider CJ, VanDerWal J, Rahbek C, Kozak KH,  
561 Sanders NJ (2014) The origin and maintenance of montane diversity: Integrating  
562 evolutionary and ecological processes. *Ecography*, **37**, 711–719.
- 563 Gutenkunst RN, Hernandez RD, Williamson S H, Bustamante CD (2009). Inferring the  
564 Joint Demographic History of Multiple Populations from Multidimensional SNP

- 565 Frequency Data. *PLOS Genetics*, 5(10), e1000695.
- 566 Hackspacher PC, Ribeiro LFB, Ribeiro MCS, Fetter AH, Hadler Neto JC, Tello Saenz CA,  
567 Dantas EL (2004) Consolidation and break-up of the South American platform in  
568 Southeastern Brazil: tectonothermal and denudation histories. *Gondwana Research*, 7,  
569 91–101.
- 570 Hooghiemstra H, Van der Hammen T (2004) Quaternary ice-age dynamics in the Colombian  
571 Andes: developing an understanding of our legacy. *Philosophical Transactions of the*  
572 *Royal Society B: Biological Sciences*, 359, 173–181.
- 573 Karl M, Glasmacher UA, Kollenz S, Franco-Magalhaes AOE, Stockli DF, Hackspacher PC  
574 (2013) Evolution of the South Atlantic passive continental margin in southern Brazil  
575 derived from zircon and apatite (U–Th–Sm)/He and fission-track data. *Tectonophysics*,  
576 604, 224–244.
- 577 Knowles LL, Massatti R. (2016). Distributional shifts – not geographic isolation – as a  
578 probable driver of montane species divergence. *Ecography*, 40, 1475–1485.
- 579 Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a  
580 new method for the analysis of genetically structured populations. *BMC genetics*, 11, 94.
- 581 Ledru M-P, Mourguiart P, Riccomini C (2009) Related changes in biodiversity, insolation  
582 and climate in the Atlantic rainforest since the last interglacial. *Palaeogeography*,  
583 *Palaeoclimatology, Palaeoecology*, 271, 140–152
- 584 Leigh JW, Bryant D. (2015). popart: full-feature software for haplotype network construction.  
585 *Methods in Ecology and Evolution*, 6, 1110–1116.
- 586 Li H (2013) Aligning sequence reads, clone sequences and assembly contigs with  
587 BWA-MEM. *arXiv preprint arXiv:1303.3997*.
- 588 Mastretta-Yanes A, Xue AT, Moreno-Letelier A, Jorgensen TH, Alvarez N, Piñero D,  
589 Emerson BC (2018). Long-term in situ persistence of biodiversity in tropical sky islands  
590 revealed by landscape genomics. *Molecular Ecology*, 27, 432–448.
- 591 Maldonado-Coelho M (2012) Climatic oscillations shape the phylogeographical structure of  
592 Atlantic Forest fire-eye antbirds (Aves: Thamnophilidae). *Biological Journal of the*  
593 *Linnean Society*, 105, 900–924.
- 594 Mayr E, Diamond JM (1976) Birds on islands in the sky: Origin of the montane avifauna of  
595 Northern Melanesia. *Proceedings of the National Academy of Sciences of the United*  
596 *States of America*, 73, 1765–1769.
- 597 McCormack JE, Huang H, Knowles LL (2009) Sky islands. *Encyclopedia of Islands*, 4,  
598 841–843.

- 599 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K,  
600 Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The genome analysis toolkit: A  
601 MapReduce framework for analyzing next-generation DNA sequencing data. *Genome*  
602 *Research*, **20**, 1297–1303.
- 603 Meisling KE, Cobbold PR, Mount VS (2001) Segmentation of an obliquely rifted margin,  
604 Campos and Santos basins, southeastern Brazil. *AAPG Bulletin*, **85**, 1903–1924.
- 605 Melo MS, Riccomini C, Hasui Y, Almeida FFMA, Coimbra AM (1985) Geologia e evolução  
606 do sistema de bacias tafrogênicas continentais do sudeste do Brasil. *Revista Brasileira de*  
607 *Geociências*, **15**, 193–201.
- 608 Modenesi-Gauttieri MC, Hiruma ST, Riccomini C (2002) Morphotectonics of a high plateau  
609 on the northwestern flank of the Continental Rift of southeastern Brazil *Geomorphology*,  
610 **43**, 257–271.
- 611 Moritz C, Patton JL, Conroy CJ, Parra JL, White GC, Beissinger SR (2008) Impact of a century  
612 of climate change on small-mammal communities in Yosemite National Park, USA.  
613 *Science (New York, N.Y.)*, **322**, 261–264.
- 614 Nadachowska-Brzyska K, Li C, Smeds L, Zhang G, Ellegren H (2015). Temporal Dynamics of  
615 Avian Populations during Pleistocene Revealed by Whole-Genome Sequences. *Current*  
616 *Biology*, **25**, 1375–1380.
- 617 Oliveira MAT, Behling H, Pessenda LCR (2008) Late-Pleistocene and midHolocene  
618 environmental changes in highland valley head areas of Santa Catarina State, Southern  
619 Brazil. *Journal of South American Earth Sciences*, **26**, 55-67.
- 620 Oliveira MAT, Porsani JL, Lima GL, Jeske-Pieruschka V, Behling H (2012) Late Pleistocene  
621 to Holocene peatland evolution in southern Brazilian highlands as depicted by radar  
622 stratigraphy, sedimentology and palynology. *Quaternary Research*, **77**, 397-407.
- 623 Patton JL, Smith MF (1992) mtDNA phylogeny of Andean mice: a test of diversification  
624 across ecological gradients. *Evolution* **46**, 174–183.
- 625 Peres EA, Sobral-Souza T, Perez MF, Bonatelli IA, Silva DP, Silva MJ, Solferini VN (2015)  
626 Pleistocene niche stability and lineage diversification in the subtropical spider *Araneus*  
627 *omnicolor* (Araneidae). *PloS one*, **10**, e0121543.
- 628 Pie MR, Faircloth BC, Ribeiro LF, Bornschein MR, McCormack JE (2018). Phylogenomics of  
629 montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands  
630 followed by climatic stability. *Biological Journal of the Linnean Society*, **125**, 72–82.
- 631 Rodrigues MT, Cassimiro J, Pavan D, Curcio FF, Verdade VK, Pellegrino KCM (2009). A  
632 New Genus of Microteiid Lizard from the Caparaó Mountains, Southeastern Brazil, with a

633 Discussion of Relationships among Gymnophthalminae (Squamata). *American Museum*  
634 *Novitates*, **3673**, 1–27.

635 Stotz, D. F., Fitzpatrick, J. W., Parker III, T. A., & Moskovits, D. K. (1996). Neotropical birds:  
636 ecology and conservation. University of Chicago Press.

637 Tello Saenz CA, Hackspacher PC, Hadler Neto JC, Iunes PJ, Guedes S, Paulo SR, Ribeiro LFB  
638 (2003) Recognition of cretaceous, paleocene and neogene tectonic reactivation, through  
639 apatite fission-track analysis, in Precambrian areas of the Southeast Brazil: association  
640 with the South Atlantic Ocean opening. *Journal of South American Earth Science*, **15**,  
641 137–142.

642 Thomé MTC, Zamudio KR, Haddad CFB, Alexandrino J (2014) Barriers, rather than  
643 refugia, underlie the origin of diversity in toads endemic to the Brazilian Atlantic Forest.  
644 *Molecular Ecology*, **23**, 6152–6164.

645 Thomé MT, Zamudio KR, Giovanelli JG, Haddad CF, Baldissera FA Jr, Alexandrino J (2010)  
646 Phylogeography of endemic toads and post-Pliocene persistence of the Brazilian Atlantic  
647 Forest. *Molecular Phylogenetics and Evolution*, **55**, 1018–1031.

648 Winger BM, Bates JM (2015) The tempo of trait divergence in geographic isolation: avian  
649 speciation across the Marañón valley of Peru. *Evolution*, **69**, 772–782.

650 Zamudio KR, Bell RC, Mason NA (2016). Phenotypes in phylogeography: Species' traits,  
651 environmental variation, and vertebrate diversification. *Proceedings of the National*  
652 *Academy of Sciences of the United States of America*, **113**, 8041–8048.

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666 **Figures and tables**

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

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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
<b>polytomy_gene_flow</b>	<b>-969,301</b>	<b>11</b>	<b>4485,80</b>	<b>7,68E-01</b>
(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

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680 **Table 2.** Parameter values of simulated models with the best likelihood in Fastsimcoal2. The  
 681 name of the models represent the topology, in newick format and the demographic syndromes  
 682 tested (see Figure 3). In bold: best model.

Model	Ne					NMc		NM		NM	
	Ne cp	Ne ml	mc	Ne a1	a2	p/ml	p	p/mc	c/cp	ml/c	ml/m
				5425							
(cp(mc,ml)_no_gene_flow	7862	17744	36326	5	1610	n/a	n/a	n/a	n/a	n/a	n/a
				1858							
(mc(ml,cp)_no_gene_flow	5249	32714	75535	93	5687	n/a	n/a	n/a	n/a	n/a	n/a
(ml(mc,cp)_no_gene_flow	4485	23123	44924	9689	7660	n/a	n/a	n/a	n/a	n/a	n/a
polytomy_no_gene_flow	4125	17300	33859	n/a	9138	n/a	n/a	n/a	n/a	n/a	n/a
(cp(mc,ml)_gene_flow	7562	20728	38539	8463	6663	0,36	1,70	0,67	0,01	2,35	
(ml(mc,cp)_gene_flow	7254	23247	47682	6049	7555	0,52	1,44	0,08	0,62	3,70	
				1127							
(mc(ml,cp)_gene_flow	9519	40571	61488	3	5941	0,21	1,68	0,15	0,47	3,98	
<b>polytomy_gene_flow</b>	<b>3866</b>	<b>10944</b>	<b>18842</b>	<b>n/a</b>	<b>4552</b>	<b>0,32</b>	<b>1,15</b>	<b>0,49</b>	<b>0,98</b>	<b>3,15</b>	
(cp(mc,ml)_bottleneck_gene_flow	8065	22007	51902	8229	2018	0,48	1,06	0,38	0,48	3,01	
(mc(ml,cp)_bottleneck_gene_flow	7305	22418	41563	4746	2553	0,48	0,18	0,45	0,52	3,43	
(ml(mc,cp)_bottleneck_gene_flow	6053	18163	41631	6229	5336	0,38	0,63	0,43	0,01	2,74	
polytomy_bottleneck_gene_flow	4161	14562	30245	n/a	3160	0,41	0,02	0,35	0,01	3,78	
<b>polytomy_gene_flow lo</b>											
<b>95% CI</b>	<b>1243</b>	<b>7319</b>	<b>20345</b>	<b>n/a</b>	<b>7839</b>	<b>0,19</b>	<b>0,02</b>	<b>0,22</b>	<b>0,00</b>	<b>1,77</b>	
<b>polytomy_gene_flow up</b>					<b>4163</b>						
<b>95% CI</b>	<b>4337</b>	<b>19877</b>	<b>40176</b>	<b>n/a</b>	<b>5</b>	<b>0,78</b>	<b>0,84</b>	<b>1,55</b>	<b>0,56</b>	<b>2,95</b>	

<b>Model</b>	<b>NMm c/ml</b>	<b>TDIV 1</b>	<b>TDIV 2</b>	<b>SCcp</b>	<b>SCm l</b>	<b>SCm c</b>	<b>TSC cp</b>	<b>TSC ml</b>	<b>TSC mc</b>
(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
<b>polytomy_gene_flow</b>	<b>2,65</b>	<b>n/a</b>	<b>8742</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>
(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
<b>polytomy_gene_flow lo 95% CI</b>	<b>0,68</b>	<b>n/a</b>	<b>10140</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>
<b>polytomy_gene_flow up 95% CI</b>	<b>4,25</b>	<b>n/a</b>	<b>47404</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>

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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

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

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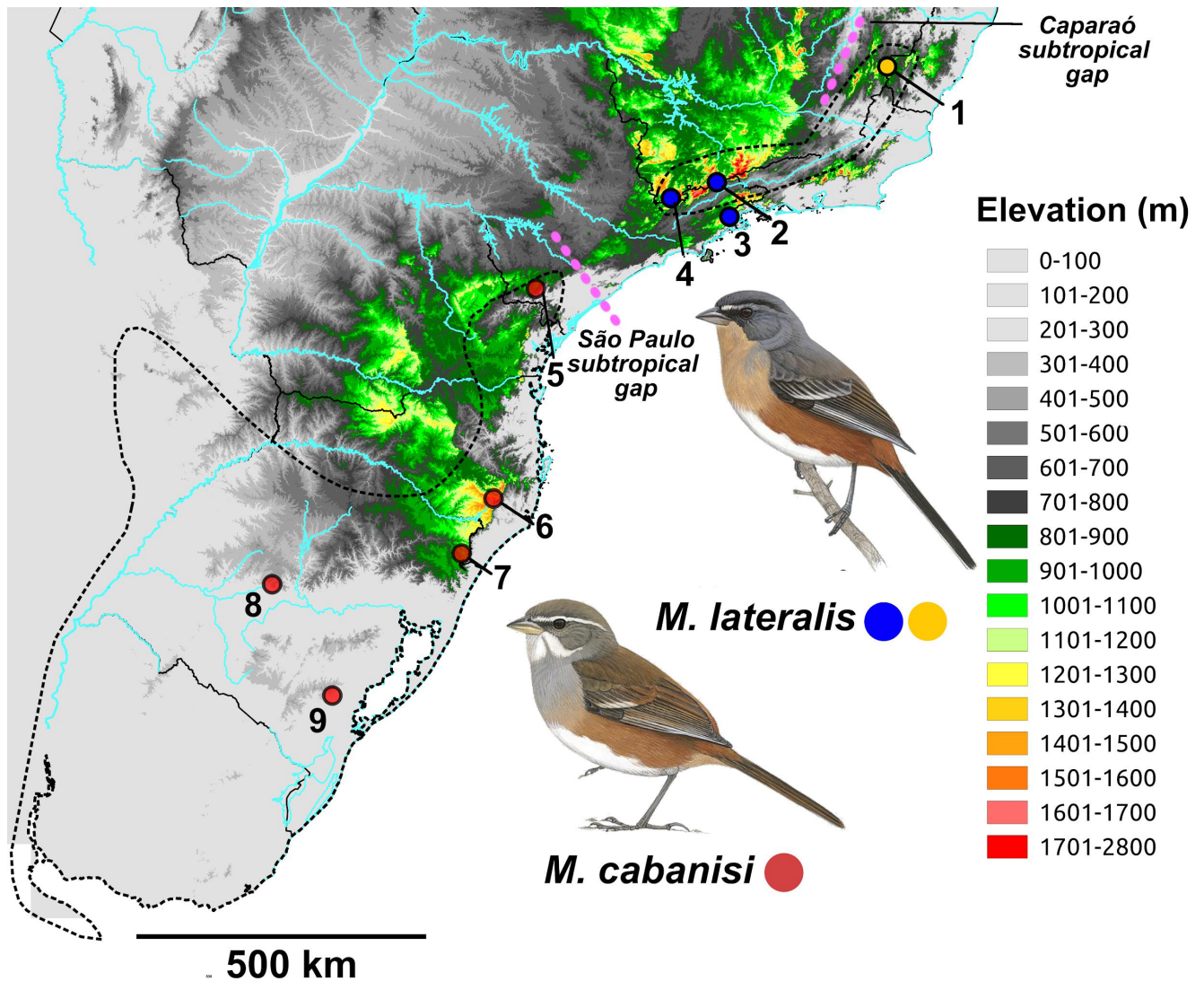
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707 **Figure 1.** Localities sampled. Blue and yellow circles represent *Microspingus lateralis* while  
708 red circles represent *Microspingus cabanisi*. Major highlands include Caparaó (1), Northern  
709 Serra do Mar (2), Mantiqueira (3 and 4),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

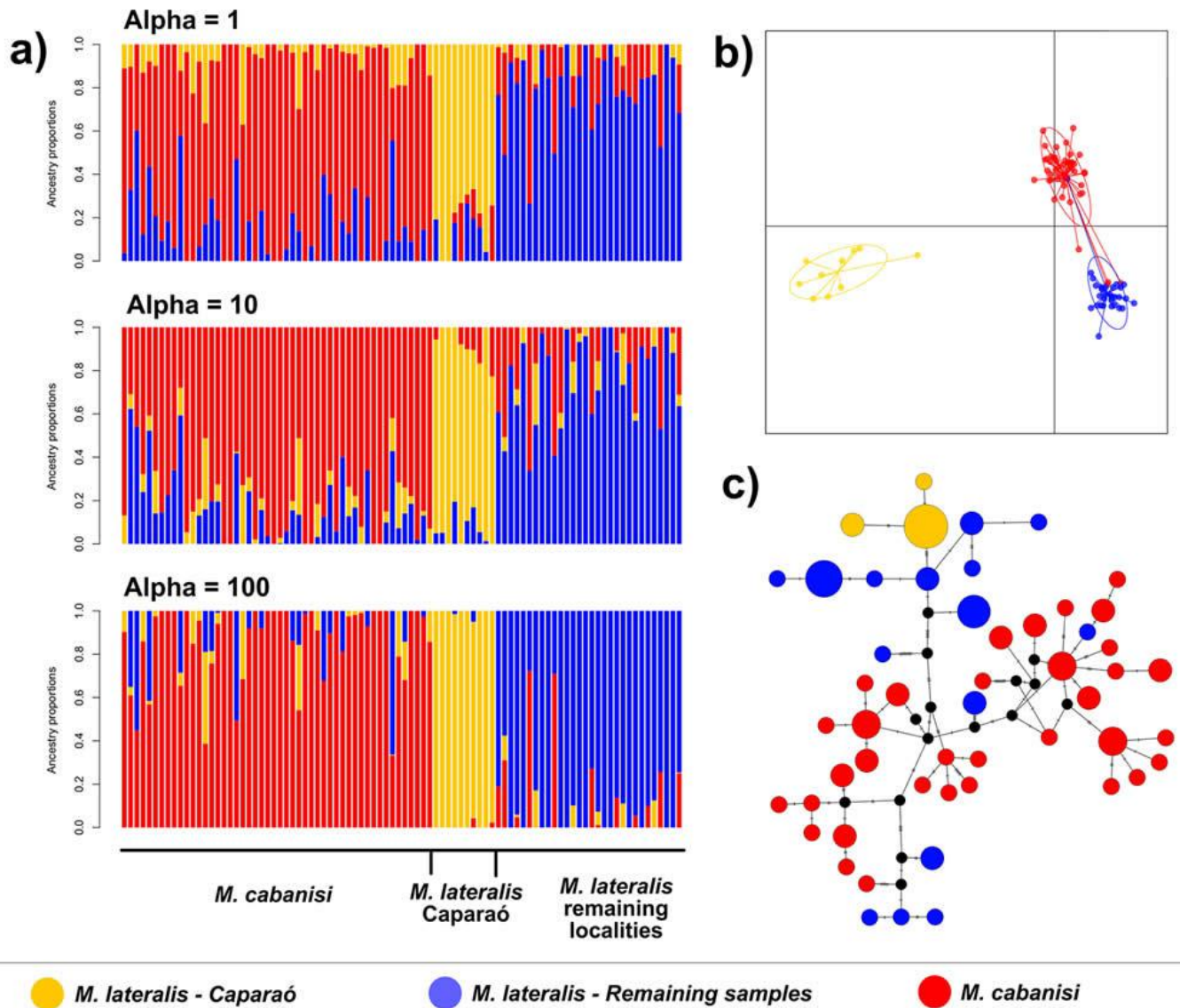
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720 **Figure 2.** Plots depicting population structure according to (a) the best fitting sNMF model

721  $K=3$  based on different values of the regularization parameter alpha (1, 10, 100) and (b) the

722 three clusters inferred with DAPC and (c) cytochrome-b median-joining network. Black dots in

723 the haplotype network indicate inferred haplotypes.

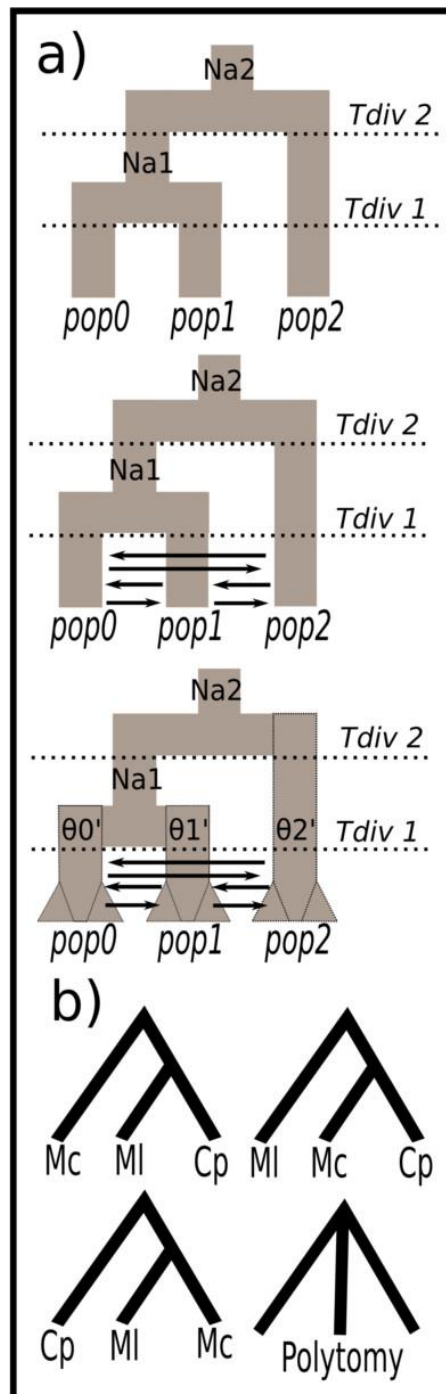
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730 **Figure 3.** Demographic models simulated in Fastsimcoal2 for (a) three populations and (b)

731 alternative topologies tested under these demographic scenarios, in a total of 12 simulated

732 models. Na – ancestral effective population size;  $\theta'$  – effective population size before

733 population size change; Tdiv – divergence time; Mc – *Microspingus cabanisi*; MI –

734 *Microspingus lateralis*; Cp - Caparaó population of *Microspingus lateralis*

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736 **Supplementary Information**

737 **Table S1.** Samples used in the present study.

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

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



FRA76	<i>Poospiza cabanisi</i>	8	Santa Maria	-29,5974	-53,7006	pending	pending
FRA77	<i>Poospiza cabanisi</i>	8	Itaara	-29,5896	-53,7934	pending	pending
FRA78	<i>Poospiza cabanisi</i>	8	Itaara	-29,5920	-53,7521	pending	pending
FRA80	<i>Poospiza cabanisi</i>	8	Itaara	-29,5892	-53,7933	pending	pending
FRA81	<i>Poospiza cabanisi</i>	8	Itaara	-29,5929	-53,7598	pending	pending
FRA82	<i>Poospiza cabanisi</i>	8	Itaara	-29,5929	-53,7598	pending	pending
FRA84	<i>Poospiza cabanisi</i>	8	Itaara	-29,5937	-53,7343	pending	pending
FRA85	<i>Poospiza cabanisi</i>	8	Itaara	-29,5819	-53,8062	pending	pending
FRA61	<i>Poospiza cabanisi</i>	9	Pelotas	-31,6277	-52,5227	pending	pending
FRA62	<i>Poospiza cabanisi</i>	9	Pelotas	-31,6088	-52,5188	pending	pending
FRA64	<i>Poospiza cabanisi</i>	9	Pelotas	-31,6317	-52,5416	pending	pending
FRA65	<i>Poospiza cabanisi</i>	9	Pelotas	-31,6071	-52,5486	pending	pending
FRA66	<i>Poospiza cabanisi</i>	9	Pelotas	-31,6071	-52,5486	pending	pending
FRA67	<i>Poospiza cabanisi</i>	9	Pelotas	-31,5265	-52,5505	pending	pending
FRA68	<i>Poospiza 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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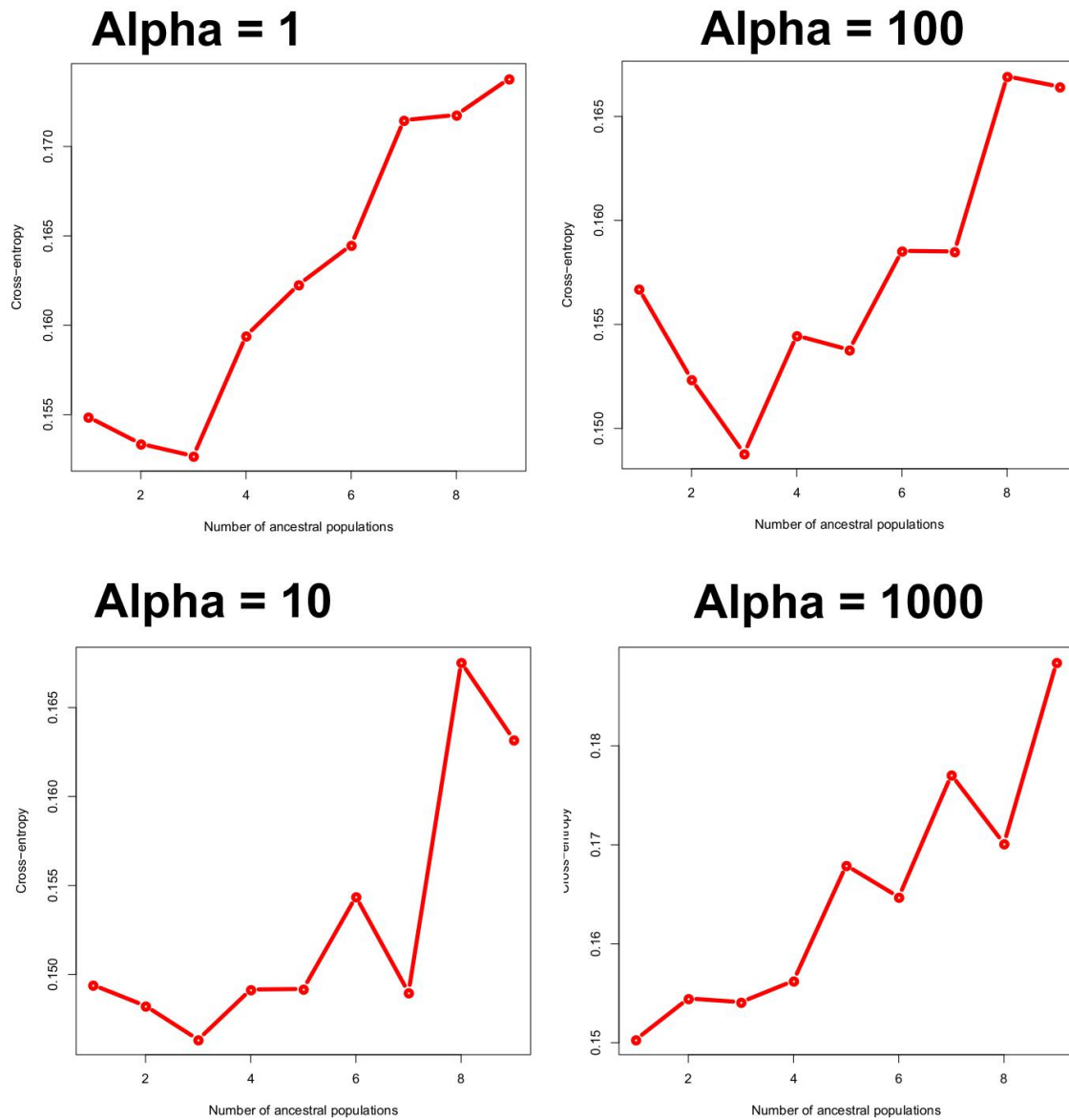
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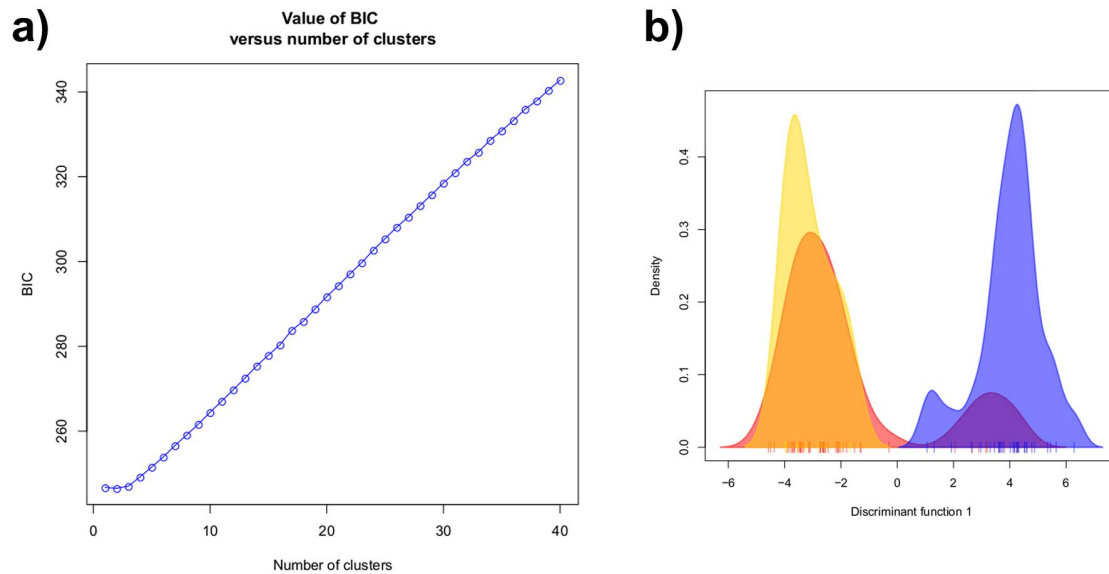
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769 **Figure S1.** Cross-entropy sNMF runs for alpha values of 1, 10, 100 and 1,000.

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774 **Figure S2.** BIC values of (a) K-clustering method and (b) DAPC plot for two populations.

775 Colors in the DAPC plot follow the ones assumed for Figures 1 and 2.

#### 776 **Data Accessibility**

777 Dryad XXXX and SRA XXXX

778

779 Raw Illumina files, Set of SNPs, Joint SFS

780

#### 781 **Author contributions**

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783 FRA, DFA and GT designed the study. FRA, JACM and GT performed field work. FRA,

784 DFAS and GT performed data analysis. FRA, KCMP, CYM and MJH contributed funds for lab

785 or field work. All authors participated in the discussion of the results. FRA, MMC and GT

786 wrote the manuscript with input from the other authors. All authors approved the final version

787 of the manuscript.