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## POPULATION GENETIC STRUCTURE OF THE ATLANTIC FOREST ENDEMIC CONOPOPHAGA LINEATA (PASSERIFORMES: CONOPOPHAGIDAE) BASED ON NUCLEAR SEQUENCE REVEALS A CONTACT ZONE IN THE ATLANTIC FOREST

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ENDEMIC *CONOPOPHAGA LINEATA* (PASSERIFORMES:  
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CONTACT ZONE IN THE ATLANTIC FOREST**

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**ABSTRACT**

The Rufous Gnateater, *Conophaga lineata*, is a small, insectivorous understory bird, endemic to and widely distributed in tropical and subtropical Atlantic Forest in South America. The Rufous Gnateater's distribution makes it ideally suited for testing two major hypotheses for the origin of biodiversity, the riverine barrier and forest refugia hypotheses. A previous study using Rufous Gnateater mitochondrial DNA data showed a phylogeographic break in northern São Paulo state (SP), close to the valley of Rio Paraíba do Sul. Here, we sequenced a nuclear marker for some of the same individuals previously analyzed and obtained a similar population genetic structure: two clades and a phylogeographic break in northern SP. We consistently detected signals of demographic expansion for both clades. The estimated date of this expansion is the Pleistocene (250,000 years ago), providing support for the forest refugia hypothesis as a potential driver of Rufous Gnateater diversification. We also found evidence of gene flow between the two clades, with a contact zone occurring in the states of Minas Gerais and SP.

Key-Words: Atlantic Forest, nuclear intron, passeriformes, refugia, Pleistocene, secondary contact.

## INTRODUCTION

The Rufous Gnateater (*Conopophaga lineata*) (Conopophagidae: Passeriformes) (Weid 1831) is a small, insectivorous understory bird. This species is distributed in tropical and subtropical forests of South America (Sigrist 2005), from Paraguay and northeast Argentina to northeast Brazil (Sick 1997; Whitney 2003). Despite its large distribution, and remarkable geographical variation in song and plumage (Whitney 2003), few studies have investigated this species (Willis et al. 1983; Dantas et al. 2007; Dantas et al. 2009). Three subspecies are recognized: *C. l. cearae* is found in northeastern Brazil, from Ceará to Pernambuco; *C. l. lineata* occurs from Pernambuco to southern Bahia, Central Brazil, in southern Goiás and northern Mato Grosso do Sul; and *C. l. vulgaris* is found in southeastern Brazil, from southern Espírito Santo to Rio Grande do Sul; eastern Paraguay, Misiones and eastern Uruguay (Whitney 2003). A phylogeny of the genus *Conopophaga* based on mitochondrial data indicated that *C. l. cearae* did not form a monophyletic clade with the other two subspecies (*C. l. lineata* and *C. l. vulgaris*; Pessoa 2007). A phylogeographic study of *C. l. lineata* and *C. l. vulgaris* based on mitochondrial data resulted in four groups that included individuals from: 1) a northern clade that included south Bahia and northeast of Minas Gerais (clade BA); 2) a clade including Minas Gerais, Rio de Janeiro, and northeast of São Paulo (Clade MG); 3) a clade including São Paulo and Paraná (Clade SP); and 4) a clade including individuals from Rio Grande do Sul to Misiones, Argentina (Clade RS) (Pessoa 2007). Sari (2005) described one phylogeographic break in northern São Paulo State and southern Minas Gerais. However, in southern Minas Gerais and northern São Paulo, Sari (2005) and Pessoa (2007) found individuals from two clades, from the clade MG and clade SP, suggesting that this region should be a secondary contact zone between populations that expanded after being isolated and evolving allopatrically. This break is concordant with the Rio Paraíba do Sul valley, the path of a river that originates in Serra da Bocaina, north of São Paulo, and flows into Rio de Janeiro.

*C. lineata* is an Atlantic Forest endemic species, representing a good model for evolutionary studies in this biome. The Atlantic Forest is distributed along eastern Brazil, eastern Paraguay, and northeastern Argentina (Gusmão Câmara 2003). This biome is considered one of the most important areas for conservation in the world, due to its high biodiversity, high levels of endemism, and degradation (Ribeiro et al. 2009). Although only approximately 11% of the primary forest still remains, in a highly fragmented state (Ribeiro et al. 2009), many species are still being described in this area (Weber et al. 2005; Miranda et al. 2006). Its biota is the result of a complex evolutionary history, but the processes shaping it are

poorly understood (Mustrangi and Patton 1997; Costa et al. 2000; Geise et al. 2001; Pellegrino et al. 2005). The evolutionary processes involved in the formation of the Atlantic Forest can be indirectly studied based on phylogeographic analyses of endemic species, also important for conservation purposes (Moritz 2002). Knowledge of the range limits of lineages and species is fundamental for effective conserving biodiversity.

There are several hypotheses for the origin of extreme biodiversity. Among them is the riverine barriers model (Wallace 1852) and the Pleistocene refuge model (Haffer 1969; Vanzolini and Williams 1970). The riverine barrier hypothesis (Wallace 1852) predicts that some rivers limit regional faunas and therefore may represent gene flow barriers, reshaping much of the biota distribution. In the Atlantic Forest, the Doce river (Costa et al. 2000; Silva et al. 2004; Brown 2005), Jequitinhonha river (Lacerda et al. 2007), São Francisco and Paraíba do Sul rivers (Silva and Straube 1996; Pellegrino et al. 2005) may be barriers to dispersal of many animals. Additionally, the tectonic activity associated with the formation of geographical landmarks could also have influenced the distribution of biodiversity, mainly in the southern Atlantic Forest, where complex relief exists with many mountain ranges and valleys, as in the region of Serra do Mar (Petri and Fulfaro 1983; Riccomini et al. 1989; Suguio et al. 2005; Cabanne et al. 2008).

Isolation in forest refugia during the climatic change cycles of the Pleistocene may also help explain speciation, mainly in the Amazon basin (Haffer 1969; Vanzolini and Williams 1970; Brown and Ab'Saber 1979; Haffer and Prance 2001). The Pleistocene refuge hypothesis proposes that during glacial maxima, rainforests were reduced to patches (refugia) isolated by open areas, and organisms isolated in these refugia diverged into new species by drift and/or divergent selection. In the following interglacial period, the forest expanded, resulting in secondary contact among the now reproductively-isolated new clades. Brown and Ab'Saber (1979) proposed that open areas dominated the current Atlantic Forest's landscape during the maximum of Late Pleistocene glaciations, suggesting that the refuge theory could be important to understand the biological diversification of the biome.

A paleoclimate model for the distribution of the Atlantic Forest predicted that at glacial maximum during the Pleistocene there were two large and stable forest refugia, one in the state of Pernambuco, north of the Atlantic Forest, and another in Bahia (Carnaval and Moritz 2008). According to this model, throughout the Pleistocene, the forest was less stable in the south of the Atlantic Forest, south of Rio Doce, where several smaller refuge forests were predicted, than in the north. This model is partially congruent with phylogeographic or population genetic data from Passeriformes (Cabanne et al.

2007, 2008; d'Horta et al. 2011; Maldonado-Coelho 2012), bats (Pavan et al. 2011), lizards (Pellegrino et al. 2005), and frogs (Carnaval et al. 2009; Amaro et al. 2012).

In this context, the present study aims to investigate a diversity break found in northeastern São Paulo and southeastern Minas Gerais for *C. lineata* populations, detected with mtDNA data by Sari (2005) and Pessoa (2007). To achieve this objective, we analyzed data from a nuclear intron (FIB5) in order to test whether the formation of Paraíba Valley or a Pleistocene refuge could be responsible for the patterns found with mitochondrial markers. We tested the following predictions: first, if the lineages found by Sari (2005) and Pessoa (2007) are the result of barriers promoted by rivers, the estimated date of clade separation should be concordant with the formation of the Paraíba Valley, which began in the Miocene-Pliocene (approximately 15 million years ago, Mya) and lasted until the Early Pleistocene (Petri and Fulfaro 1983). Alternatively, if these two clades are the result of vicariance during Pleistocene cycles of glaciations that generated forest refugia, the estimated date of clade separation should be more recent, observed through shallow levels of DNA divergence and a signal of demographic expansion since the last glacial maximum (LGM), about 21,000 years ago.

## **METHODOLOGY**

### *Study area and samples*

Samples (blood or muscle,  $N=146$ , Supplementary Material) were collected between 1999 and 2009 in the Brazilian states of Minas Gerais and São Paulo (Fig. 1). Blood was collected (approximately 0.1 mL) with insulin syringes from the largest vein in the right cervical region and muscle was obtained from specimens, which were deposited at the Museu de Zoologia da Universidade de São Paulo and the Taxonomic Collection Center at the Universidade Federal de Minas Gerais. Tissue samples are deposited at the Laboratório de Biodiversidade e Evolução Molecular (Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil), or at the Laboratório de Genética e Evolução Molecular de Aves (Instituto de Biociências, Universidade de São Paulo, Brazil). Total DNA was obtained from blood or muscle samples by a conventional proteinase K–SDS digestion, organic extraction with phenol–chloroform, and ethanol precipitation (Bruford et al. 1992).

### *Nuclear sequences*

The nuclear intron 5 from the  $\beta$ -Fibrinogen gene was amplified with the primers FIB5 and FIB6 (Marini and Hackett 2002). The PCR reaction (10  $\mu$ l) contained 20–40 ng of total DNA, 1X of *Taq*

buffer, 200  $\mu$ M of each dNTP, 0.5  $\mu$ M of each primer, and 0.5 U of *Taq* polymerase. Amplifications were performed with an initial step at 95 °C for 4 min and 37 cycles of 45 s at 94 °C, 45 s at 53.5 °C, and 1 min at 72 °C, followed by a final extension of 10 min at 72 °C.

PCR products were cleaned by precipitation using 20% Polyethyleneglicol with 2.5 M NaCl. The sequences were obtained in a MegaBACE 1000 sequencer (Amersham-Biosciences). The alignments were made by eye in Bioedit version 5.06 (Hall 2001). The heterozygous nucleotide positions were identified by high quality double peaks in the electropherograms. All resolved positions received a posterior probability of one in the phasing analyses. To identify both haplotypes of heterozygous individuals with multiple variable sites, we used a Bayesian approach in the program PHASE 2.0 (Stephens et al. 2001; Stephens and Donnelly 2003), implemented in DNAsp 5.1 software (Librado and Rozas 2009).

#### *Analytical Methods*

Descriptive analyses including haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) were done in DNAsp v.5.10.01 (Librado and Rozas 2009). We used the Network software version 4.6 ([www.fluxus-technology.com](http://www.fluxus-technology.com)) to obtain the network for intron 5 from the  $\beta$ -Fibrinogen gene (410 bp) with a median joining method (Bandelt et al. 1999; Polsin and Daneschmand 2003). Additionally, we calculated Tajima's (1989), Fu's (1993) and Ramos-Onsins and Rozas  $R^2$  statistics neutral deviation parameters with DNAsp v.5.10.01 (Librado and Rozas 2009). We selected these test statistics due to their power to detect population expansion scenarios in specific sampling conditions and with specified population expansion rate, time since the expansion, sample size and number of segregation sites (Ramos-Onsins and Rozas 2002). We used the likelihood ratio test as implemented in the software Modeltest, version 3.7 (Posada and Crandall, 1998), to select the evolutionary model that best fit FIB5 (JC) with a proportion of invariable sites (I) of 0.352 and a discrete gamma distribution ( $\alpha = 0.3860$ ).

In addition, to analyze population size dynamics through time, we used the Extended Bayesian Skyline Plot method (EBSP; Heled and Drummond 2008) implemented in BEAST 1.6.1 (Drummond and Rambaut 2007). We reconstructed the phylogenies with a Bayesian approach implemented in BEAST (Drummond and Rambaut 2007). We performed four independent runs of 10,000,000 steps, logged every 2,000 steps, and burn-in of 1,000,000 runs. To evaluate the convergence of parameters between runs and the performance of analysis (ESS values  $> 200$ ), we used TRACER 1.5 (<http://beast.bio.ed.ac.uk/Tracer>) (Drummond and Rambaut 2007). To check the level of population genetic structure among localities, we

performed an analysis of molecular variance (AMOVA) with two hierarchical levels using ARLEQUIN 3.5 (Excoffier et al. 2010). Additionally, we performed a Bayesian analysis of population structure, using sequences of FIB5, in BAPS 5.4 (The Bayesian Analysis of Population Structure, Corander et al. 2007). BAPS estimates hidden population substructure by clustering sampled populations into panmictic groups. The analysis is performed considering each individual and the probability it fits in each cluster, uninformed by the population origin of the individual. We tested the mixture analysis with 2 through 20 populations, with the population allele frequencies and structure parameters automatically set to be uninformative. This produced a joint posterior distribution of partitions of the sampled populations into panmictic groups, along with their respective allele frequencies. We ran BAPS 10 times for  $10^5$  interactions after a burn-in period of 20,000 steps. The resulting partitions were averaged based on their plotted posterior probabilities.

We performed divergence time estimates between pairs of *C. lineata* lineages with Isolation\_Migration software (IM) (Nielsen and Wakeley 2001; Hey and Nielsen 2004). We then estimated the effective population sizes for current ( $\theta_1$ ,  $\theta_2$ ) and ancestral ( $\theta_a$ ) populations, the divergence time between lineages, and migration between populations ( $m_1$ ,  $m_2$ ) using the IM model. To define the priors, we carried out initial runs followed by five posterior runs for each analysis, applied a burn-in of 2,000,000 steps, and finished with at least 20,000,000 steps in the chain after the burn-in stage and a minimum effective sample size value of 50. To obtain the divergence times in years, we considered  $t = t_u$  (Hey and Nielsen 2004). To calculate the dates, we assumed a divergence of 0.53% per million years for FIB5 (Axelsson et al. 2004; Cabanne et al. 2008).

## RESULTS

We obtained FIB5 sequences (410 bp) from 120 individuals of *C. lineata* (Will be submitted the sequences to GenBank after accepted the manuscript). There were no indels and no evidence of recombination. We identified 80 haplotypes and 70 polymorphic sites were identified, showing high genetic diversity (haplotype diversity  $h = 0.929$  and nucleotide diversity  $\pi = 0.00942$ ,  $\theta_s = 10.47$ ) (Table 1, Fig. 2).

### *Demographic history*

The neutrality test for intron 5 of the  $\beta$ -Fibrinogen gene showed a significant bias, with  $F_s = -75.52$  ( $p = 0.00$ ); Tajima  $D = -1.91$  ( $p = 0.001$ );  $R_2 = 0.0301$  ( $p = 0.010$ ) and raggedness statistic  $r =$



0.0141 ( $p = 0.033$ ), suggesting a recent demographic expansion scenario. This outcome is congruent with the mismatch distribution that showed a wave signal, which is consistent with population expansion (estimated initial theta ( $\theta_0$ ) 1.45 and tau 2.36) (Fig. 3). The haplotype network showed two clades, one corresponding to Minas Gerais and Bahia states (MG clade) and another to São Paulo state (SP clade) (Appendix I). The two clades show star shapes, indicating population expansion, with one abundant haplotype and several haplotypes at low frequencies (Fig. 2). This star-like network configuration corroborated the neutrality test results (Tajima  $D$ ,  $F_s$ ,  $R_2$ ). The skyline plot analyses we generated to reconstruct the effective population size through time also indicated population expansion starting at 250,000 years ago for FIB5 (Fig. 4).

#### *Phylogeography, date estimates and population structure*

The haplotype network based on FIB5 sequences showed clear genetic structure in *C. lineata*. This structure is concordant with a phylogeographic break in the north of São Paulo (Fig. 2). The  $F_{ST}$  between these regions was 0.422 with  $p < 0.001$  (Table 2). When the two geographic regions were analyzed separately, the genetic diversity and expansion signal were maintained (Table 1). The time to the most recent common ancestor of these two lineages was not confinable because ESS was 37. The effective population size of the SP clade was 315,320, the MG clade was 140,253, and an ancestral population was estimated to be 30,835. The estimated migration rate from SP to MG ( $N_m = 0.165$ ) was higher than from MG to SP ( $N_m = 0.055$ ) (Fig. 5). The BAPs outcome showed two clear groups that correspond to the SP and MG clades, with some individuals collected in one place but genetically related to another. For example, three individuals sampled in Minas Gerais State were related to the SP clade and 9 individuals sampled in São Paulo State were related to the MG clade (Fig. 6).

## **DISCUSSION**

Nucleotide diversity found for *C. lineata* in FIB5 ( $\pi = 0.009$ ) was similar to diversity values obtained for other species of Passeriformes from the Atlantic Forest. For the same locus in *Xinphorhynchus fuscus*, Cabanne et al. (2008) found  $\pi = 0.0063$ , while Batalha-Filho et al. (2012) found  $\pi = 0.00403$  for FIB5 in *Basileuterus leucoblepharus*, and d'Horta et al. (2011) found  $\pi = 0.0043$  for FIB7 in *Sclerurus scansor*. Furthermore, *C. lineata* showed a clear signal of population expansion based on all neutrality tests ( $D$  Tajima = -1.92,  $F_u$ 's = -75.52,  $R_2 = 0.03$ ). This outcome was corroborated by Skyline plot analyses that showed a population expansion starting 250,000 years ago, possibly associated with

climate changes during the Late Pleistocene. Recent population expansions were also observed for other vertebrates in the Atlantic Forest, such as frogs (Amaro et al. 2012), lizards (Carnaval et al. 2009), snakes (Grazziotin et al. 2006), birds (Cabanne et al. 2007, 2008; d'Horta et al. 2011; Maldonado-Coelho 2012; Batalha-Filho et al. 2012) and mammals (Martins et al. 2009). Thus, the consensus is that the Atlantic Forest went through geological or climatological events that marked the genetics of several groups. However, studies vary in their explanations for the events that allowed these lineages to separate and consequently expand as genetically distinct sub-populations. On the other hand, expansion due to a vicariant event caused by a barrier depends upon the dating of barrier formation.

Our study of *C. lineata* using FIB5 revealed significant population genetic structure, with two main lineages. One lineage was associated with specimens collected from northern São Paulo and Paraná, and another lineage appeared in the Minas Gerais ( $F_{ST} = 0.422$ ) (Fig. 2), which is concordant with a possible phylogeographic break in northern São Paulo. This break was also observed for this species based on mitochondrial data (Sari 2005; Pessoa 2007) and for several other species of forest vertebrates: frogs (*Proceratophrys boie*; Amaro et al. 2012, *Thoropa miliaris* complex; Fitzpatrick et al. 2009), snakes (*Bothrops jararaca*; Graziotin et al. 2006), and birds (*Xinphorhynchus fuscus*; Cabanne et al. 2008) also show well-defined phylogeographic breaks.

Recently, studies using palaeoclimatic dating revealed that the Atlantic Forest passed through many changes during the Pleistocene (Behling 2002; Behling and Pillar 2007). In addition, studies of phylogeography in Passeriformes confirmed that the climatic changes of the Pleistocene affected the distribution of lineages in the region (Cabanne et al. 2007; Maldonado-Coelho 2012). It is expected that species living in isolated forest habitats will have passed through a recent population expansion after a bottleneck during the Pleistocene period. These demographic oscillations should mark the genome of the species, and we found such signs in our study. Evidence including mismatch distribution analysis, Tajimas  $D$ ,  $F_u$ 's and  $R_2$  tests, and Bayesian skyline plots showed that a relatively recent demographic event, such as population growth, caused most of the coalescent events that occurred before the expansion and, consequently, samples of these populations have gene genealogies stretched near the external nodes and compressed near the root (i.e., star-like genealogies) (Ramos-Onsins and Rozas 2002).

Cabanne et al. (2008) proposed that populations in the southern Atlantic Forest (which has low forest stability) that were affected by Pleistocene refugia would exhibit low genetic diversity and strong signatures of demographic expansion. Fitzpatrick et al. (2009) affirmed that demographic expansion in

the southern Atlantic Forest, south of the Doce river, should be attributed to demographic and geographical expansions of populations that occupied very small refugia. *Conopophaga lineata* lineages showed a strong signature of demographic expansion and genetic diversity, comparable to other Passeriformes of the Atlantic Forest, such as *X. fuscus* (Cabanne et al. 2007, 2008), *B. leucoblepharus* (Batalha-Filho et al. 2012) and *S. scansor* (d'Horta et al. 2011).

Alternatively, other studies have corroborated the hypothesis of rivers as barriers (ex. Pellegrino et al. 2005; Lacerda et al. 2007). Pellegrino et al. (2005) found two lineages that diverged between southern Minas Gerais and São Paulo in geckos (*Gymnodactylus darwini* complex), suggesting that this divergence resulted from the formation of the Paraíba do Sul valley. If the Paraíba do Sul river was responsible for the splitting of *C. lineata* lineages, we would expect that these lineages would be older than the Pleistocene period, accumulating mutations for the last 15 million years (the date of the formation of the Paraíba valley), showing a remarkable differentiation and low gene flow.

In contradiction to this prediction, our results demonstrate recent demographic expansion for both lineages (the MG and SP clades), associated with date estimates of expansion occurring in the Pleistocene period (250,000 years ago), and seem to indicate that refugia affected *C. lineata* evolutionary history in the southern Atlantic Forest. We believe that the lineages from Minas Gerais were most likely the result of Bahia refugia, and the lineages from São Paulo derived from São Paulo refugia. In addition, BAPs results confirm the structure in two clades, but indicated shared individuals among them, mainly from 12 individuals in the regions Buri, Teodoro Sampaio and Euclides da Cunha in São Paulo State and the regions Nova Lima, Santa Barbara and Caratinga in Minas Gerais state. These results corroborate the hypothesis, proposed by Sari (2005) and Pessoa (2007), that southeastern Minas Gerais and northern São Paulo state could be a region of secondary contact between lineages. However, the recent migration of individuals indicated by BAPS suggests that this could be merging of lineages. Similar patterns of divergence and secondary contact were suggested by Cabanne et al. (2007) for *Xiphorhynchus fuscus* in the valley of the Paraíba do Sul river, estimating the divergence between lineages to have occurred approximately 70,000 years ago through mitochondrial DNA.

Our study with FIB5 confirmed the structure observed using mitochondrial DNA in *C. lineata* by Sari (2005) and Pessoa (2007); the divergence time between lineages estimated by those authors was 200,000 years ago. In our study, it was not possible to estimate the divergence between lineages; however, our estimate for expansion of populations was similar to this value (250,000 years ago; Fig. 4),

and suggested that divergence in the nuclear locus occurred before this time. This difference is expected because the nuclear locus (FIB5) was less variable, but presented longer coalescence time than mtDNA. Because these genetic markers have different effective population sizes and modes of inheritance, it is expected that demographic events will shape the variation in the markers in distinct ways (Zink & Barrowclough 2008). However, the high congruence between nuclear and mitochondrial marker results gives us confidence to infer that *C. lineata* evolutionary history split during the Pleistocene following a population expansion.

Cabanne et al. (2008) proposed that minor geographic features that are traditionally considered inefficient barriers could become important to explain some biogeographic patterns in the Atlantic Forest, where climatic changes appear as a central factor modulating the effect of barriers to gene flow. In this context, the Paraíba do Sul valley and the mountain ranges of Serra da Mantiqueira and Serra do Mar could be modified due to climatic alterations, such as changes in water flow and vegetation. This partial barrier effect may be considered together with more widespread changes in the forest distribution during glaciations to explain divergence and transitions of lineages in the southern Atlantic Forest. Consequently, the pattern of *C. lineata* genetic structure we documented could be generated by vicariance events in the Pleistocene followed by secondary contact in the Holocene. Batalha-Filho et al. (2012) studied *B. leucoblepharus* in southern Atlantic Forest in Brazil, and did not find significant structure, suggesting that species with different habitat requirements could show distinct patterns of phylogeographic distribution. Thus, barriers observed for some endemic taxa from the Atlantic Forest may have acted as selective ecological filters, wherein species with higher tolerance to habitat fragmentation were able to maintain gene flow or disperse across these barriers. Contrary to expected, *C. lineata* is a species relatively tolerant of habitat fragmentation (Dantas et al. 2007) that showed a clear signal of population structure. On the other hand, Cabanne et al. (2012) suggested that canopy birds tend to be less genetically differentiated than understory birds, a likely explanation for the genetic structure observed in *C. lineata*.

Evolution in isolation and secondary contact provides a possible explanation for the current phylogeographic structure of *C. lineata*. Costa (2003) suggested that speciation in the Neotropics could not be explained by any single model of vicariance or climatic change. Thus, it is possible that no general pattern for organisms' diversification in the Atlantic Forest will arise, but a complex range of scenarios shall be described. The refuge hypothesis, the influence of geography, and river barriers are among the

most discussed models in the study of Neotropical diversification. Our data did not support a primary influence of rivers on the divergence between the two main mitochondrial lineages of *C. lineata* in the south-central Atlantic forest. Instead, the data supported the influence of isolation by distance in separated forest relicts as a main vicariant event, followed by subsequent demographic expansions, on shaping the current phylogeographical structure of *C. lineata*. Thus, vicariance and expansions leading to secondary contact appear to be related to recent natural forest landscape dynamics affecting population of birds, and perhaps other organisms endemic to this region.

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#### Author Contributions

Gisele Dantas sequenced, analyzed the FIB5 data and wrote the paper. Miguel Ângelo Marini, Rodrigo, Cristina Miyaki, Gustavo Cabanne are responsible for field collection. Fabrício R. Santos, Gustavo Cabanne and Gisele Dantas have done statistical analyses. All authors contributed with discussion and raising of ideas for this manuscript.

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Table I.: Summary statistic of Fib5 for the Minas Gerais clade and São Paulo clade.

Localities	Minas Gerais	São Paulo	Total
N	128	82	210
Nh	40	39	71
Hd	0.885	0.892	0.929
Pi	0.0066	0.0072	0.0094
Theta	0.0166	0.0186	0.0259
Fu's	<b>-36.05</b>	<b>-34.43</b>	<b>-75.52</b>
D Tajima	<b>-1.910</b>	<b>-1.94</b>	<b>-1.92</b>
R2	<b>-0.030</b>	<b>-0.037</b>	<b>0.031</b>

In bold values that were significant  $p < 0.05$ .

Table II.: Analysis of molecular variance (AMOVA) for *Conopophaga lineata* using two populations MG and SP Clades.

Source of Variation	d.f.	Sum of squares	Variance components	of	Percentage of variation
Among Population	1	108.016	1.03551 Va		42.22
Within Populations	210	297.592	1.41710 Vb		57.78
Total	211	405.608	2.45262		

FST : 0.42221.

## FIGURES LEGENDS

Figure 1.: Sampling localities of *Conopophaga lineata* in the Atlantic Forest, eastern Brazil.

Figure 2.: Mismatch distribution of FIB5 *Conopophaga lineata* from the Atlantic Forest, eastern Brazil.

Figure 3.: Median joining network based on 410 bp of  $\beta$ -Fibrinogen intron 5 of *Conopophaga lineata* individuals from Minas Gerais (grey) and São Paulo (black).

Figure 4.: Bayesian skyline plot based on the analysis of intron 5-  $\beta$ Fibrinogen from *Conopophaga lineata* with a log-normal restricted clock. The y-axis is the effective number of individuals; the thick solid line is the median estimate and the thin line (gray) shows the 95% highest posterior density limit. The x-axis is scaled as million years ago (mya).

Figure 5. □ Distributions of migration events for each pairwise comparison between geographically contiguous lineages of *Conopophaga lineata* obtained from the IM program: a) São Paulo to Minas Gerais and b) Minas Gerais to São Paulo.

Figure 6.: The color-coded image of the *Conopophaga lineata* matrix based on the DNA sequence at intron B-Fibrinogen. Columns correspond to the individual strains (number below). The data matrix is portioned into two clusters: red corresponds to São Paulo and green corresponds to Minas Gerais.

Appendix I.: Samples of *Conopophaga lineata* used in nuclear intron (FIB5) analysis.

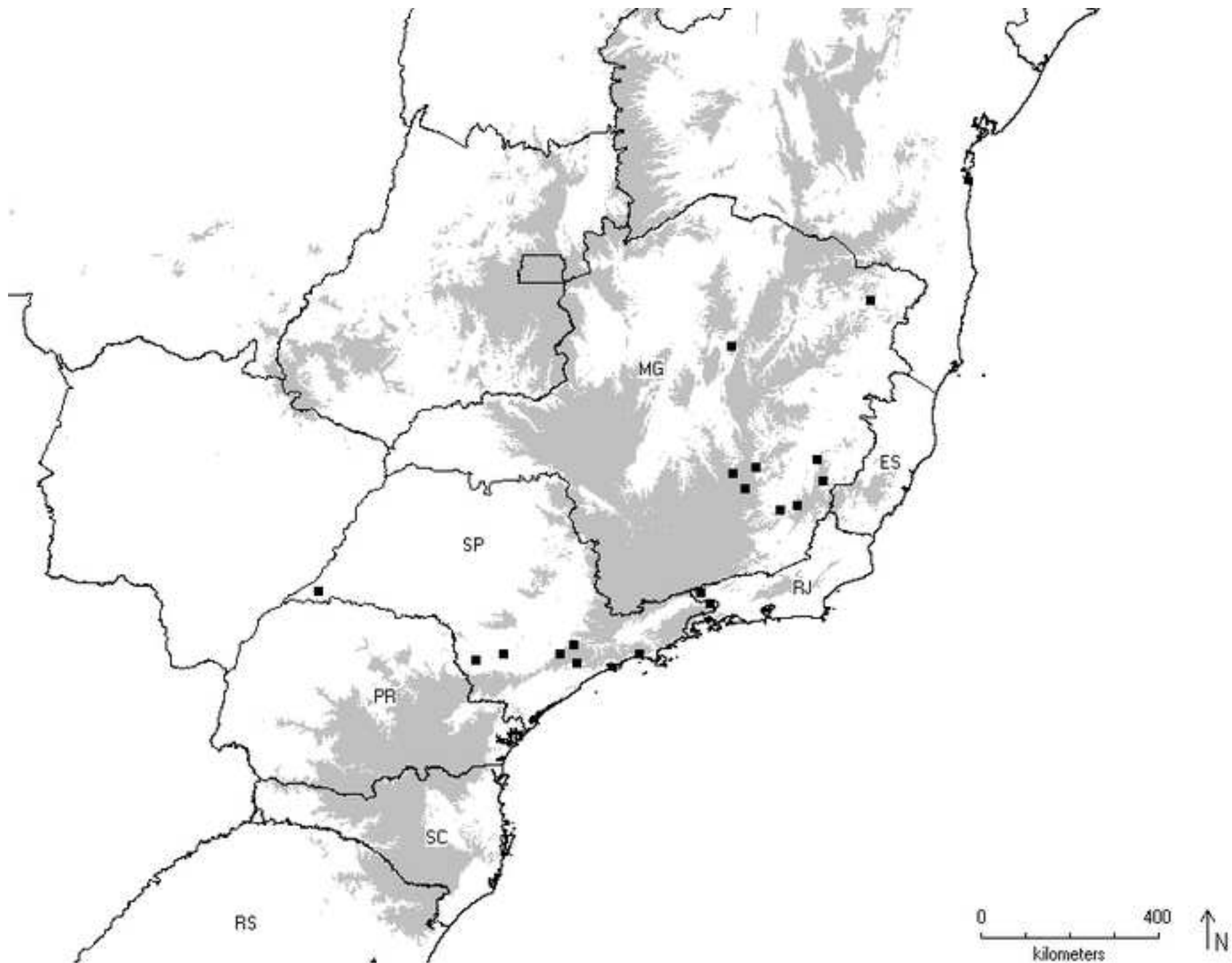
<b>ID</b>	<b>Localities</b>	<b>State</b>	<b>Clade</b>
LGEMA 819	Morro Grande	São Paulo	SP
LGEMA 864	Morro Grande	São Paulo	SP
LGEMA 894	Morro Grande	São Paulo	SP
LGEMA 896	Morro Grande	São Paulo	SP
LGEMA 909	Morro Grande	São Paulo	SP
LGEMA 934	Morro Grande	São Paulo	SP
LGEMA 982	Morro Grande	São Paulo	SP
LGEMA995	Morro Grande	São Paulo	SP
LGEMA1000	Morro Grande	São Paulo	SP
LGEMA1064	Piedade	São Paulo	SP
LGEMA1349	Wenceslau Braz	Paraná	SP
LGEMA 1415	São Roque	São Paulo	SP
LGEMA 1416	São Roque	São Paulo	SP
LGEMA 1423	São Roque	São Paulo	SP
LGEMA 1538	Itaberá	São Paulo	SP
LGEMA 1547	Buri	São Paulo	SP
LGEMA 1553	Buri	São Paulo	SP
LGEMA 1558	Buri	São Paulo	SP
LGEMA 1568	Bananal	São Paulo	SP
LGEMA 1627	Bananal	São Paulo	SP
LGEMA 1633	Belo Horizonte	Minas Gerais	MG
LGEMA 1668	Pontal de Paranapanema	São Paulo	SP
LGEMA 1770	Pontal de Paranapanema	São Paulo	SP
LGEMA 1775	Pontal de Paranapanema,	São Paulo	SP
LGEMA 1776	Pontal de Paranapanema	São Paulo	SP
LGEMA 1874	Pontal de Paranapanema	São Paulo	SP
LGEMA 1879	Pontal de Paranapanema	São Paulo	SP
LGEMA 2049		São Paulo	SP
LGEMA 2269	Teodoro Sampaio	São Paulo	SP
LGEMA 2292	Euclides da Cunha	São Paulo	SP
LGEMA 2301	Teodoro Sampaio	São Paulo	SP
LGEMA 2302	Teodoro Sampaio	São Paulo	SP
LGEMA 2313	Teodoro Sampaio	São Paulo	SP
LGEMA 2419	Juquitiba	São Paulo	SP
LGEMA 2519	Morro Grande	São Paulo	SP
LGEMA 2915	Morro Grande	São Paulo	SP
LGEMA 2969	Buri	São Paulo	SP
LGEMA 3178	Morro Grande	São Paulo	SP
LGEMA 10494	Euclides da cunha	São Paulo	SP
LGEMA 10507	Teodoro Sampaio	São Paulo	SP

B0251	Simonésia	MG	MG
B0253	Simonésia	MG	MG
B0255	Simonésia	MG	MG
B0256	Simonésia	MG	MG
B0257	Simonésia	MG	MG
B0260	Nova Lima	MG	MG
B0262	Araponga	MG	MG
B0264	Caratinga	MG	MG
B0265	Caratinga	MG	MG
B0267	Jequitinhonha	MG	MG
B0268	Nova Lima	MG	MG
B0299	Araponga	MG	MG
B0300	Araponga	MG	MG
B0301	Araponga	MG	MG
B0302	Araponga	MG	MG
B0305	Araponga	MG	MG
B0306	Araponga	MG	MG
B0309	Araponga	MG	MG
B0310	Araponga	MG	MG
B0312	Araponga	MG	MG
B0313	Araponga	MG	MG
B0314	Araponga	MG	MG
B0316	Araponga	MG	MG
B0375	Araponga	MG	MG
B0377	Viçosa	MG	MG
B0381	Nova Lima	MG	MG
B0382	Caratinga	MG	MG
B0383	Caratinga	MG	MG
B0386	Caratinga	MG	MG
B0387	Caratinga	MG	MG
B0388	Caratinga	MG	MG
B0389	Caratinga	MG	MG
B0391	Simonésia	MG	MG
B0393	Simonésia	MG	MG
B0395	Nova Lima	MG	MG
B0396	Nova Lima	MG	MG
B0397	Nova Lima	MG	MG
B0398	Nova Lima	MG	MG
B0400	Nova Lima	MG	MG
B0402	Nova Lima	MG	MG
B0403	Nova Lima	MG	MG
B0404	Nova Lima	MG	MG
B0407	Nova Lima	MG	MG



B0408	Nova Lima	MG	MG
B0409	Nova Lima	MG	MG
B0410	Nova Lima	MG	MG
B0411	Nova Lima	MG	MG
B0412	Nova Lima	MG	MG
B0414	Nova Lima	MG	MG
B0415	Nova Lima	MG	MG
B0416	Nova Lima	MG	MG
B0419	Jequitinhonha	MG	MG
B0420	Jequitinhonha	MG	MG
B0421	Jequitinhonha	MG	MG
B0422	Jequitinhonha	MG	MG
B0423	Jequitinhonha	MG	MG
B0424	Jequitinhonha	MG	MG
B0425	Nova Lima	MG	MG
B0841	Bocaiúva	MG	MG
B0958	Santa Bárbara	MG	MG

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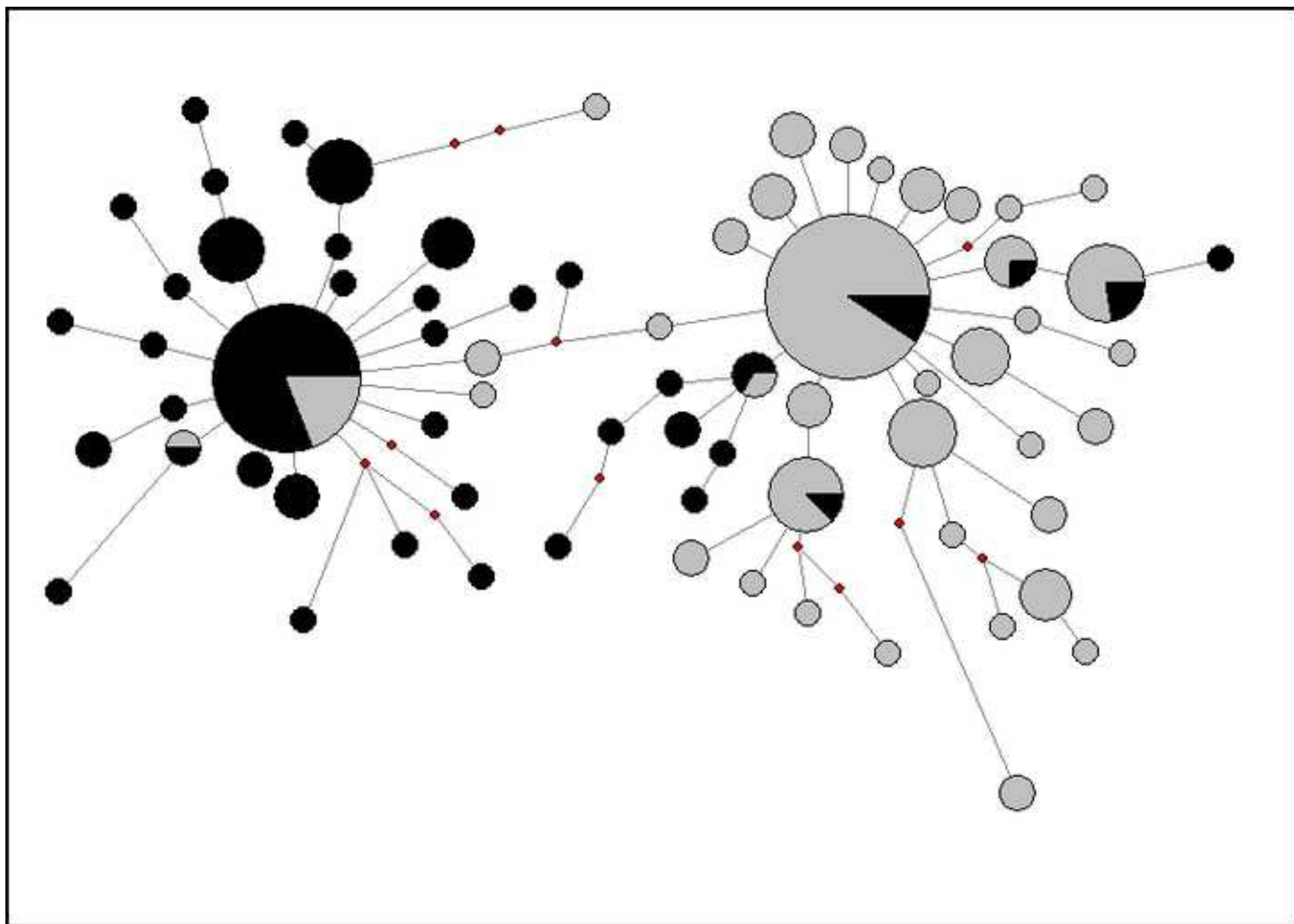


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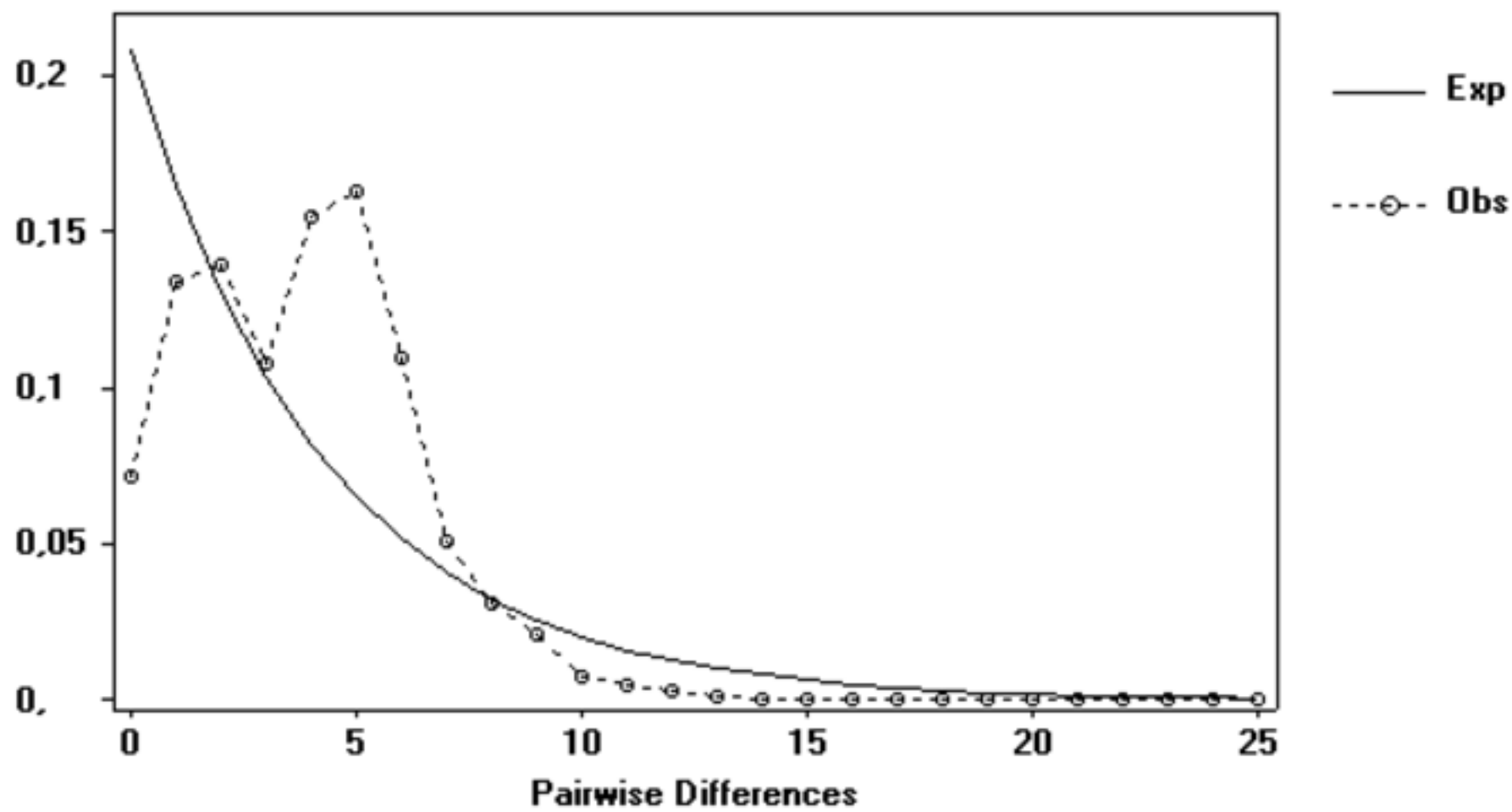
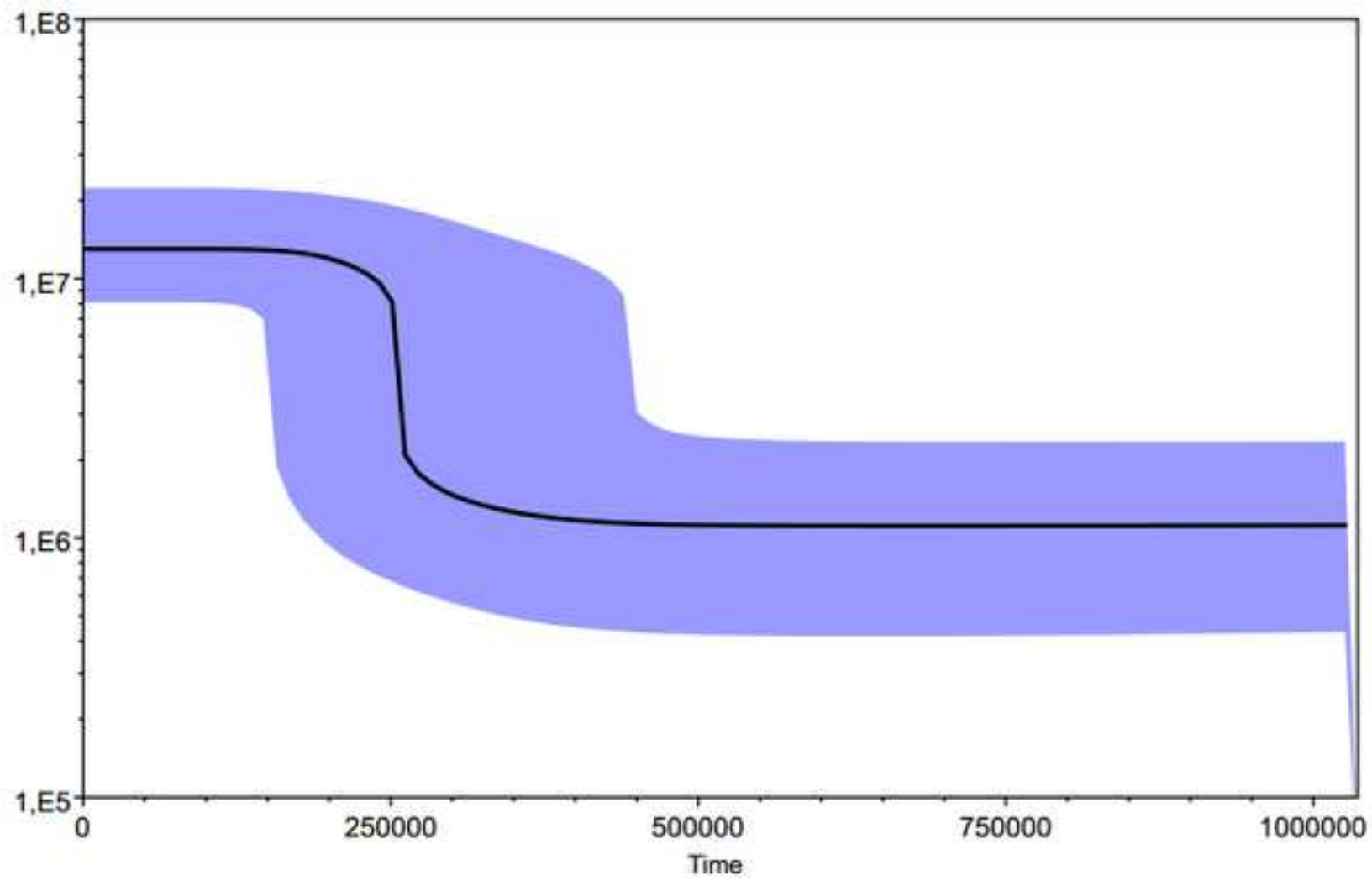


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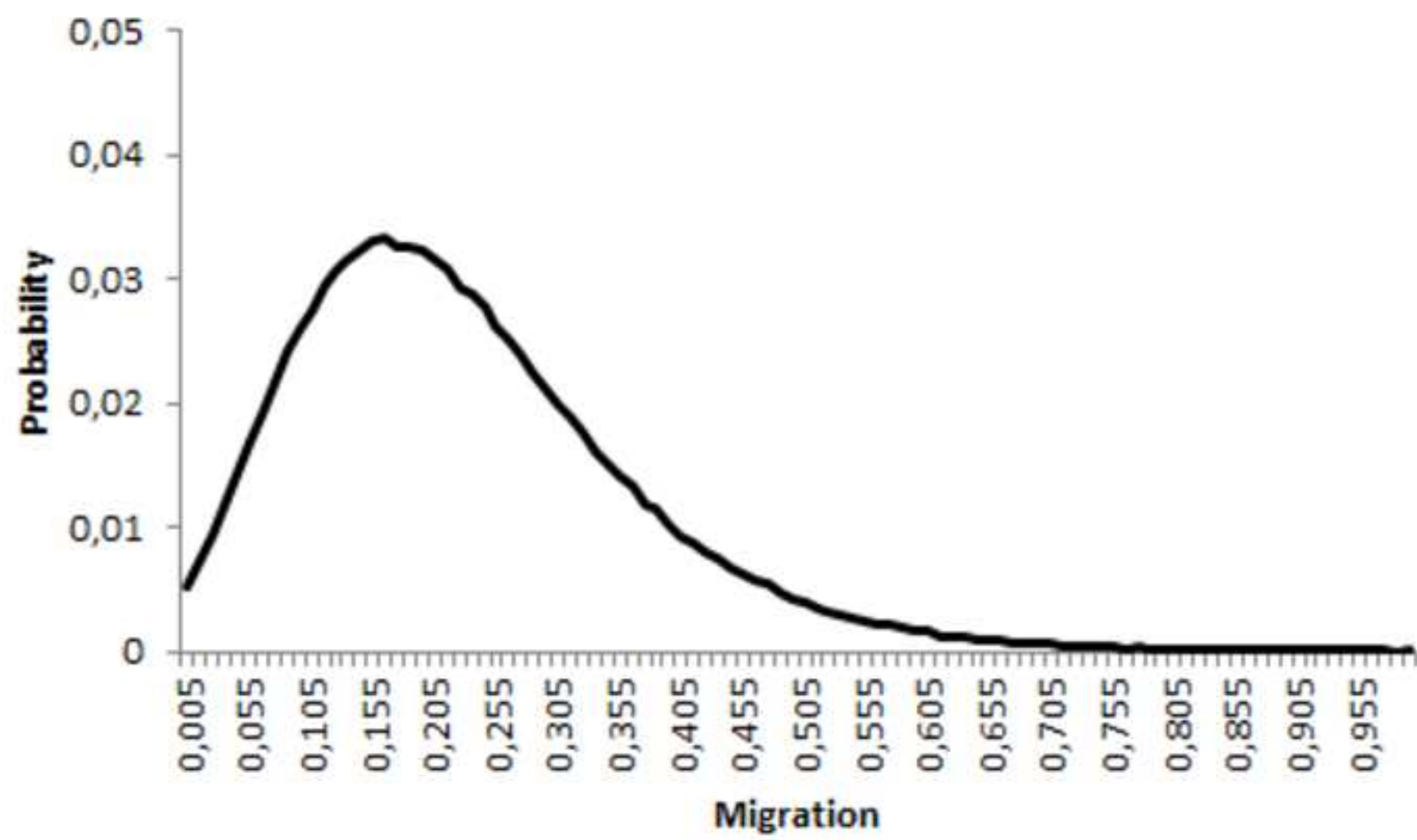
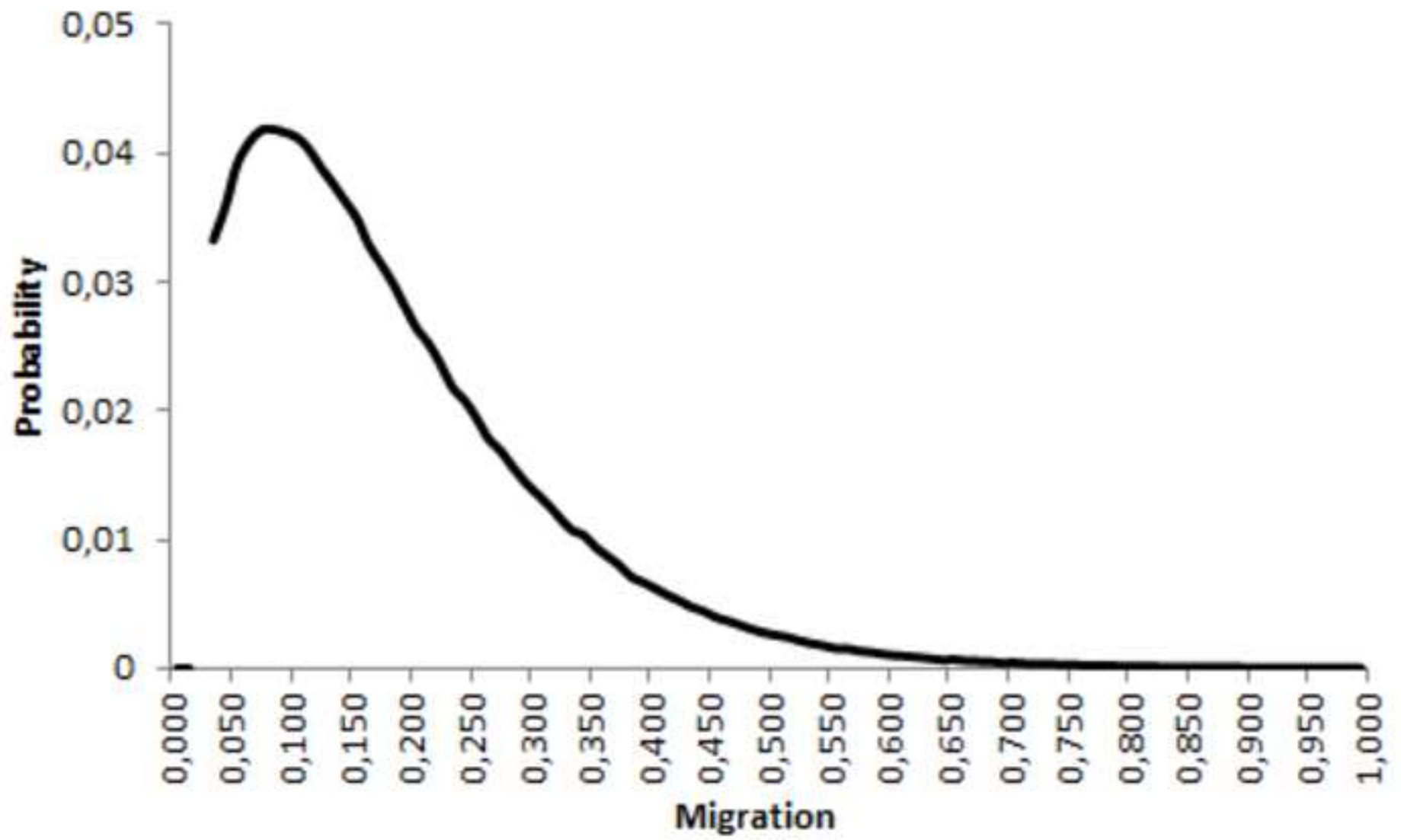


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