

**Eloisa Helena Reis Sari**

**Análise Genética de Populações Fragmentadas  
de *Conopophaga lineata* (Passeriformes) da  
Mata Atlântica de Minas Gerais**

Dissertação apresentada ao Curso de Pós-Graduação do Departamento de Biologia Geral do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Mestre em Genética.

Orientador: Prof. Fabrício Rodrigues dos Santos

Belo Horizonte - MG  
Universidade Federal de Minas Gerais  
Instituto de Ciências Biológicas  
Departamento de Biologia Geral  
2005

# **Livros Grátis**

<http://www.livrosgratis.com.br>

Milhares de livros grátis para download.

Ao Juninho e ao Giovanni,  
que iluminaram minha vida por um  
tempo efêmero mas deixaram uma  
infinita saudade.

## AGRADECIMENTOS

Aos meus pais, que são para mim o mais perfeito exemplo de vida e meus maiores incentivadores a seguir nesta carreira e projeto de vida, sempre me apoiando em todas as momentos e decisões (e várias vezes ajudando a escolher quais eram as mais sensatas).

À minha linda irmã, minha maior companheira e amiga durante estes dois anos, que muitas vezes ouviu, calada, broncas injustificáveis e continuou cuidando de mim.

Ao Chefito Fabrício, que, mesmo sem me conhecer, me aceitou em seu laboratório, confiou em mim e me deu a oportunidade de realizar o projeto com que sempre sonhei. Que me ensinou tanto e, apesar dos seus compromissos impreteríveis (e na maioria, chatos!), sempre dava um jeito de me ouvir quando percebia que eu precisava de conselhos.

Ao Rodrigo, a pessoa mais prestativa que conheci no ICB, por sempre discutir comigo todos as minhas dúvidas técnicas e profissionais. Mais do que isto, por ter se mostrado um verdadeiro amigo e compreensivo colega de trabalho.

À Dani, minha tutora, sempre atenciosa e dedicada, a quem procurei seguir os passos por ser uma profissional tão competente e uma pessoa tão doce.

Aos verdadeiros amigos de Viçosa (agora quase todos em BH!): Tintia, Lê, Vevs, Lózinha, Gustavo, Dana, Lila, Binha, Débora, Nandinho e tantos outros, pelo carinho, cumplicidade, compreensão e, muitas vezes, pelo colo.

Aos tantos companheiros de laboratório que passaram pelo LBEM nestes dois anos ou continuam lá e, de alguma forma, me ajudaram com uma extração, PCR, placa ou só por tornar o ambiente mais descontraído mesmo. Agradeço em particular a: Poca, Paulinha, Leandro e Ju; Sibelle e Mariana, companheiras com os passarinhos; Tiago, por ressuscitar minhas PCRs; Gabi, pelas primeiras placas; Simons, pela amizade e bom humor; e ao Paco, um recente mas tão fiel e amado amigo.

Aos amigos ecólogos, por terem me adotado e me divertido tanto. Em especial à querida Bets, à sarcástica dupla Paçoca e Maldinei, à Angel, ao Ioiô, pelo companheirismo e pelas pizzas de madrugada no ICB, e ao Rafa, que esteve ao meu lado durante grande parte desta caminhada deixou-a muito mais aprazível.

Aos amigos, colegas, professores e funcionários do "corredor da Genética", pelas construtivas discussões acadêmicas, seminários e também pelas festinhas e lanches. Em especial a Carol, Renata, Bernadete, Marina e Ferdí, com quem compartilhei o sufoco dos últimos meses.

Aos professores de Viçosa que me ajudaram a descobrir minha paixão pela genética: Lúcio Campos, Pedro Carneiro, Cosme Cruz, Mara Tavares e Paulo DeMarco.

Aos diversos ornitólogos que entraram em minha vida: Rômulo Ribon, um dos maiores responsáveis pela minha paixão pelos pássaros, por ter me apresentado a Ornitologia e o

campo (com direito a muitos tombos) com tanto entusiasmo; Gisele Dantas, por me "presentear" com as conopophaguinhas e sempre ser solícita em responder minhas dúvidas; Gustavo, pela amizade e ajuda com suas mirabolantes idéias argentinas; Nescau, pela empolgação e intercâmbio de informações sobre as *Conopophaga*; Iara, pela animada companhia nos congressos; Marcos Rodrigues, por emprestar livros, redes, anilhas, peles, viagens (e alunos!); todos do Laboratório de Ornitologia da UFMG, pela disponibilidade em coletar amostras em levar geneticistas "PPs" pra campo, em especial a Fred, Lucas, Chris e Érica; Miguel Marini e alunos, pela maioria das coletas.

A todos os meus tios e primos, principalmente aqueles residentes em BH, que me acolheram afetosamente e não deixaram com que eu me sentisse "órfã" nesta cidade. Também por serem tão eruditos e incentivarem intensamente minha carreira acadêmica.

À Cidinha, pelas deliciosas comidas enviadas de Viçosa.

A todos os outros que de alguma forma colaboraram para a realização do meu projeto e a produção da minha dissertação.

A Deus, que me acolhe, consola, guia, ampara e que permitiu com que tudo isto acontecesse e que todas estas pessoas participassem da minha vida.

*"Toda la gloria es nada, toda vida es sagrada (...)  
No dejaremos huella, sólo polvo de estrellas.  
Vale una vida lo que un sol vale"  
(Jorge Drexler)*

# SUMÁRIO

<b>LISTA DE ANEXOS</b> _____	<b>vii</b>
<b>RESUMO</b> _____	<b>1</b>
<b>INTRODUÇÃO</b> _____	<b>2</b>
<b>ARTIGO:</b> Historical and anthropic events affecting genetic structure in populations of the Atlantic forest bird <i>Conopophaga lineata</i> _____	<b>5</b>
<b>Abstract</b> .....	<b>7</b>
<b>Introduction</b> .....	<b>8</b>
<b>Materials and Methods</b> .....	<b>11</b>
Field sampling _____	11
Molecular methods _____	11
Analyses of Sequences, Intraspecific Phylogenies and Population Structure _____	12
Analyses of habitat fragmentation effects _____	13
<b>Results</b> .....	<b>14</b>
Mitochondrial Control Region Sequences _____	14
Intraspecific Phylogenies _____	14
AMOVA and regressions between genetic and geographical distances _____	15
Habitat fragmentation effects _____	16
<b>Discussion</b> .....	<b>17</b>
Intraspecific Diversity and Phylogenies _____	17
Population's Diversity, AMOVA and Geographical Correlations _____	20
Analyses of Anthropic Fragmentation Effects _____	21
Implications for Conservation _____	23
<b>Acknowledgements</b> .....	<b>25</b>
<b>References</b> .....	<b>26</b>
<b>Tables and Figures</b> .....	<b>31</b>
<b>CONCLUSÕES</b> _____	<b>37</b>
<b>REFERÊNCIAS BIBLIOGRÁFICAS</b> _____	<b>38</b>

## **LISTA DE ANEXOS**

**ANEXO 1:** Lista de indivíduos utilizados, por população, com números das anilhas e do Banco de DNA do LBEM.

**ANEXO 2:** Tabela de haplótipos de região controle do DNA mitocondrial (1174 pb analisados) encontrados para *Conopophaga lineata* (excluindo-se os gaps de alinhamento) e número de indivíduos em cada população que os apresentam.

**ANEXO 3:** Primers utilizados para amplificação e seqüenciamento da região controle do DNA mitocondrial de *Conopophaga lineata* e *C. melanops*.



## RESUMO

A Mata Atlântica é um bioma majoritariamente brasileiro que apresenta altos níveis de biodiversidade e avançada degradação. Assim estudos visando a sua conservação são urgentes. A filogeografia é uma abordagem genética que permite o delineamento de estratégias para a preservação de espécies, associando a distribuição espacial da variabilidade genética com aspectos históricos (distribuição temporal). No presente trabalho relatamos o uso de seqüências da região controle do mtDNA (1074 pb) para analisar aspectos históricos que influenciaram a distribuição das linhagens maternas (haplótipos) em populações de *Conopophaga lineata* em fragmentos da Mata Atlântica de Minas Gerais (MG). Foram coletadas amostras de sangue de 104 indivíduos de *C. lineata* em 12 fragmentos florestais de diferentes tamanhos. As análises sugeriram uma diversidade genética específica alta em comparação às de outros passeriformes neotropicais. As populações só se mostraram fortemente estruturadas entre regiões geográficas muito distantes (nordeste e sudeste de MG – 500 km). Também foi observada a existência de dois clados de haplótipos ocorrendo em simpatria no sudeste de MG. A divergência entre estes dois clados foi de 5,92%, comparável ao normalmente encontrado para comparações entre espécies congênicas de passeriformes neotropicais. O clado menos freqüente mostrou-se geneticamente mais parecido com indivíduos do estado de São Paulo do que com os de MG. Esta região poderia representar um contato secundário entre duas linhagens genéticas que se divergiram em alopatria há, no mínimo, 180 mil anos. Não foi observada correlação direta entre o nível de diversidade nucleotídica e o tamanho do fragmento. Entretanto, a estruturação da diversidade genética encontrada em fragmentos pequenos e em fragmentos grandes foi diferente.

# INTRODUÇÃO

A destruição ambiental e a perda de habitats, causadas principalmente pela expansão das atividades do homem, como agricultura e pecuária, são graves ameaças à biodiversidade e principais causas da atual onda de extinção (Scharlemann *et al.* 2004). Nos últimos 300 anos as áreas de aves endêmicas ("Endemic Bird Areas" - EBAs) foram as mais afetadas pela transformação de áreas de florestas tropicais em plantações comerciais (Scharlemann *et al.* 2004). O Brasil destaca-se entre os países que apresentam mais alta biodiversidade e dois de seus biomas – a Mata Atlântica e o Cerrado – são considerados pontos mundialmente prioritários para conservação ("hotspots") devido ao seu elevado grau de endemismo e avançada degradação (Myers *et al.* 2000). A Mata Atlântica passou por um forte processo de fragmentação florestal e ocupa hoje o equivalente a apenas 7,5% de seu território original (Myers *et al.* 2000).

A variabilidade genética tem um importante papel na sobrevivência de populações e espécies a médio e longo prazo, pois se acredita que uma baixa variação possa restringir o surgimento de futuras adaptações evolutivas (Milligan *et al.* 1994). Isto aumentaria o risco de extinção da espécie, que ficaria mais susceptível aos efeitos da estocasticidade ambiental, demográfica ou genética (Frankham 1995, Turner, 1996). A fragmentação ambiental pode ser determinante na perda de variabilidade intra-populacional, reduzindo os tamanhos populacionais e favorecendo processos como endogamia e deriva genética. Além disso, a fragmentação leva a uma diminuição do fluxo gênico, decorrente do resultante isolamento geográfico e, portanto, reprodutivo (Frankham *et al.* 2002).

Assim, o conhecimento dos efeitos da fragmentação florestal sobre as populações é de grande importância para a conservação da biodiversidade. Entretanto, um pequeno número de estudos analisando os efeitos da fragmentação tem sido realizado em florestas tropicais e estes são principalmente com aves (Turner, 1996).

Os Suboscines são pássaros que ocorrem majoritariamente na região Neotropical. Dentre as 938 espécies de Passeriformes que ocorrem no Brasil, 609 pertencem à Subordem Suboscines, sendo 102 espécies endêmicas no país (Sick 1997). Entretanto, este é um grupo pouco conhecido do ponto de vista genético. A maioria dos trabalhos abordando estrutura genética populacional em aves foi realizada no hemisfério Norte, com Passeriformes da Subordem Oscines. Desta forma, a maioria das seqüências nucleotídicas e dos marcadores genéticos de passeriformes publicados até o momento é de Oscines, o que dificulta a padronização de técnicas moleculares para analisar espécies de Suboscines.

*Conopophaga lineata*, conhecida popularmente como chupa-dente ou cuspidor, é uma espécie de pássaro que ocorre na Mata Atlântica e pertence à família Conopophagidae, da Subordem Suboscines (Passeriformes). Ela se distribui do Ceará ao Rio Grande do Sul (no

Brasil) e no nordeste da Argentina (Misiones), leste do Uruguai e leste do Paraguai (Whitney 2003; Sick 1997). Por ocupar uma extensa área de distribuição, *C. lineata* reconhecidamente apresenta variações no canto e plumagem, mas que ainda não foram delimitadas geograficamente (Pinto 1978; Whitney 2003). Esta espécie é dependente florestal (Andrade e Marini 2001), possui hábitos insetívoros e generalistas e consegue utilizar hábitat de bordas e florestas secundárias, o que facilita a sua vida em ambientes perturbados (Ribon 1998; Whitney 2003). Por estar presente em vários fragmentos de mata de distintos tamanhos, *C. lineata* pode ser um bom modelo para o conhecimento do efeito da fragmentação sobre a diversidade genética das populações.

Dados obtidos através do uso de marcadores genéticos podem revelar muitas informações acerca da subdivisão das populações (Haig 1998; Templeton 1998), favorecendo estudos sobre fragmentação florestal antrópica, vicariância e outros eventos históricos antigos. Além disso, se forem utilizados marcadores que possibilitem a contextualização da distribuição da variabilidade não só em âmbito geográfico, mas também em âmbito temporal, estudos filogeográficos também podem ser realizados (Avice *et al.* 1987). A filogeografia lida com a filogenia de linhagens ou haplótipos e sua distribuição espacial e pode ser feita baseada em análises de seqüências de DNA mitocondrial (Avice *et al.* 1987).

O DNA mitocondrial (DNAm<sub>t</sub>) de vertebrados, por sua vez, é uma molécula circular simples (15-20 Kb), que apresenta alta taxa evolutiva se comparada ao DNA nuclear e herança materna, sem recombinação, favorecendo seu uso em estudos microevolutivos (Avice *et al.* 1987). A região controle ou hipervariável do DNAm<sub>t</sub> é uma região não codificadora que apresenta taxa evolutiva maior que o restante da mitocôndria. Como consequência, esta região apresenta uma maior quantidade de variações nucleotídicas e é muito utilizada em estudos de variabilidade intra-específica (Baker e Marshall 1997; Milot *et al.* 2000), que podem ser utilizados em projetos para conservação da biodiversidade.

Neste trabalho, o seqüenciamento da região controle do mtDNA foi utilizado para analisar a diversidade genética de *C. lineata* em populações de fragmentos da Mata Atlântica de Minas Gerais. Este é um dos três estados brasileiros que possuem menor proporção de áreas protegidas de Mata Atlântica; apenas 3,37 % do total da área ocupada por Mata Atlântica no estado encontram-se dentro de parques e reservas (Ayres *et al.* 1997). Além disto, o que resta é principalmente composto por pequenos fragmentos florestais isolados, muitas vezes de matas secundárias. Assim, estratégias para conservação deste bioma em Minas Gerais são necessárias.

Considerando-se estes fatos, surgiu a idéia do estudo aqui relatado. Este projeto começou a partir de capturas de aves em fragmentos grandes (maiores que 1000 ha) e pequenos (menores que 50 ha) de Mata Atlântica em Minas Gerais, isolados entre si e de

outras matas por monoculturas ou pastagens por uma distância mínima de um quilômetro. As capturas foram realizadas entre os anos de 2000 e 2003 pela equipe do Prof. Miguel Â. Marini e em 2004, por alunos do Prof. Marcos Rodrigues. *Conopophaga lineata* foi escolhida como a espécie a ser utilizada para as análises genéticas por ter sido abundantemente capturada em muitos dos fragmentos amostrados. Além disto, nenhum trabalho populacional tinha sido antes realizado com seqüenciamento de DNAm de esta espécie. Outras características da espécie, como sua dependência florestal e a variação geográfica de seu canto e plumagem, também foram importantes para sua escolha.

Os objetivos deste trabalho foram:

- 1) padronizar a amplificação e o seqüenciamento da região controle do mtDNA de *C. lineata*;
- 2) analisar a variabilidade genética e a distribuição hierárquica desta nas populações de *C. lineata* amostradas em fragmentos de Mata Atlântica de Minas Gerais;
- 3) inferir a distribuição geográfica das linhagens de haplótipos traçando a filogeografia da espécie;
- 4) verificar se a diversidade genética e a sua distribuição são significativamente diferentes em fragmentos grandes e pequenos;

Para isto foram analisados 104 indivíduos de *C. lineata* coletados em 12 fragmentos florestais de nove localidades de Minas Gerais (Fig. 1). Todas as amostras de DNA extraídas destes espécimes encontram-se depositadas no banco de DNA do Laboratório de Biodiversidade e Evolução Molecular do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (fiel depositário de amostras de componentes do patrimônio genético através da deliberação nº 46, de 18 de dezembro de 2003, do CGEN/MMA). As análises genéticas foram feitas sob autorização especial do IBAMA/MMA de acesso e remessa de amostra de componente do patrimônio genético nº 03/2004 e as capturas realizadas com as seguintes licenças concedidas pelo IBAMA/MMA: 011/2000, processo nº 1835/2000; 053/2001, processo nº 1835/00-07; 070/2002, processo nº 02015.001835/00-07; 207/2003, processo nº 02015.023482/98-38.

Este estudo visa ainda obter resultados que apoiem estratégias de conservação da espécie estudada e, conseqüentemente, da Mata Atlântica. Áreas prioritárias para conservação podem ser propostas por possuírem populações com uma identidade genética única, altos índices de diversidade ou por representarem focos de manutenção da variabilidade genética de *C. lineata*.

ARTIGO:

**Historical and Anthropogenic Events Affecting Genetic  
Structure in Populations of the Atlantic Forest Bird  
*Conopophaga lineata***

# **Historical and Anthropogenic Events Affecting Population Genetic Structure of the Rufous Gnateater, *Conopophaga lineata*, in the Brazilian Atlantic Forest**

E. H. R. Sari<sup>1</sup>, R. A. F. Redondo<sup>1</sup>, M. Â. Marini<sup>2</sup>, and F. R. Santos<sup>1</sup>

<sup>1</sup> Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. <sup>2</sup> Departamento de Zoologia, Universidade de Brasília, Brasília, DF, Brazil.

Date of receipt:

**Keywords:** *Conopophaga lineata*, Atlantic forest, phylogeography, habitat fragmentation, mitochondrial control region

**Corresponding author:**

Fabício R. Santos, Prof. Dr.  
Av. Antônio Carlos, 6627, C.P. 486  
Departamento de Biologia Geral  
Instituto de Ciências Biológicas  
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil  
Email : [fsantos@icb.ufmg.br](mailto:fsantos@icb.ufmg.br)  
FAX: 55 31 3499 2570

Running title: Phylogeography of *Conopophaga lineata*

1 **Abstract**

2 The Brazilian Atlantic forest is one of the five leading world *Hotspots for Conservation*  
3 *Priorities* that presents high biodiversity levels and has been strongly destroyed. Then  
4 researches concerning its conservation are urgently required. Phylogeographic surveys can  
5 give support to species' preservation policies, associating the spatial distribution of genetic  
6 variability with historical events, like vicariance, and anthropic ones, as habitat  
7 fragmentation. This study investigated *Conopophaga lineata* phylogeography in recently  
8 fragmented populations in the Atlantic forest. Mitochondrial control region sequences of  
9 1074 bp were used to analyse 104 individual from 12 forest fragments, differently sized, of  
10 Minas Gerais State (MG), Brazil. The species presented genetic diversity much higher than  
11 other Neotropical passerine species. Strong genetic variability structure was found only  
12 between distant geographic regions (southeastern and northeastern, 500 km apart). In  
13 southeastern region, two clades, occurring sympatrically in three populations, were identified.  
14 Divergence between clades was 5.92%, comparable to values found between Neotropical  
15 congeneric bird species. The less common clade (EN) was more similar to individuals from  
16 São Paulo State than to those from MG. This finding might indicates that southeastern MG is  
17 a region of secondary contact between two populations that were separated until 180,000  
18 years ago, when the clades evolved allopatrically. No direct correlation was found between  
19 nucleotide diversities and fragment/population sizes. However, genetic structure and  
20 isolation by distance were found to be different among large fragments and among small  
21 fragments.

## 1 **Introduction**

2 In recent years, the contribution of genetics to conservation biology has become  
3 widespread, since molecular markers are now able to precisely reveal many features about  
4 interactions within and among populations (O'Brien 1994; Haig 1998). Therefore some  
5 genetic markers indirectly inform about historical events that has lead populations to the  
6 current distribution and its outcomes (Haig & Avise 1996, Haig 1998). Ancient events, as  
7 vicariance and dispersal, and more recent ones, like anthropic habitat fragmentation can be  
8 verified by analysing a specific distribution of the genetic variability (Avise *et al.* 1987; Haig  
9 & Avise 1996; Cunningham & Moritz, 1998; Segelbacher *et al.* 2003; Brown *et al.* 2004;  
10 Cabanne 2004).

11 Phylogeographical surveys allow evaluating a species genetic diversity distribution in  
12 its geographical range and also in an evolutionary time scale, *i.e.* they incorporate  
13 intraspecific phylogenies into species geographical distribution (Avise *et al.* 1987).  
14 Mitochondrial DNA (mtDNA) is a molecular marker that has been successfully used to infer  
15 the phylogeography of many vertebrate species, correlating the findings to historical events  
16 (Cunningham & Moritz, 1998; Milá *et al.* 2000; Milot *et al.* 2000; Pope *et al.* 2000; Zink &  
17 Blackwell-Rago 2000). Mitochondrial sequences can be used to establish association  
18 between microevolutionary processes (*e.g.* mutation, genetic drift, populational genetic  
19 structure) and phylogenetics and macroevolution, making this genetic marker suitable for  
20 phylogeographical studies (Avise *et al.* 1987).

21 Habitat destruction can lead populations to fragmentation, reducing their effective  
22 size and usually the genetic flow among them, due to geographic isolation (Frankham 1995;  
23 Turner 1996). As a consequence, fragmented populations become more susceptible to  
24 genetic drift and inbreeding, frequently resulting in a reduction of genetic variability within  
25 populations and a marked structure among populations. Nevertheless consequences of  
26 habitat fragmentation depend on the previous population structure and sometimes it can be  
27 inferred by phylogeography (Turner 1996; Frankham *et al.* 2002;). It is known that genetic  
28 diversity is very important to populations and species persistence in nature, since low  
29 genetic variation could restrict the emergence of future evolutionary adaptations (Milligan *et*  
30 *al.* 1994). As a result, species and populations with low variability levels may not be able to  
31 cope with stochastic environmental changes and could go extinct (Frankham 1995).

32 A striking example of habitat destruction is the Brazilian Atlantic Forest, which has  
33 been strongly devastated particularly in the last 50 years (Hirota 2003). Today it occupies  
34 approximately only 7.5% of its original area (Myers *et al.* 2000) and the majority of the  
35 forest remnants are isolated and smaller than 100 ha (Chiarello 2000). This Neotropical  
36 rainforest also presents high concentration of endemic species, and therefore is considered



1 as one of the five leading world hotspots and should be a priority for conservation,  
2 management and scientific research (Myers *et al.* 2000). This biome has 1020 bird species,  
3 from which 188 are endemic and 104 are threatened (Conservation International *et al.*  
4 2000), making it the most endangered group of the Neotropical bird assemblage (Stotz *et al.*  
5 1996). Habitat destruction is recognised as a major cause of biodiversity loss,  
6 notwithstanding there are few studies on tropical rainforests analysing fragmentation  
7 effects, and most of them are focused in the Amazon Forest (Turner, 1996). There are even  
8 fewer considering genetics of bird species in fragmented populations (Dantas 2003; Cabanne  
9 2004; Brown *et al.* 2004). Therefore, it is a critical priority to carry out surveys on genetic  
10 effects of anthropic fragmentation for Atlantic Forest birds.

11 *Conopophaga lineata* (Passeriformes: Conopophagidae), the Rufous Gnateater, is a  
12 small (12cm length; 20-25g) Suboscine bird with generalist insectivorous habits (Ribon 1998;  
13 Whitney 2003). It is widely distributed in Brazilian Atlantic Forest, also occurring in  
14 northeastern Argentina (Misiones), eastern Paraguay and eastern Uruguay (Sick 1997;  
15 Whitney 2003). This is an understory species that lives in areas with dense herbaceous and  
16 woody plants, in secondary growth forest and in fragment borders (Ribon 1998; Sick 1997;  
17 Whitney 2003). Besides, it can be found even in small forest patches (Ribon 1998; Willis *et*  
18 *al.* 1983), making *C. lineata* capture accessible in many locations along its distribution range.

19 Conopophagidae is one of the least known Neotropical bird families; published  
20 information is vague and relationships between species and subspecies are undefined  
21 (Whitney 2003). *Conopophaga lineata* presents remarkable geographical variation in song and  
22 plumage (Pinto 1978; Whitney 2003), but the “distributional limits of these vocalizations, and  
23 the extent to which they correspond to named populations, requires much additional  
24 research” (Whitney 2003).

25 Currently three subspecies are officially recognised, but with largely indefensible  
26 range limits along Brazilian States: *C. l. cearae* Cory (NE Brazil, from Ceará to Pernambuco  
27 and northeastern Bahia, where it requires confirmation), *C. l. lineata* Wied (NE Brazil, from  
28 Pernambuco to southern Bahia; Central Brazil, in the south of Goiás and northern Mato  
29 Grosso do Sul), *C. l. vulgaris* Ménériès (south-centre and SE Brazil, from southern Mato  
30 Grosso do Sul and southern Espírito Santo to Rio Grande do Sul; eastern Paraguay, Misiones  
31 and eastern Uruguay) (Whitney 2003). However, there are many regions inside *C. lineata*  
32 distribution where the subspecies to be found is not predictable. The southeastern Brazilian  
33 State, Minas Gerais, is one of them, with a large area and situated in the middle of *C. l.*  
34 *lineata* and *C. l. vulgaris* geographical distributions, according to Whitney (2003). Minas  
35 Gerais is also included in the distributional range of three biomes: Atlantic Forest, Caatinga  
36 and Brazilian Cerrado (another world hotspot for conservation priorities) (Myers *et al.* 2000),  
37 consequently presenting interesting transition areas between them.

1 Here we report the use of mtDNA control region sequences to assess genetic variability in 12  
2 populations of *C. lineata* from Minas Gerais State. We tested if populations' genetic lineages  
3 are associated to its geographical distribution and if recent habitat fragmentation has  
4 affected the distribution of this genetic diversity.

# 1 **Materials and Methods**

## 2 ***Field sampling***

3 All *Conopophaga lineata* specimens (n = 104) were captured with mist nets in 12 Atlantic  
4 Forest fragments (in nine different localities) of the Minas Gerais State, southeast Brazil  
5 (Fig.1). A single individual was sampled into the locality of Ouro Preto and its sequence was  
6 used only for phylogeographical analysis. Large and small fragments were sampled (Table 1).  
7 At three localities (Araponga, Simonésia and Nova Lima) birds were captured in two  
8 differently sized fragments (one larger than 999 ha and the other smaller than 30 ha),  
9 separated by at least five kilometres. Birds were numbered with aluminium leg bands,  
10 punctured on the brachial or tarsal vein to get blood samples, morphologically measured and  
11 released. Blood samples were stored in microtubes with absolute or 70% ethanol and kept at  
12 4°C until DNA isolation. Some specimens were collected and vouchers are deposited at the  
13 zoological collections of Universidade Federal de Minas Gerais and Universidade de Brasília.  
14

## 15 ***Molecular methods***

16 Total genomic DNA extraction was made following standard phenol-chloroform method  
17 (Sambrook *et al.* 1989). A fragment of approximately 1200 bp of the mitochondrial control  
18 region (CR) was amplified by Polymerase Chain Reaction (PCR) using primers LEC1 (5'  
19 TCCACACTCGACATCTCATT 3') and H16137 (5' AAAATRYCAGCTTTGGGAGTTG 3'). LEC1 was  
20 designed in this study for *C. lineata* and it hybridizes in the beginning of CR, approximately  
21 80 bp downstream of the tRNA<sup>Thr</sup> gene. H16137, located at tRNA<sup>Pro</sup> gene, was modified for  
22 Thamnophilidae (Lacerda 2004, based on primers designed by M. D. Sorenson). PCR reaction  
23 mixtures were performed in 12,5 µl with 1 U Taq Polymerase (Phoneutria), 1X Taq Buffer  
24 (Phoneutria), 200 µM dNTPs, 0.4 µM of each primer and approximately 10-50 ng of genomic  
25 DNA. Amplification cycling protocol was: initial denaturation at 94°C for 2 min; 36 cycles at  
26 94°C for 30 seg, 57°C for 40 seg, 72°C for 2 min and a final extension step at 72°C for 10  
27 min. Products concentration were verified on an ethidium bromide-stained 0.8% agarose gel.  
28 PCR products were purified with 20% PEG (polyethylene glycol)/ 2.5 M NaCl precipitation.  
29 Both strands of purified DNA were sequenced using the primers above and also internal  
30 primers: LEC2 (5' TGTACCTGTTGTGGTCGGCGTA 3'), designed specifically for *C. lineata* and  
31 H4 (5' TTGACGAGGTAAAATATGTCT 3'), made for Thamnophilidae (Lacerda 2004). Purified  
32 PCR products were cycle-sequenced with ET DYE Terminator Kit (Amersham Biosciences)  
33 according to manufacturer's instructions with 35 cycles at 95°C for 25 s, 50°C for 15 s and  
34 60°C for 3 min. The runs were done in MegaBACE (Amersham Biosciences) automatic  
35 sequencer. In order to produce unambiguous sequences, two independent PCR reactions

1 were made for each individual and both products were sequenced. A mtDNA CR sequence of  
2 *Conopophaga melanops* was produced to be used as outgroup in phylogenetic analysis. The  
3 aligned sequences are deposited in GenBank under Accession number XXX.  
4

#### 5 ***Analyses of Sequences, Intraspecific Phylogenies and Population Structure***

6 Consensus sequences (of the two PCR products, each sequenced with four primers) were  
7 made for every individual using computer programs: Phred v. 0.20425 (Ewing & Green 1998;  
8 Ewing *et al.* 1998), Phrap v. 0.990319 (www.phrap.org) and Consed 12.0 (Gordon *et al.*  
9 1998). Clustal W was used for sequence alignment of different consensus from distinct  
10 individuals using *default* parameters. Model testing analysis (Posada & Crandall 1998) was  
11 made with Hyphy program (Pond *et al.* 2004) to determine the evolutionary mutation model  
12 of these sequences. Based on this analysis' results, the Tamura-Nei model with gamma  
13 distribution ( $\alpha$  parameter = 0.786272) was chosen to estimate genetic distances in further  
14 procedures.

15 Nucleotide diversity ( $\pi$ ), haplotype diversity (h), average number of nucleotide  
16 differences ( $k$ ) and transition/transversion rates (R) were calculated with programs DnaSP  
17 (version 4.0; Rozas *et al.* 2003), MEGA (version 3.1; Kumar *et al.* 2004) and Arlequin (version  
18 2.000; Schneider *et al.* 2000). Relationships among unique haplotypes were assessed by  
19 building a network using the median-joining algorithm (Bandelt *et al.* 1999) with the program  
20 NETWORK (version 4.1.0.8; www.fluxus-engineering.com). Additional phylogenies were  
21 constructed with both Maximum Parsimony (MP) and Neighbor Joining (NJ) algorithms using  
22 MEGA (version 3.1; Kumar *et al.* 2004). Both trees were tested with 1000 bootstrap  
23 replicates. MP bootstrap consensus tree was produced by heuristic search using CNI (Close  
24 Neighbor Interchange) with 10 repetitions of random additions of sequences (complete  
25 deletion of gaps/ missing data). NJ tree was made with Tamura-Nei model with gamma  
26 distribution ( $\alpha$  parameter = 0.786272). Sequences used in all phylogenetic analysis were  
27 uncorrected.

28 Divergence time of 17.44% was used to estimate separation between phylogroups, as  
29 employed by Milot *et al.* (2000) for the first domain of mitochondrial control region of the  
30 Oscine passerine *Dendroica petechia*.

31 Hierarchical distribution of genetic diversity was verified through Analysis of Molecular  
32 Variance (AMOVA; Excoffier *et al.* 1992) with Arlequin (version 2.000; Schneider *et al.*  
33 2000). Each fragment sampled was considered as a population. In order to test the  
34 phylogeographical structure, two different tests were made: (1) three-level AMOVA,  
35 considering **fragments** (n = 11) as **populations** and **localities** (n = 8) as **groups**; and (2)  
36 three-level AMOVA, also considering **fragments** (n = 11) as **populations** but **groups**

1 represented by **geographical regions** (n = 2: northeast and southeast). More details are  
2 described in table 2. Significance of  $\Phi$  statistics, which estimates the percentage of genetic  
3 variation within populations, among populations within groups and among groups, was tested  
4 with 1000 nonparametric permutations.

5 To test whether geography define population genetic structure, simple linear  
6 regressions were made to correlate geographical distances with two genetic distance  
7 indexes:  $\Phi_{st}$  between pairs of populations, calculated with Arlequin, and log of Nei distance  
8 (average number of nucleotide substitutions per site between populations; Nei 1987),  
9 calculated with DnaSP. Log of Nei distance was used because neither Nei values nor their  
10 residuals from regressions presented a normal distribution. Great circle geographical  
11 distances between populations were calculated with the program Earth (Jonh A. Byers –  
12 [www.wcrl.ars.usda.gov/cec/moregen.htm](http://www.wcrl.ars.usda.gov/cec/moregen.htm)) using coordinates for each fragment.

13

#### 14 ***Analyses of habitat fragmentation effects***

15 In order to verify whether effects of anthropic fragmentation are expressed over genetic  
16 diversity, a simple linear regression between population nucleotide diversity ( $\pi$ ) and log of  
17 fragment size was made. Also, ANOVA was used to compare average  $\pi$  in populations among  
18 fragment size classes. Three different arrangements of fragment classes were tested: (1)  
19 large ( $\geq 1000$  ha; n = 4) *versus* small ( $\leq 100$  ha; n = 5); (2) large ( $\geq 1000$  ha; n = 4) *versus*  
20 medium (between 1000 ha and 30 ha; n = 3) *versus* small ( $\leq 30$  ha; n = 4); and (3)  
21 considering only large and small fragments sampled on same localities, *i.e.* large ( $\geq 1000$  ha;  
22 n = 3) *versus* small ( $\leq 30$  ha; n = 3).

23 Then, populations from small and large fragments were considered separately to perform two  
24 kinds of analyses: simple linear regressions between  $\Phi_{st}$  and geographical distances  
25 separating pairs of populations and two-level AMOVA (Excoffier *et al.* 1992; performed with  
26 Arlequin by Schneider *et al.* 2000) (Table 2). In these analyses, fragments considered were  
27 those from the following localities: Araponga, Nova Lima, Simonésia, Bocaiúva and  
28 Jequitinhonha (four small and four large fragments). AMOVA tests also were performed  
29 without northeastern populations (Bocaiúva – BO – and Jequitinhonha –JE), only with  
30 populations from the three localities where both one small and one large fragment were  
31 sampled.

# 1 **Results**

## 2 ***Mitochondrial Control Region Sequences***

3 The complete sequence alignment of 104 individuals resulted in 1074 bp of the mitochondrial  
4 control region, with the beginning approximately 150 bp downstream tRNA<sup>Thr</sup> and the end 30  
5 bp upstream tRNA<sup>Pro</sup>. Fifty-one haplotypes were found, with 106 mutations in 98 variable  
6 sites, from which 81 were parsimony informative. Gaps were present at 13 sites. As  
7 expected, the light (L) strand presented low Guanine content (G = 14.4%) and transitions  
8 were higher than transversions (R = 3.0). As described by Baker and Marshall (1997), most  
9 differences between sequences were observed in domains I and III, but mainly into third  
10 domain. The preserved boxes F, D, C (Baker & Marshall 1997) and B, and the bird similarity  
11 box (Ruokonen & Kvist 2002) could be identified. Total nucleotide diversity ( $\pi$ ) was estimated  
12 as  $0.0128 \pm 0.0021$ , haplotype diversity (h) as  $0.973 \pm 0.006$  and average number of  
13 nucleotide differences (k) as  $12.6250 \pm 5.7396$ . Values of  $\pi$ , h and k for each population are  
14 depicted in table 1. It is noticeable the high  $\pi$  and k values in populations AJ, NLJ, NLP and  
15 BO.

16

## 17 ***Intraspecific Phylogenies***

18 When median-joining network was drawn, two very different clusters were identified (Fig. 2).  
19 Most specimens (n = 97) belonged to one cluster and seven to a different one. However  
20 these two clusters are not geographically correlated and individuals from both clusters occur  
21 sympatrically in three populations (AJ, NLJ and NLP). Trees constructed with both Maximum  
22 Parsimony (MP) and Neighbor Joining (NJ) algorithms strongly supported the existence of two  
23 clades (Fig 3). Average percentage of nucleotide differences between them was 5.92%  
24 (uncorrected sequences). In order to clarify results explanation, clades were named as TY,  
25 for the bigger clade (with 97 individuals) and as EN, for the smaller (with 7 individuals).

26 Afterwards, all sequences were compared to the mtDNA CR sequences of two  
27 individuals from northeast of São Paulo State (SP; unpublished sequences produced by R. O.  
28 Pessoa), located at the southern boundary of Minas Gerais State (MG). SP sequences were  
29 shorter than the obtained in this study. Then, nucleotide alignment of all *C. lineata* plus *C.*  
30 *melanops* sequences resulted in 1055 bp. Using this new alignment, 49 haplotypes were  
31 identified. In NJ and MP trees, two main branches were identified: one with all individuals  
32 from TY clade and another with EN clade and SP individuals; this last branch was strongly  
33 supported by bootstrap replicates (100% for NJ – data not shown – and 99% for MP – Fig.  
34 3). Hence, all populational analysis done considering data as a whole (n = 103) were also  
35 done excluding individuals from EN clade (n = 7; more details on the discussion).

1 Besides that, within the TY clade, two haplotype lineages were identified on MJ  
2 network and NJ and MP trees (Figs. 2 and 3): one from northeastern Minas Gerais State (BO  
3 and JE) and the other from southeastern regions (mainly composed of haplotypes from  
4 southeastern populations). JE population presented unique haplotypes, but BO population  
5 presented one haplotype from the same lineage of JE and two belonging to southeastern  
6 haplotype lineage. Average percentage of nucleotide differences between those two  
7 haplotype lineages was 1.3%.

### 9 ***AMOVA and regressions between genetic and geographical distances***

10 AMOVA revealed no geographic structure of genetic diversity among localities because the  
11 majority of the variation, 74.20%, was found within populations/fragments and the remaining  
12 was detected almost entirely among populations within localities (25.54%;  $\Phi_{st} = 0.258$ ,  $p =$   
13  $0.000$ ). When comparison was made between northeastern and southeastern regions, a  
14 stronger geographic structure was found ( $\Phi_{st} = 0.422$ ,  $p = 0.000$ ), but most variation was  
15 still within populations/fragments (57.8%). Considering only the TY clade, higher  $\Phi_{st}$  values  
16 were obtained: for localities comparison,  $\Phi_{st}$  was 0.360 ( $p = 0.000$ ), with most percentage  
17 of variation within populations/fragments (64.0%); for regions contrast,  $\Phi_{st}$  was 0.613 ( $p =$   
18  $0.000$ ) and most part of diversity (55.24%) was between northeastern and southeastern  
19 regions and not within populations (Table 2).

20 In  $\Phi_{st}$  pairwise comparisons, JE population presented highest significant values of  $\Phi_{st}$   
21 against all other populations (from 0.394, against BO, to 0.681, against SS), considering all  
22 individuals or just TY clade. For pairwise percentage sequence divergences, considering TY  
23 and EN, values ranged from 0.39% (CA x SB) to 4.4% (NLJ x JE) and highest values were  
24 those from all comparisons with NLJ population; excluding EN clade, values ranged from  
25 0.33% (NLP x SB) to 1.44% (JE x AB), and highest values were those from comparisons with  
26 JE population.

27 Determination coefficients from all simple linear regressions between geographical and  
28 genetic distances were significant. Considering all individuals, geographical distance could  
29 explain 26.26% of genetic differences measured as  $\Phi_{st}$  values ( $R^2 = 0.263$ ,  $p = 0.000$ ) and  
30 10.52 % of log of Nei distance ( $R^2 = 0.105$ ,  $p < 0.016$ ). Excluding individuals from the EN  
31 clade, geographical distances explained a greater part of genetic distances: 45.10% of  $\Phi_{st}$   
32 values ( $R^2 = 0.451$ ,  $p = 0.000$ ) and 73.24% of log of Nei distance ( $R^2 = 0.732$ ,  $p = 0.000$ ).  
33 Genetic distances' residuals from all regressions presented normal distribution.

## 1 **Habitat fragmentation effects**

2 Simple linear regression between population nucleotide diversity ( $\pi$ ) and log of fragment size  
3 provided small and non-significant correlation coefficients (R). All ANOVA tests also  
4 presented non-significant results.

5 AMOVA revealed that diversity among large fragments was geographically structured,  
6 considering individuals from both clades ( $\Phi_{st} = 0.535$ ,  $p = 0.000$ ) or excluding the EN clade  
7 ( $\Phi_{st} = 0.573$ ,  $p = 0.000$ ). On the other hand, diversity structure among small fragments was  
8 noticeably weaker, considering all individuals ( $\Phi_{st} = 0.011$ ,  $p \geq 0.326$ ; not significant) or not  
9 ( $\Phi_{st} = 0.132$ ,  $p = 0.000$ ).

10 Contrasting to the results with four fragments, AMOVA tests (without EN clade)  
11 considering only three localities (Nova Lima, Araponga and Simonésia) revealed a stronger  
12 structure among small fragments ( $\Phi_{st} = 0.148$ ,  $p = 0.002$ ) than among large fragments ( $\Phi_{st}$   
13  $= 0.079$ ,  $p = 0.085$ , marginally significant).

14 Simple linear regressions between genetic and geographic distances showed that in  
15 large fragments, geographic distances can explain a high percentage of genetic divergence  
16 between pairs of populations: 72.49% ( $R^2 = 0.725$ ,  $p = 0.031$ ) and 82.48% ( $R^2 = 0.825$ ,  $p =$   
17  $0.012$ ) excluding EN clade. For small fragments, there was no association of genetic and  
18 geographic distances between pairs of populations, since regressions presented small and  
19 not significant correlation coefficients.



# 1 Discussion

## 2 *Intraspecific Diversity and Phylogenies*

3 Nucleotide diversity found for *C. lineata* (1.28%) was similar to diversity values obtained  
4 using mitochondrial control region (CR) of passerine species sampled along larger areas in  
5 temperate regions (Milot *et al.* 2000; Kvist *et al.* 2001). Nevertheless, *C. lineata* diversity is  
6 higher than some values recently obtained for Neotropical passerines with mitochondrial  
7 assays. Using restriction enzymes over 2800 bp of mitochondrial DNA (control region,  
8 cytochrome *b* and ND6). Brown *et al.* (2004) registered diversity values of 0.52% for  
9 *Henicorhina leucosticta* and 0.45% for *Eucometis penicillata*. Lacerda (2004) estimated that  
10 intraspecific diversity for three *Thamnophilidae* species varied from 0.43% to 0.78% and  
11 Cabanne (2004) found 0.82% (with great SD: 0.45) for *Xiphorhynchus fuscus fuscus*. In  
12 these two latter studies, nearly 600 bp of the control region were used to investigate  
13 Atlantic Forest populations with geographical distributions compatible to the one considered  
14 in this work. Therefore, the higher value found for *C. lineata* may be related to the use of  
15 longer sequences of mitochondrial control region. This region is divided into three domains: a  
16 central and more conserved one, flanked by two variable domains (Baker & Marshall 1997).  
17 Sequences obtained for this work included all three domains and, for *C. lineata*, the third  
18 domain was noticeable more variable than the first one. The two latter studies mentioned  
19 before included only first domain and part of the central domain. Hence, more variation was  
20 already expected to be found within the 1074 bp analysed for *C. lineata*. When only the first  
21 600 bp were considered for *C. lineata*, a nucleotide diversity of 0.88% was found.

22 Furthermore, high total diversity can be justified by the existence of two very  
23 divergent clades: EN and TY. Divergence between these clades (5.92%) was similar to  
24 divergence among some different neotropical passerine species within *genera*, as described  
25 for *Xiphorhynchus* (3.4% - 10.0%; Aleixo 2002), *Cranioleuca* (0.5% - 4.7%; García-Moreno *et al.*  
26 *al.* 1999), *Thamnophilus* (2.7% - 7.4%; Lacerda 2004), *Ramphocelus* (0.1% - 9.5%; Hackett  
27 1996) and two *Drymophila* species (7.2%; Bates *et al.* 1999). Once more, the divergence  
28 value between clades could be explained by the size of *C. lineata* sequences, although Aleixo  
29 (2002) analysed sequences with 2430 bp length (from cytochrome *b*, ND2 and ND3) and  
30 Hackett (1996) analysed sequences with 1412 bp length (cyt *b* and ND2) on their surveys  
31 and found comparable divergence values between species. Also, Zink and Blackwell-Rago  
32 (2000) investigated 1115 bp (CR, cyt *b* and ND2) of the thrasher *Toxostoma curvirostre* and  
33 found specific nucleotide diversity of 1.1%. In this latter example, since the percentage of  
34 sequence divergence between two *T. curvirostre* subspecies' groups diverge in 2% and  
35 there were morphological differences between them, the authors suggested that both  
36 groups warrant a species status.

1           Then what could explain the existence of so divergent clades within *C. lineata*, not  
2 geographically separated? One explanation proposed for this phylogeographic pattern would  
3 be that southeastern Minas Gerais State (MG) is a region of secondary contact between  
4 populations that expanded after being isolated for a long time, when clades have evolved  
5 allopatrically (Avice *et al.* 1987). The grouping of EN clade with São Paulo State (SP)  
6 sequences supports this model. Also, TY clade was much more frequent, indicating that  
7 southeastern MG is closer to the region where this clade evolved. In contrast, EN clade  
8 seems to have evolved more to the south of Brazil, because SP location is under the south  
9 boundary of MG.

10           The separation time between clades TY and EN was estimated as approximately  
11 180,000 years, meaning that the populations where they evolved were unconnected at least  
12 for this period. This time is coincident with recent Pleistocene, when repeated glaciations  
13 affected neotropical forests' distribution, resulting on vicariant effects over species diversity  
14 (Ab'Saber 1979). It is suggested that the mountain chain called Serra do Mar was an  
15 Atlantic Forest refugee during last glacial periods (Ab'Saber 1979). The Serra do Mar is  
16 situated in northeastern SP and southern Rio de Janeiro, near to the coast. The analysed  
17 samples from São Paulo State were collected very close to this mountain region (in the city  
18 of Bananal) and might indicate that this area could have been where EN clade evolved.  
19 Nevertheless, this divergence time estimative might be heedfully interpreted, since the  
20 mutation rate used was calculated for the first domain of control region and *C. lineata*  
21 sequences presented almost no variation in central domain, but most part of variation was  
22 into third domain.

23           Furthermore, specimens from EN clade were observed only in three populations (AJ,  
24 NLJ and NLP) within two localities, but also these localities presented the largest population  
25 sizes. So, this could have been affected by unequal sampling, suggesting that more EN  
26 individuals could be found in distinct populations if more birds were captured. Even though, if  
27 this clade really evolved allopatrically in the south of Brazil, the proportion of EN clade is  
28 expected to diminish towards northern MG down to null in JE locality, as it was detected. A  
29 similar phylogeographical pattern was previously described for *Thamnophilus caerulescens* on  
30 a study that explored the same geographic range area (Lacerda 2004). In this study,  
31 Investigating 721 bp of mitochondrial control region the existence of two sympatric haplotype  
32 lineages (presenting divergence of 1.38%) was identified on the same localities (Araponga  
33 and Nova Lima). Coincidentally, these places also had bigger sample sizes. On the other  
34 hand, Zink (1997) explains that compatible phylogeographies can point towards the same  
35 historical event.

36           An alternative explanation presented by Avice *et al.* (1987) for the phylogenetic  
37 discontinuity without spatial separation, is that reproductively isolated sibling species were

1 misinterpreted as belonging to a single species. This is a reasonable possibility for *C. lineata*  
2 taking into account its unknown variations in song and plumage. There are three recognized  
3 subspecies, but their geographical ranges are not clearly defined. The subspecies *C. l.*  
4 *lineata* was described in Bahia State, which is at the northern boundary of MG; *C. l. vulgaris*  
5 was described in Rio de Janeiro State, which makes southeastern frontier of MG (Pinto  
6 1978).

7 Hence, MG is close to both places where these two subspecies were described and it  
8 would not be unforeseen the presence of both subspecies in this State. SP sequences  
9 certainly belong to *C. l. vulgaris* even though, as it was explained above, the divergence  
10 between two clades is not only compatible with two subspecies, but with species  
11 divergence. Unfortunately, only four vouchers were collected, because we did not have a  
12 license to collect all specimens, and they all belong to TY clade. However, if these clades  
13 representing subspecies prove to be reproductively isolated, they will deserve a species'  
14 status. Future fieldwork, to compare songs and collect vouchers, and investigation of  
15 morphology, plumages and DNA from museum samples are essentially required.

16 All of the analyses were also made without the EN clade to allow an independent  
17 estimate excluding species ascertainment biases. If the existence of two species is to be  
18 confirmed, then our population comparisons would be ineffective.

19 Nucleotide diversity without EN clade was 0.6%, a value comparable to other  
20 Neotropical passerines (Bates *et al.* 2003; Brown *et al.* 2004; Cabanne 2004; Lacerda 2004)  
21 and lower than diversity levels calculated for temperate birds (Milot *et al.* 2000; Kvist *et al.*  
22 2001).

23 Particularly considering TY clade, it is clear the existence of a lineage with individuals  
24 only from northeastern MG (JE and BO populations). All samples from JE belong to this  
25 lineage, but only one from BO belongs to it. This could be a consequence of low gene flow  
26 resulting from the long distance between southeastern and northeastern populations (until  
27 520 km), as suggested by Avise *et al.* (1987) to explain this kind of phylogeographical  
28 structure. It seems that JE might be isolated from southeastern populations due to an  
29 ancient extrinsic barrier to gene flow (Avise *et al.* 1987). Although, TY clade is a  
30 polyphyletic group, what makes it inaccurate to estimate the divergence time between  
31 lineages.

32 A similar phylogeographical pattern was also described for *Xiphorhynchus fuscus*  
33 *fuscus* (Cabanne 2004), *Thamnophilus ambiguus* and *Pyriglena leucoptera* (Lacerda 2004) in  
34 works that surveyed some of the localities considered here, within the same geographic  
35 range. However, none of these studies analysed populations on east-central MG, and the  
36 two lineages could be explained simply by geographical distance (correlation between  
37 genetic and geographic distances will be discussed afterwards). Besides, occurrence of both

1 lineages in BO could indicate gene flow between this population and the others. Another  
2 explanation by Avise *et al.* (1987) would be the extinction of intermediate haplotypes, what  
3 is congruous with the wide distribution and the habitat destruction of all these species.  
4

#### 5 **Population's Diversity, AMOVA and Geographical Correlations**

6 Not surprisingly, populations that revealed higher diversity values ( $\pi$  and  $k$ ) were those with  
7 individuals from TY and EN clades (AJ, NLJ and NLP) and BO, which had haplotypes from the  
8 two TY lineages. In addition, highest values of pairwise percent sequence divergence  
9 between populations were those that included populations NLJ. High values were also found  
10 in all pairwise divergences including JE population, due to its unique haplotype lineage. Bates  
11 *et al.* (2003) found for some populations of Cerrado birds, separated by 1800 km, comparable  
12 genetic distances: 0.29% (in *Lepidocolaptes angustirostris*) and 1.76% (in *Formicivora*  
13 *rufa*), using 379 bp of *cyt b*. Lacerda (2004) also found similar values for *T. ambiguus*,  
14 varying from 0.23% (between two northeastern MG populations) to 1.2% (between  
15 populations from two MG regions). However, all these values were smaller than the  
16 divergence found between NLJ and other populations considering both clades.

17 Hierarchical structure among populations showed that genetic variation was not  
18 arranged among localities, but between geographical regions. Although  $\Phi_{st}$  values were high  
19 in all AMOVA tests, a great part of the diversity was usually found within populations.  
20 Considering only the TY clade, higher  $\Phi_{st}$  values were obtained and it was expected ever  
21 since populations with individuals from both clades present high diversity values, increasing  
22 variation within populations and among populations within groups. In addition, lower pairwise  
23 differences between southeastern populations might have been caused by one or more  
24 characteristics, such as lack of obvious geographical barriers, increased gene flow into this  
25 region or recent population range expansion (as we suggested above).

26 Opposing to the results found here for mitochondrial control region sequences,  
27 10.77% of genetic diversity was observed among these *C. lineata* populations (except SB  
28 and BO) with RAPD markers, showing that variation was mostly within rather than among  
29 populations or regions (Dantas 2003). Although, strong structure between northeastern and  
30 southeastern MG was also described for *T. ambiguus* (75.67%) but it was not that strong  
31 for *P. leucoptera* (16.96%; Lacerda 2004). Furthermore, four populations of *Gymnopithys*  
32 *leucapsis* and *Henicorhina leucosticta* separated by at most 110 km in Costa Rica showed  
33 lower  $\Phi_{st}$  values (25.6% and 25.1%, respectively). All these species inhabit understory  
34 humid forests and have insectivorous habits and this might indicate that population genetic  
35 structure is more related to a species origin and expansion history than to its ecological  
36 features.

1 In agreement with hierarchical structure of genetic diversity, regressions between  
2 genetic and spatial distances were significant, showing higher values without individuals from  
3 EN clade (until 73.24%). The strong effect of geography over genetic distances indicates  
4 isolation by distance, corroborating that reduced gene flow between regions was important  
5 to the diversification of the northeastern lineage.  
6

### 7 ***Analyses of Anthropic Fragmentation Effects***

8 Genetic diversity structure and association between genetic and geographic distances are  
9 correlated to equilibrium between gene flow and genetic drift in populations (Hutchinson &  
10 Templeton 1999). Habitat fragmentation limits gene flow and reduces population size,  
11 increasing genetic drift. Hence, expected genetic consequences of fragmentation are: loss  
12 of genetic variability within populations, stronger population structure ( $\Phi_{st}$ ) and weaker  
13 correlation between geographic and genetic distances (Cunningham & Moritz 1998).

14 There are very few studies exploring effects of anthropic fragmentation over genetic  
15 diversity, especially on tropical forests. In Costa Rica, three passerine species presented  
16 significantly decrease of mtDNA restriction polymorphisms in smaller forest fragments,  
17 isolated for only 50 years (Brown *et al.* 2004). Also, an European Cappercaillie (*Tetrao*  
18 *urugallus*) presented more allelic variability (measured with microsatellites) in larger forest  
19 areas compared to smaller ones (Segelbacher *et al.* 2003). Moreover, in continuous areas *T.*  
20 *urugallus* presented significant correlation between geographic and genetic distance  
21 opposing to smaller fragments, where no pattern of isolation by distance was identified.

22 On the other hand, as described here, some surveys could not find a direct  
23 correlation between fragment size or connectivity and diversity (ANOVA and regressions) but  
24 also used analyses of genetic structure and isolation by distance to contrast small and large  
25 fragments (or continuous areas). Two different works (studies) analysed the genetic  
26 diversity of an Australian rain forest lizard in fragmented populations. In the first work,  
27 Cunningham & Moritz (1998) did not find relationship between diversity and fragment size  
28 using mtDNA restrictions, and they found a higher  $G_{st}$  value in some continuous areas than  
29 in fragments. Otherwise, they registered that diversity distribution in this species was more  
30 affected by older historical processes. Then, Sumner *et al.* (2004) compared differently sized  
31 fragments and continuous areas within a smaller region where the lizard presented a uniform  
32 history, as described by the former study. They also did not find association between  
33 number of microsatellite alleles or heterozigosity and size of forest area. In addition,  $F_{st}$   
34 value was slightly smaller in fragments and the effect of geography on genetic distances  
35 was stronger in continuous forest.

1           This same pattern was described here for *C. lineata* when northeastern populations  
2 were considered in fragmentation analysis: although there was no predictable stronger  
3 diversity structure ( $\Phi_{st}$ ) in small fragments, the effects of their isolation were detectable by  
4 the disruption of the strong correlation between geographical and genetic distances. This  
5 could be explained by increased gene flow among southeastern fragments and between  
6 these and BO population, as proposed before because of low pairwise  $\Phi_{st}$  values identified  
7 for these comparisons. It is relevant to mention that JE, considered in AMOVA of large  
8 fragments, was the only population that presented significant  $\Phi_{st}$  against all other  
9 populations. The corresponding population used in AMOVA for small fragments was BO, which  
10 has only three individuals – contrasting to the big sample size of populations from other small  
11 fragments – and presents only one haplotype from the northeastern lineage.

12           When AMOVA was performed considering only populations from localities where both  
13 one small and one large fragment were sampled, small fragments presented slightly stronger  
14 genetic structure ( $\Phi_{st} = 0.148$ ,  $p = 0.002$ ) than large did ( $\Phi_{st} = 0.079$ ,  $p = 0.085$ ), as an  
15 expected outcome of fragmentation. It supports the idea that stronger genetic structure  
16 found among large fragments when JE and BO were considered might represent a result of an  
17 asymmetrical test for different sizes of fragments, due to higher genetic distance of JE to  
18 the other three populations. This shows that even not so conspicuous historical events like  
19 a possible natural extrinsic barrier to gene flow can interfere on analyses of genetic effects  
20 of habitat fragmentation.

21           It was expected that both small and large fragments compared, presented the same  
22 amount of gene flow, as they are much similarly distributed (on the same locality, small and  
23 large fragment are separated by five to 20 km). The difference between them would indicate  
24 that smaller fragments were under stronger genetic drift. Although, this could not be assured  
25 from the present data because of the small number of populations analysed. Also, there was  
26 a great difference in number of individuals analysed in large and small fragments.

27           Nevertheless, habitat fragmentation might be too recent a process so that its effects  
28 can be assessed, because it takes time for the genetic structure to return to equilibrium  
29 after changes in landscape. Thus, populations that have declined rapidly due to habitat loss  
30 could have normal levels of genetic variation while low genetic variability could be found in  
31 populations with long histories of small population sizes and inbreeding (Frankham 1995).  
32 Fragmentation puts animals into a drastically new situation, and how they will cope with the  
33 fragmented landscape depends not only on inherent characteristics, such as whether they  
34 are territorial within the fragments and whether they are able to move among different  
35 fragments (Pires & Fernandez, 1999) but also on the distance and kind of matrix between  
36 forest patches. Then, another drawback in estimating effects of recent fragmentation  
37 habitat is that previous genetic diversity distribution is not known.

1 Other explanation for the absence of direct association between diversity and  
2 fragment size would be that small fragments might not contain smaller populations since  
3 more birds were captured in them. Nonetheless, the minor relation between sizes of a small  
4 and a large fragment was 1/10 (BO, with 100 ha and SS and NLJ, with 1000 ha) and this  
5 would be not so a plausible suggestion. Even so, *C. lineata* is a generalist insectivorous and  
6 could be more frequent in small fragments due to specialists (potential competitors)  
7 decreasing in these areas (Kattan 1994). Besides, both small and large fragments did not  
8 present biased sexual rate, indicating that the species is reproductively successful,  
9 independently of the forest patch size (Dantas 2003).

10 In addition, loss of diversity could have been imperceptible because of the molecular  
11 marker used. The control region has a higher evolution rate if compared to the rest of  
12 mitochondrial DNA, but random nuclear markers like short tandem repeats (STRs) or RAPD  
13 usually gauge higher levels of variability within populations. However, RAPD markers were  
14 used to analyse most of these *C. lineata* populations but also could not reveal diversity loss  
15 in small fragments (Dantas 2003).

### 16 17 **Implications for Conservation**

18 Diversity distribution patterns are determined not only by recent events, like anthropic  
19 fragmentation, but also by long term processes, like vicariance, dispersal and population  
20 expansions. This can potentially confound the understanding of populations' genetic dynamic  
21 but can be more clearly investigated by using haplotype trees (Templeton 1998). In this  
22 study an obvious historical event determined the variability distribution of *C. lineata*  
23 populations in Minas Gerais State. Because it was possible to identify the results of this  
24 event (existence of two clades) analyses of fragmentation effects were not blurred. Also,  
25 the existence of a less striking event, like the slightly divergent northeastern lineage could  
26 have affected fragmentation analysis.

27 As no remarkable anthropic effect was identified, conservation of this bird should  
28 focus on populations with high diversity or with singular identity, which might represent  
29 evolutionary significant units (Moritz 1994). In this sense, localities where EN clade was  
30 identified can be considered important for conservation of *C. lineata* genetic variability for  
31 their high diversity and for the preservation of the rarer clade. Likewise, JE population  
32 represent another significant unit because of its particular identity, a recurrent singularity  
33 observed in other suboscines species (Cabanne 2004; Lacerda 2004)

34 *C. lineata* is not a threatened species, but it is a dependant species from the  
35 endangered Atlantic forest. This bird could be useful as a model to estimate effect of  
36 fragmentation over more rare species living in the same area with similar habits and to

- 1 identify regional historical events. Furthermore, the identification of evolutionary significant
- 2 units of this species and others may enforce the need for preservation of important areas for
- 3 the Atlantic forest conservation.



1 **Acknowledgements**

2 We thank D. R. Lacerda and G. S. Cabanne for the technical help and discussions. We also  
3 thank people who helped in field work and CEMAVE/IBAMA. This work received grants from  
4 FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais) and CNPq (National Research  
5 Council) from Brazil. E.H.R.S. was supported by CAPES (Comissão de Aperfeiçoamento de  
6 Pessoal de Nível Superior) and R.A.F.R. by CNPq from Brazil. All bird samples were collected  
7 with the government permits from 011/2000, 053/2001, 070/2002, 207/2003 from IBAMA and  
8 deposited in our collection recognized by CGEN 053/2001/MMA. This work was also  
9 performed according to the special authorization for access to genetic resources in Brazil #  
10 03/2004 issued by IBAMA/MMA.

## 1 **References**

- 2 Ab'Saber AN (1979) Os mecanismos da desintegração das paisagens tropicais no Pleistoceno.  
3 *Paleoclimas*, **8**, 1-11.
- 4 Aleixo A (2002) Molecular systematics and the role of the "várzea"–"terra-firme" ecotone in  
5 the diversification of *Xyphorhynchus* woodcreepers (Aves: Dendrocolaptidae). *Auk*, **119**,  
6 621-640.
- 7 Avise JC *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between  
8 population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**,  
9 489-522.
- 10 Baker AJ, Marshall HD (1997) Mitochondrial control region sequence as tools for  
11 understanding evolution. In: *Avian molecular evolution and systematics* (ed. Mindell DP),  
12 pp. 51-82. Academic Press, San Diego.
- 13 Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific  
14 phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.
- 15 Bates JM, Hackett SJ, Goerck J (1999) High levels of mitochondrial DNA differentiation in two  
16 lineages of antbirds (*Drymophila* and *Hypocnemis*). *Auk*, **116**, 1093-1106.
- 17 Bates JM, Tello JG, Silva JMC (2003) Initial assessment of genetic diversity in ten bird  
18 species of South America Cerrado. *Studies on Neotropical Fauna and Environment*, **38**,  
19 87-94.
- 20 Brown LM, Ramey RR, Tamburini B, Gavin TA (2004) Population structure and mitochondrial  
21 DNA variation in sedentary Neotropical birds isolated by forest fragmentation.  
22 *Conservation genetics*, **5**, 743-757.
- 23 Cabanne GS (2004) *Estudo da diferenciação genética populacional do Arapaçu rajado –*  
24 *Xyphorhynchus fuscus fuscus – no sudeste da floresta Atlântica pela análise de*  
25 *seqüências da região controladora do DNA mitocondrial*. Master Degree Thesis,  
26 Universidade de São Paulo.
- 27 Chiarello AG (2000) Influência da caça ilegal sobre mamíferos e aves das matas de tabuleiro  
28 do norte do estado do Espírito Santo. *Boletim do Museu de Biologia Mello Leitão*, **11/12**,  
29 229-247.

- 1 Conservation International Do Brasil *et al.* (2000). *Avaliação e Ações Prioritárias para a*  
2 *Conservação da Biodiversidade da Mata Atlântica e Campos Sulinos*. Ministério do Meio  
3 Ambiente, Brasília.
- 4 Cunningham M, Moritz C (1998) Genetic effects of forest fragmentation on a rainforest  
5 restricted lizard (Scincidae: *Gnytoscincus queenslandiae*). *Biological Conservation*, **83**,  
6 19-30.
- 7 Dantas GPM (2002) *Efeito da fragmentação florestal na razão sexual e variabilidade*  
8 *genética de Conopophaga lineata (Aves: Conopophagidae) em áreas de Mata Atlântica*.  
9 Master Degree thesis, Universidade Federal de Minas Gerais.
- 10 Ewing B, Green P (1998) Basecalling of automated sequencer traces using Phred II: error  
11 probabilities. *Genome Research*, **8**, 186-194.
- 12 Ewing B, Hillier L, Wendi M, Green P (1998) Basecalling of automated sequencer traces using  
13 Phred I: accuracy assessment. *Genome Research*, **8**, 175-185.
- 14 Excoffier L, Smouse P E, Quattro JM (1992) Analysis of molecular variance inferred from  
15 metric distances among DNA haplotypes: application to human mitochondrial DNA  
16 restriction data. *Genetics*, **131**, 479-491.
- 17 Frankham R (1995) Conservation genetics. *Annual Review of Genetics*, **2**, 305-327.
- 18 Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to conservation genetics*. Cambridge  
19 University Press, Cambridge.
- 20 García-Moreno J, Arctander P, Fjeldsä J (1999) A case of rapid diversification in the  
21 Neotropics: phylogenetic relationships among *Cranioleuca spinetails* (Aves, Furnariidae).  
22 *Molecular Phylogenetics and Evolution*, **12**, 273-281.
- 23 Gordon D, Abajian C, Green P (1998) Consed: A graphical tool for sequence finishing.  
24 *Genome Research*, **8**, 195-202.
- 25 Hackett SJ (1996) Molecular phylogenetics and biogeography of tanagers in the genus  
26 *Ramphocelus* (Aves). *Molecular Phylogenetics and Evolution*, **5**, 368-382.
- 27 Haig SM, Avise JC (1996) Avian Conservation Genetics. In: *Conservation Genetics: Case*  
28 *Histories from Nature* (eds. Avise JC and Hamrick JR), pp. 160-189. Chapman and Hall,  
29 New York.

- 1 Haig SM (1998) Molecular contributions to conservation. *Ecology*, **79**, 413-425.
- 2 Hirota MM (2003) Monitoring the Brazilian Atlantic forest cover. In: *The state of the*  
3 *hotspots: the Atlantic Forest* (eds. Galindo-Leal C, Câmara GI). Center for Applied  
4 Biodiversity Science. Conservation International, Washington D.C.
- 5 Hutchinson DW, Templeton AR (1999) Correlation of pairwise genetic and geographic  
6 distance measures: inferring the relative influence of gene flow and drift on the  
7 distribution of genetic variability. *Evolution*, **53**, 1898-1914.
- 8 Kattan GH, Álvarez-Lopez H, Giraldo M (1994) Forest fragmentation and bird extinctions: San  
9 Antônio eighty years later. *Conservation Biology*, **8**, 138-146.
- 10 Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary  
11 Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150-163.
- 12 Kvist L, Martens J, Ahola A, Orell M (2001) Phylogeography of a Palearctic sedentary  
13 passerine, the willow tit (*Parus montanus*). *Journal of Evolutionary Biology*, **14**, 930-941.
- 14 Lacerda DR (2004) *Filogeografia comparada e filogenia de espécies de Thamnophilidae*  
15 *(Aves: Passeriformes) de Mata Atlântica de Minas Gerais*. PhD thesis, Universidade Federal  
16 de Minas Gerais.
- 17 Milá B, Girman DJ, Kimura M, Smith TB (2000) Genetic evidence for the effect of a postglacial  
18 population expansion on the phylogeography of a North America songbird. *Proceedings of*  
19 *the Royal Society of London*, **267**, 1033-1040.
- 20 Milligan BG, Leebens-Mack J, Strand AE (1994) Conservation genetics: beyond the  
21 maintenance of marker diversity. *Molecular Ecology*, **3**, 423-435.
- 22 Milot E, Gibbs HL, Hobson KA (2000) Phylogeography and genetic structure of northern  
23 populations of the yellow warbler (*Dendroica petechia*). *Molecular Ecology*, **9**, 667-681.
- 24 Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology*  
25 *and Evolution*, **9**, 373-375.
- 26 Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots  
27 for conservation priorities. *Nature*, **403**, 853-858.
- 28 O' Brien SJ (1994) A role for molecular genetics in biological conservation. *Proceedings of the*  
29 *National Academy of Sciences USA*, **91**, 5748-5755

- 1 Pinto O (1978) *Novo catálogo das aves do Brasil*. Revista da Imprensa dos Tribunais, São  
2 Paulo.
- 3 Pires AS and Fernandez FAS (1999) Use of space by the marsupial *Micoureus demerarae* in  
4 small Atlantic Forest fragments in south-eastern Brazil. *Journal of Tropical Ecology*, **15**,  
5 279–290.
- 6 Pond SLK, Frost SDW, and Muse SV (2004) HyPhy: hypothesis testing using phylogenies.  
7 *Bioinformatics Advance Access* DOI 10.1093/bioinformatics/bti079.
- 8 Pope LC, Estoup A, Moritz C (2000) Phylogeography and population structure of an ecotonal  
9 marsupial, *Betongia tropica*, determined using mtDNA and microsatellites. *Molecular*  
10 *Ecology*, **9**, 2041-2053.
- 11 Posada D, Crandall KA (1998) Model test: testing the model of DNA substitution.  
12 *Bioinformatics*, **14**, 817-818.
- 13 Ribon R (1998) *Fatores que influenciam a distribuição de avifauna em fragmentos de Mata*  
14 *Atlântica nas montanhas de Minas Gerais*. Master Degree thesis, Universidade Federal de  
15 Minas Gerais.
- 16 Rozas J, Sánches-Delbarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism  
17 analysis by the coalescent and other methods. *Bioinformatics*, **19**, 2496-2497.
- 18 Ruokonen M, Kvist L (2002) Structure and evolution of the avian mitochondrial control  
19 region. *Molecular Phylogenetics and Evolution*, **23**, 422-432.
- 20 Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. CSHL  
21 Press, New York.
- 22 Schneider S, Roessli D, Excoffier L (2000) *Arlequin ver. 2.000: a software for population*  
23 *genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva.
- 24 Segelbacher G, Höglund J, Storch I (2003) From connectivity to isolation: genetic  
25 consequences of population fragmentation in capercaillie across Europe. *Molecular*  
26 *Ecology*, **12**, 1773-1780.
- 27 Sick H (1997) *Ornitologia brasileira*. Nova Fronteira Press, Rio de Janeiro.

- 1 Sumner J, Jessop T, Paetkau D, Moritz C (2004) Limited effect of anthropogenic habitat  
2 fragmentation on molecular diversity in a rainforest skink, *Gnypetoscincus queenslandiae*.  
3 *Molecular Ecology*, **13**, 259-269.
- 4 Stotz DF, Fitzpatrick JW, Parker III TA, Moskivits DK (1996) *Neotropical birds: ecology and*  
5 *conservation*. University of Chicago Press, Chicago.
- 6 Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses  
7 about gene flow and population history. *Molecular Ecology*, **7**, 381-397.
- 8 Turner IM (1996) Species loss in fragments of tropical rain forest: a review of the evidence.  
9 *Journal of Applied Ecology*, **33**, 200-209.
- 10 Whitney BM (2003) Family Conopophagidae. In: *Handbook of the birds of the world:*  
11 *broadbills to tapaculos v.8* (eds. Del Hoyo J, Elliot A, Christie DA) pp. 732-747. Lynx  
12 Edicions, Barcelona.
- 13 Willis E O, Oniki Y, Silva W R (1983) On the behavior of rufous gnateaters (*Conopophaga*  
14 *lineata*, Formicariidae). *Naturalia*, **8**, 67-83.
- 15 Zink RM (1997) Phylogeographic studies of North American birds. In: *Avian molecular*  
16 *evolution and systematics* (ed. Mindell DP) pp. 301-324. Academic Press, San Diego.
- 17 Zink RM, Blackwell-Rago RC (2000) Species limits and recent population history in the  
18 curved-billed thrasher. *The Condor*, **102**, 881-886.

1 **Figure Legends**

2 Fig. 1: Map of Minas Gerais State (Brazil) with the nine localities where individuals were  
3 sampled.

4  
5 Fig. 2: Median-joining network of *Conopophaga lineata* in Minas Gerais drew using control  
6 region haplotypes (1074 bp). Circle size represents haplotype frequency and different colors  
7 symbolize different populations. Lines between haplotypes are proportional to number of  
8 mutations between them. Black points represent hypothetical haplotypes.

9  
10 Fig. 3: Haplotypes Maximum Parsimony consensus tree. Haplotypes are identified by clade  
11 name (Ty or En) and the haplotype identification number. N correspond to haplotypes from  
12 northeastern lineage. In parenthesis, number of individuals of each population that show the  
13 corresponding haplotype.

**Table 1:** *Conopophaga lineata* populations (fragments) and individuals sampled in Atlantic forest fragments and genetic variation estimations: N, number of sampled individuals; h, haplotype diversity;  $\pi$ , nucleotide diversity; k, average number of nucleotide differences. Fragments' names are localities' names; when two fragments were sampled on the same locality, name is followed by the fragment identification.

Fragments	Area (ha)	N	Haplotypes	h $\pm$ SD	$\pi$ $\pm$ SD	k $\pm$ SD
<b>Northeastern</b>						
Jequitinhonha (JE)	5000	10	5	0.84 $\pm$ 0.08	0.0052 $\pm$ 0.0031	5.60 $\pm$ 2.94
Bocaiúva (BO)	100	3	3	1.00 $\pm$ 0.27	0.0102 $\pm$ 0.0080	10.94 $\pm$ 6.89
<b>Southeastern</b>						
Araponga_Brig (AB)	13000	8	8	1.00 $\pm$ 0.06	0.0052 $\pm$ 0.0032	5.55 $\pm$ 2.99
Araponga_JoRi <sup>a</sup> (AJ)	20	17	8	0.85 $\pm$ 0.07 <sup>a</sup>	0.0107 $\pm$ 0.0057 <sup>a</sup>	11.51 $\pm$ 5.49 <sup>a</sup>
Viçosa (VI)	120	5	4	0.90 $\pm$ 0.16	0.0038 $\pm$ 0.0026	4.04 $\pm$ 2.42
Simonésia_Sose (SS)	1000	6	4	0.80 $\pm$ 0.17	0.0035 $\pm$ 0.0023	3.71 $\pm$ 2.18
Simonésia_Lula (SL)	30	8	7	0.96 $\pm$ 0.08	0.0038 $\pm$ 0.0024	4.09 $\pm$ 2.28
Caratinga (CA)	30	11	6	0.91 $\pm$ 0.05	0.0043 $\pm$ 0.0026	4.60 $\pm$ 2.45
Santa Bárbara (SB)	605	8	6	0.89 $\pm$ 0.11	0.0037 $\pm$ 0.0024	3.97 $\pm$ 2.22
Nova Lima_Jamb <sup>b</sup> (NLJ)	1000	5	5	1.00 $\pm$ 0.13 <sup>b</sup>	0.0391 $\pm$ 0.0240 <sup>b</sup>	41.94 $\pm$ 22.07 <sup>b</sup>
Nova Lima_Prim <sup>c</sup> (NLP)	27	22	8	0.86 $\pm$ 0.05 <sup>c</sup>	0.0161 $\pm$ 0.0083 <sup>c</sup>	17.32 $\pm$ 8.00 <sup>c</sup>
Ouro Preto (OP)	3000	1	1	-	-	-
All		104	51	0.97 $\pm$ 0.01	0.0128 $\pm$ 0.0021	12.63 $\pm$ 5.74
All without individuals from EN clade		97	45	0.97 $\pm$ 0.01	0.0060 $\pm$ 0.0005	6.29 $\pm$ 3.01

<sup>a</sup> diversity values for Araponga\_JoRi population without the individual that belong to the EN clade: samples = 16; h = 0.8333  $\pm$  0.072 ;  $\pi$  = 0.0044  $\pm$  0.0026; k = 4.72  $\pm$  2.44; <sup>b</sup> diversity values without individuals (n = 3) from the EN clade were not calculated for Nova Lima \_Jamb population due to its small size (samples = 2); <sup>c</sup> diversity values for Nova Lima\_Prim population without individuals (n = 3) from the EN clade: samples = 19; h = 0.8129  $\pm$  0.0600;  $\pi$  = 0.0024  $\pm$  0.0015; k = 2.59  $\pm$  1.45.



**Table 2:** Hierarchical analyses of molecular variance (AMOVA) of the *Conopophaga lineata* populations. Four sets of comparisons were calculated considering populations from large and small fragments. In order to test anthropic fragmentation effects six other sets were calculated considering either large or small fragments in 5 localities (Araponga, Nova Lima, Simonésia, Bocaiúva and Jequitinhonha).

Analysis– Hierarchy (N)	$\Phi_{st}$	$\Phi_{ct}$	% molecular variance (df)		
			Among groups	Among populations within groups	Within populations
Populations and localities (103)	0.2580**	0.0026 <sup>NS</sup>	0.26 (7)	25.54 (3)	74.20 (92)
Populations and localities without EN clade (96)	0.3596**	0.2939 <sup>NS</sup>	29.39 (7)	6.57 (3)	64.04 (85)
Populations and geographic regions (103)	0.4219**	0.2949*	29.49 (1)	12.70 (9)	57.81 (92)
Populations and geographic regions without EN clade (96)	0.6133**	0.5525*	55.24 (1)	6.08 (9)	38.67 (85)
Among large fragments (29)	0.5351**	-	-	53.51 (3)	46.49 (25)
Among large fragments without EN clade (26)	0.5731**	-	-	57.31 (3)	42.69 (22)
Among small fragments (50)	0.0113 <sup>NS</sup>	-	-	1.13 (3)	98.87 (46)
Among small fragments without EN clade (46)	0.1316**	-	-	13.16 (3)	86.84 (42)
Among three southeastern large fragments without EN clade (16)	0.0786 <sup>NS</sup>	-	-	7.86 (2)	92.14 (13)
Among three southeastern small fragments without EN clade (43)	0.1484*	-	-	14.84 (2)	85.16 (40)

\*\* =  $p < 0.01$ ; \* =  $p < 0.05$ ; NS = not significant; significance of  $\Phi$  statistics was tested with 1000 nonparametric permutations; N = number of individuals analysed; df = degrees of freedom.

Fig. 1:

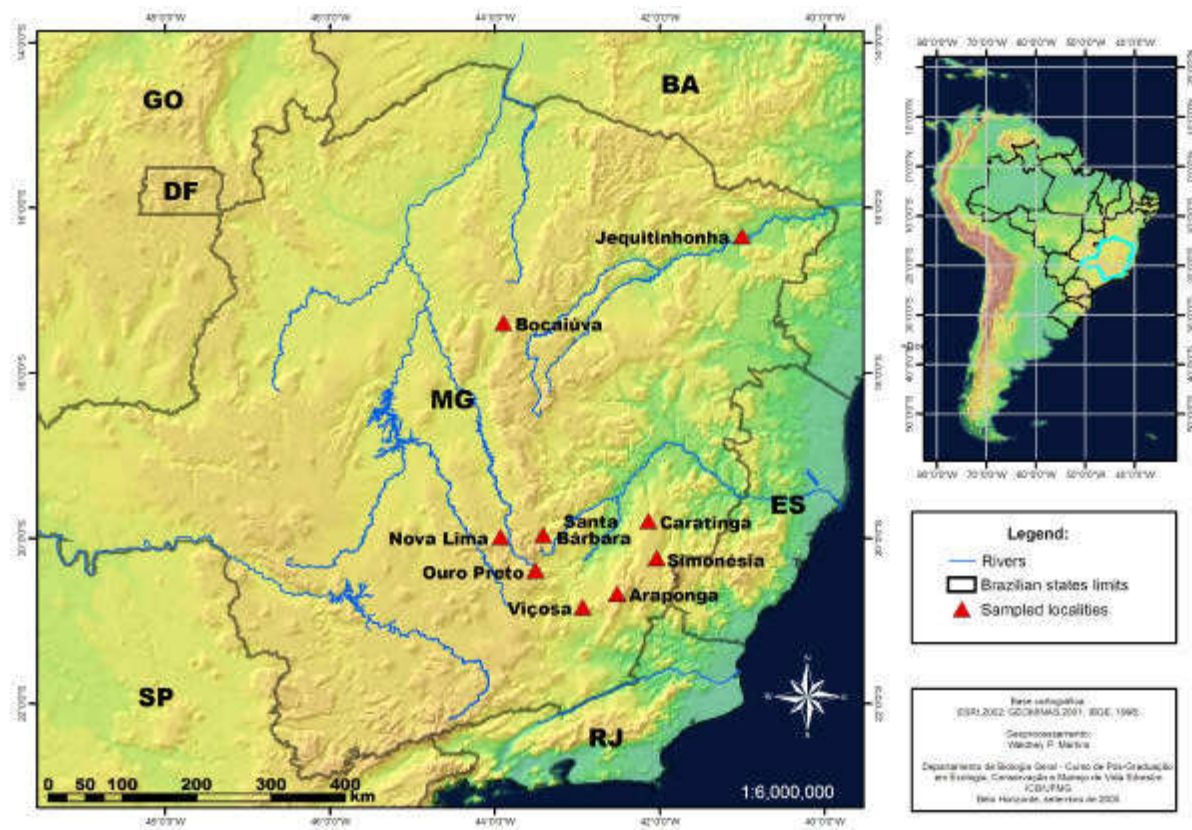


Fig. 2:

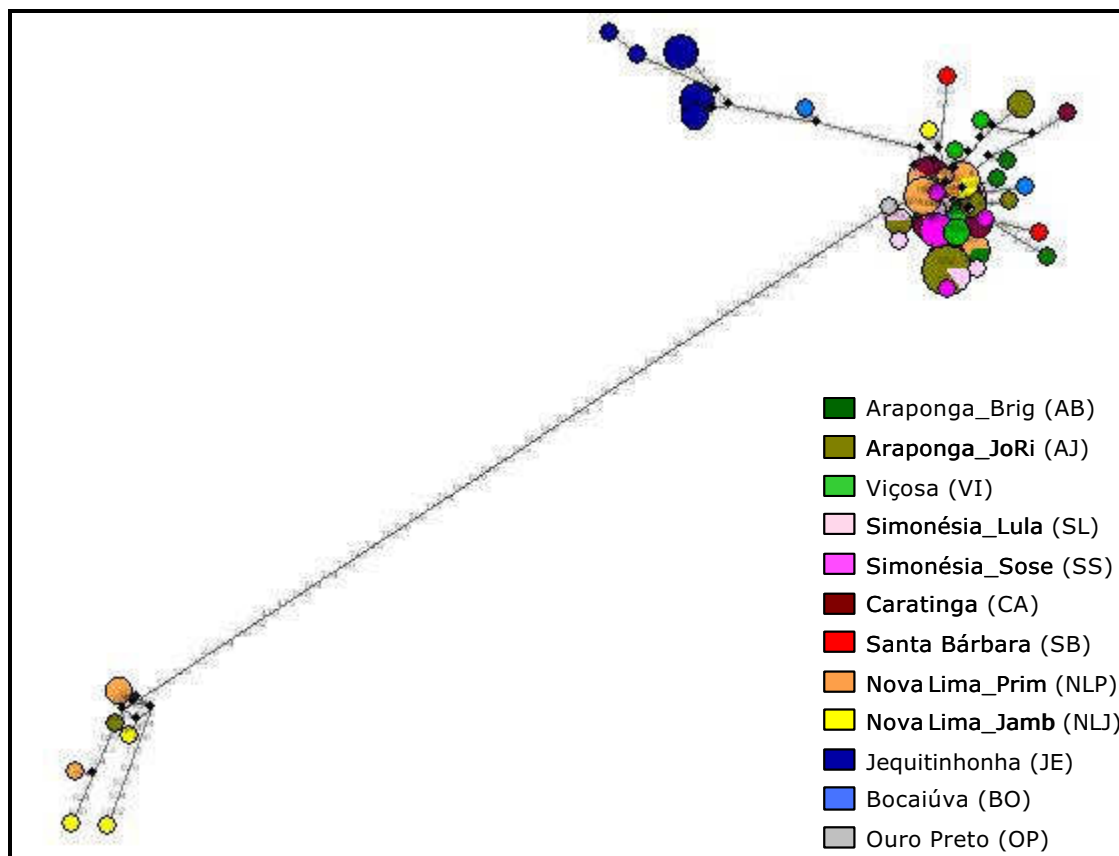
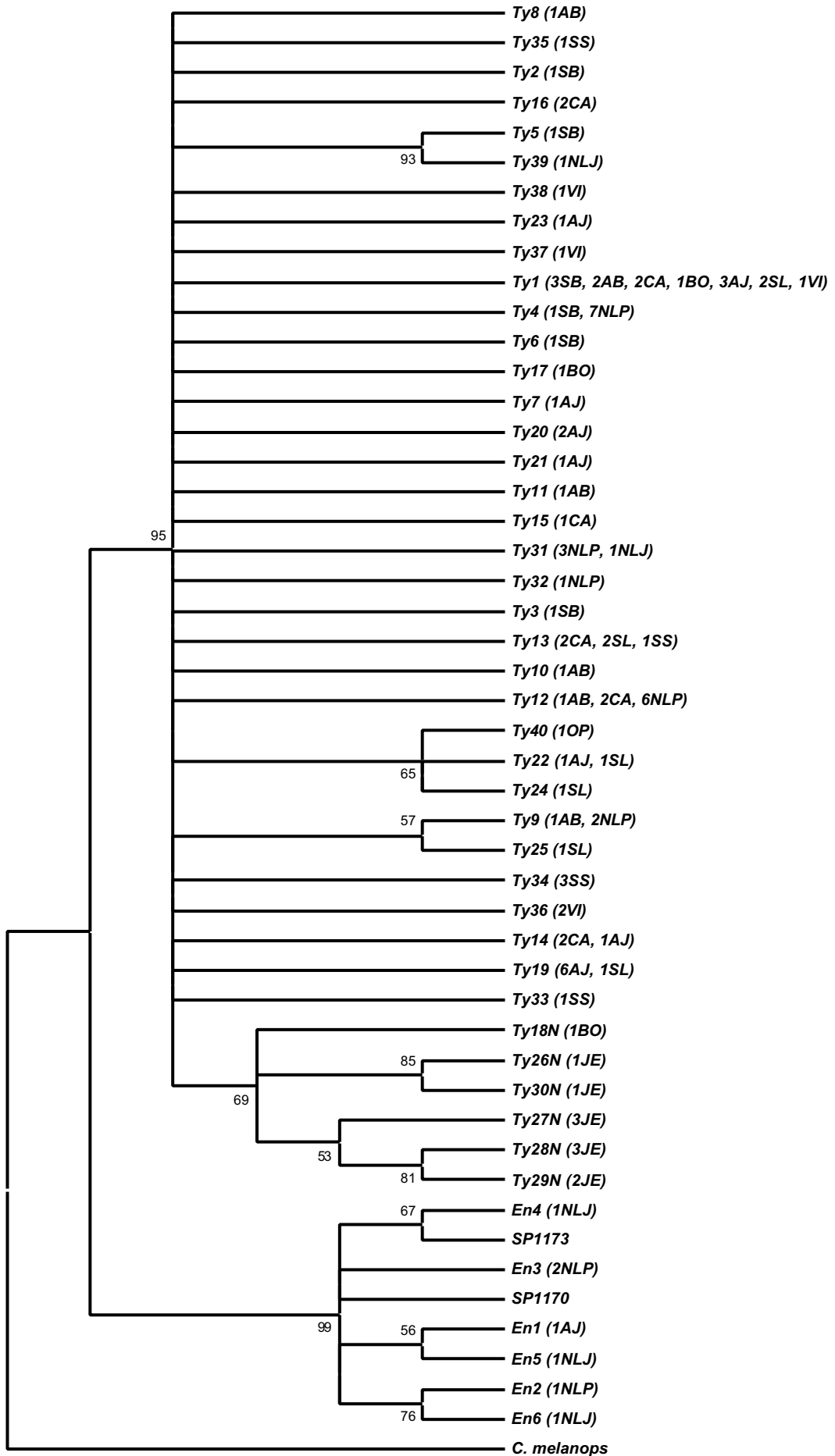


Fig. 3:



## CONCLUSÕES

- Foram eficientemente padronizados os protocolos de amplificação e seqüenciamento de região controle do DNA mitocondrial de *Conopophaga lineata*, o que possibilitou a análise de 1074 pb dos 104 indivíduos amostrados.
- A espécie *Conopophaga lineata* apresentou um alto índice de diversidade se comparada com outras espécies de passeriformes neotropicais.
- A alta diversidade da espécie justificou-se pela coexistência de dois clados em algumas populações do sudeste de Minas Gerais (MG), que apresentaram divergência compatível com a de duas espécies congênicas. Um destes clados formou um grupo monofilético com indivíduos coletados em São Paulo, na região da Serra do Mar.
- A coexistência dos dois clados pode ser explicada pela identificação errônea de duas espécies crípticas isoladas reprodutivamente, com uma só espécie. Outra explicação proposta foi que o sudeste de MG seria uma zona de contato secundário entre populações que se expandiram após um isolamento de aproximadamente 170 mil anos (época coincidente com a do Pleistoceno). Durante esta separação os dois clados teriam evoluído em diferentes refúgios pleistocênicos da Mata Atlântica, sendo um deles proposto como a Serra do Mar.
- Encontrou-se ainda uma forte estrutura filogeográfica entre as populações do sudeste e do nordeste de MG, que pode ser explicada pela grande distância geográfica e conseqüente baixo fluxo gênico. A estrutura hierárquica da diversidade genética também foi encontrada somente entre estas regiões (com um alto valor de  $\Phi_{st}$ ) e não entre todas as localidades ou populações.
- A diversidade nas populações, excluindo-se o clado EN também foi comparável ao encontrado na literatura para outros passeriformes neotropicais.
- Foi encontrado forte efeito de isolamento por distância para a espécie, observando-se uma alta correlação entre distâncias genéticas e geográficas entre populações.
- Ao contrário do que se esperava decorrente da fragmentação antrópica, não se verificou menor variabilidade nos fragmentos pequenos e a estrutura da diversidade genética entre fragmentos grandes apresentou-se mais forte do que entre os pequenos. Entretanto, o isolamento por distância só foi observado entre fragmentos grandes.
- Unidades evolutivamente significativas, importantes para a conservação de *C. lineata* e de outras espécies de suboscines da Mata Atlântica foram identificadas nas localidades de Jequitinhonha, Nova Lima e Araçuaia.

## REFERÊNCIAS BIBLIOGRÁFICAS

- Andrade, R. D. and Marini, M. Â. (2001) Bird movement between natural forest patches in southeast Brazil. In: *Ornitologia e Conservação: da ciência às estratégias*. (Albuquerque, J. L. B., Cândido Jr., J. F., Straube, F. C. and Roos, A. L. eds.) Editora Unisul, Tubarão, pp. 125-136.
- Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. and Saunders, N. C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst* 18: 489-522.
- Ayres, J.M., Fonseca, G.A.B. da, Rylands, A.B., Queiroz, H.L., Pinto, L.P. de S., Masterson, D. and Cavalcanti, R. (1997). *PP / G7 - Programa Piloto para a Proteção das Florestas Tropicais Brasileiras. Projeto Parques e Reservas - Abordagens Inovadoras para a Conservação da Biodiversidade do Brasil: Os Corredores Ecológicos do Brasil*. Versão 3.0. Vol. 2 - Mata Atlântica.
- Baker, A. J. and Marshall, H. D. Mitochondrial control region sequence as tools for understanding evolution (1997) In: *Avian molecular evolution and systematics*. (Mindell, D. P, ed.) Academic Press, San Diego, pp. 51-82.
- Frankham, R. (1995) Conservation genetics. *Ann. Rev. Genetics* 29: 305-327.
- Frankham, R., Ballou, J. D. and Briscoe, D. A. (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge.
- Haig, S. M. (1998) Molecular contributions to conservation. *Ecology* 79: 413-425
- Milligan, B.G., J. Leebens-Mack, and A.E. Strand (1994). Conservation genetics: beyond the maintenance of marker diversity. *Molecular Ecology* 3:423-435.
- Milot, E., Gibbs, H. L. & Hobson, K. A. (2000) Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). *Mol. Ecol.* 9: 667-681.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
- Pinto O (1978) *Novo catálogo das aves do Brasil*. Revista dos Tribunais, São Paulo.
- Ribon, R. (1998) *Fatores que influenciam a distribuição de avifauna em fragmentos de Mata Atlântica nas montanhas de Minas Gerais*. Dissertação apresentada para obtenção do título de Mestre ao Departamento de Biologia, UFMG, Belo Horizonte.
- Scharlemann, J.P.W., Green, R.E., and Balmford, A. (2004) Agricultural land has expanded six-fold since 1700 and Endemic Bird Areas have suffered disproportionately. In: *State of*

*the world's birds 2004: indicators for our changing world* (BirdLife International, ed) BirdLife International, Cambridge, pp. 32.

Sick, H. (1997). *Ornitologia brasileira*. Nova Fronteira, Rio de Janeiro.

Turner, I.M. (1996) Species loss in fragments of tropical rain forest: a review of the evidence. *J. Appl. Ecol.* 33: 200-209.

Whitney, B. M. (2003) Family Conopophagidae. In: *Handbook of the birds of the world: broadbills to tapaculos* v.8. (Del Hoyo, J., Elliot, A., Christie, D. A., eds.) Lynx Edicions, Barcelona, pp 732-747.

## **ANEXOS**



**ANEXO 1:** Lista de indivíduos utilizados, por população, com números das anilhas e do Banco de DNA do LBEM

População (Localidade)	Sigla	Anilha	Número no Banco de DNA
Fazenda da Mata Escura (Jequitinhonha)	JE	E40616	B0267
Fazenda da Mata Escura (Jequitinhonha)	JE	E40609	B0417
Fazenda da Mata Escura (Jequitinhonha)	JE	E40624	B0418
Fazenda da Mata Escura (Jequitinhonha)	JE	E40625	B0419
Fazenda da Mata Escura (Jequitinhonha)	JE	E40627	B0420
Fazenda da Mata Escura (Jequitinhonha)	JE	E40629	B0421
Fazenda da Mata Escura (Jequitinhonha)	JE	E40630	B0422
Fazenda da Mata Escura (Jequitinhonha)	JE	E40632	B0266
Fazenda da Mata Escura (Jequitinhonha)	JE	E40639	B0423
Fazenda da Mata Escura (Jequitinhonha)	JE	E40610	B0424
Fazenda Corredor (Bocaiúva)	BO	coletado	B0840
Fazenda Corredor (Bocaiúva)	BO	coletado	B0841
Fazenda Corredor (Bocaiúva)	BO	E62358	B1063
PE da Serra do Brigadeiro (Araponga)	AB	D39677	B0313
PE da Serra do Brigadeiro (Araponga)	AB	D39680	B0314
PE da Serra do Brigadeiro (Araponga)	AB	D39685	B0315
PE da Serra do Brigadeiro (Araponga)	AB	E41163	B0316
PE da Serra do Brigadeiro (Araponga)	AB	E41160	B0372
PE da Serra do Brigadeiro (Araponga)	AB	E41166	B0373
PE da Serra do Brigadeiro (Araponga)	AB	E41173	B0374
PE da Serra do Brigadeiro (Araponga)	AB	E41182	B0375
Mata do João Ribas (Araponga)	AJ	D39664	B0262
Mata do João Ribas (Araponga)	AJ	coletado	B0263
Mata do João Ribas (Araponga)	AJ	E41115	B0259
Mata do João Ribas (Araponga)	AJ	coletado	B0299
Mata do João Ribas (Araponga)	AJ	D39665	B0300
Mata do João Ribas (Araponga)	AJ	D39666	B0302
Mata do João Ribas (Araponga)	AJ	D39669	B0301
Mata do João Ribas (Araponga)	AJ	D39671	B0303
Mata do João Ribas (Araponga)	AJ	D39672	B0304
Mata do João Ribas (Araponga)	AJ	D39673	B0305
Mata do João Ribas (Araponga)	AJ	E41102	B0306
Mata do João Ribas (Araponga)	AJ	E41110	B0307
Mata do João Ribas (Araponga)	AJ	E41128	B0308

Mata do João Ribas (Araponga)	AJ	E41107	B0309
Mata do João Ribas (Araponga)	AJ	E51967	B0310
Mata do João Ribas (Araponga)	AJ	E51970	B0311
Mata do João Ribas (Araponga)	AJ	E51971	B0312
Viçosa - Mata 36	VI	E32952	B0376
Viçosa - Mata 36	VI	E32954	B0377
Viçosa - Mata 36	VI	E32955	B0378
Viçosa - Mata 36	VI	E32956	B0379
Viçosa - Mata 36	VI	E32961	B0380
RPPN do Sossego (Simonésia)	SS	E40584	B0394
RPPN do Sossego (Simonésia)	SS	E40572	B0247
RPPN do Sossego (Simonésia)	SS	E40575	B0258
RPPN do Sossego (Simonésia)	SS	E40587	B0248
RPPN do Sossego (Simonésia)	SS	E40591	B0251
RPPN do Sossego (Simonésia)	SS	E40651	B0252
Mata do Lula (Simonésia)	SL	B0391	B0391
Mata do Lula (Simonésia)	SL	B0392	B0392
Mata do Lula (Simonésia)	SL	B0393	B0393
Mata do Lula (Simonésia)	SL	E40659	B0253
Mata do Lula (Simonésia)	SL	E40660	B0254
Mata do Lula (Simonésia)	SL	E40661	B0255
Mata do Lula (Simonésia)	SL	E40675	B0257
Mata do Lula (Simonésia)	SL	E40677	B0256
EB de Caratinga (Caratinga)	CA	E52006	B0264
EB de Caratinga (Caratinga)	CA	E52017	B0265
EB de Caratinga (Caratinga)	CA	E52001	B0382
EB de Caratinga (Caratinga)	CA	E52002	B0383
EB de Caratinga (Caratinga)	CA	E52003	B0384
EB de Caratinga (Caratinga)	CA	E52005	B0385
EB de Caratinga (Caratinga)	CA	E52007	B0386
EB de Caratinga (Caratinga)	CA	E52014	B0387
EB de Caratinga (Caratinga)	CA	E52009	B0388
EB de Caratinga (Caratinga)	CA	E52015	B0389
EB de Caratinga (Caratinga)	CA	E52016	B0390
EPDA-PETI (Santa Bárbara)	SB	E60011	B0958
EPDA-PETI (Santa Bárbara)	SB	E60043	B0959
EPDA-PETI (Santa Bárbara)	SB	E60051	B0961
EPDA-PETI (Santa Bárbara)	SB	E60060	B0960

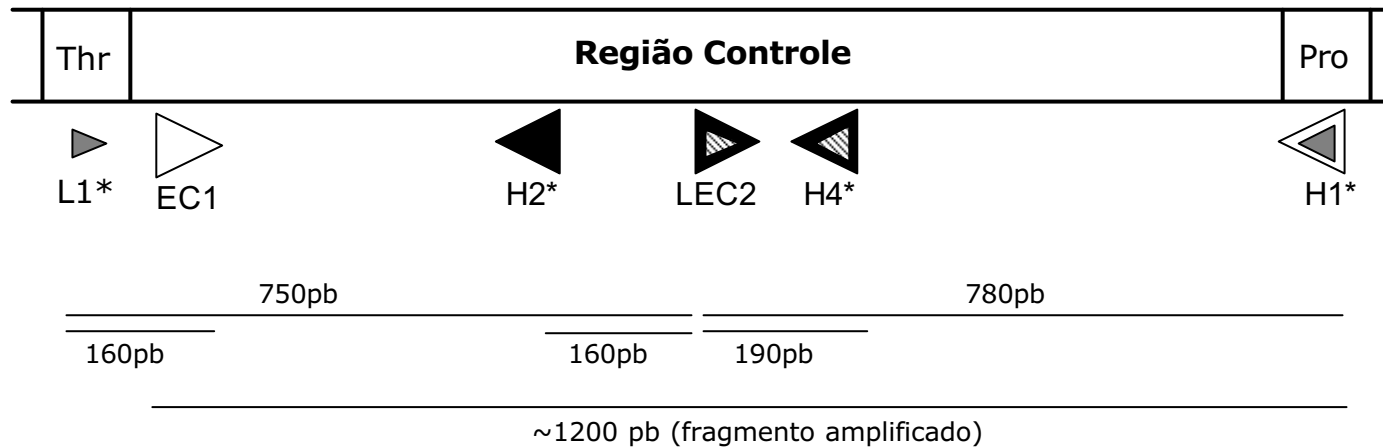
EPDA-PETI (Santa Bárbara)	SB	D34605	B0962
EPDA-PETI (Santa Bárbara)	SB	D34606	B0963
EPDA-PETI (Santa Bárbara)	SB	D34609	B1395
EPDA-PETI (Santa Bárbara)	SB	D34611	B1396
RPPN do Jambreiro (Nova Lima)	NLJ	D39691	B0413
RPPN do Jambreiro (Nova Lima)	NLJ	D39692	B0260
RPPN do Jambreiro (Nova Lima)	NLJ	D39693	B0414
RPPN do Jambreiro (Nova Lima)	NLJ	D39700	B0415
RPPN do Jambreiro (Nova Lima)	NLJ	D39696	B0416
Mata da Piedade (Nova Lima)	NLP	E32918	B0268
Mata da Piedade (Nova Lima)	NLP	E32906	B0381
Mata da Piedade (Nova Lima)	NLP	E32907	B0395
Mata da Piedade (Nova Lima)	NLP	E32908	B0396
Mata da Piedade (Nova Lima)	NLP	E32909	B0397
Mata da Piedade (Nova Lima)	NLP	E32910	B0398
Mata da Piedade (Nova Lima)	NLP	E32911	B0399
Mata da Piedade (Nova Lima)	NLP	E32912	B0400
Mata da Piedade (Nova Lima)	NLP	E32917	B0401
Mata da Piedade (Nova Lima)	NLP	E32978	B0402
Mata da Piedade (Nova Lima)	NLP	E32979	B0403
Mata da Piedade (Nova Lima)	NLP	E32980	B0412
Mata dos Primos (Nova Lima)	NLP	E32986	B0269
Mata dos Primos (Nova Lima)	NLP	E32982	B0404
Mata dos Primos (Nova Lima)	NLP	E32983	B0405
Mata dos Primos (Nova Lima)	NLP	E32985	B0406
Mata dos Primos (Nova Lima)	NLP	E32990	B0407
Mata dos Primos (Nova Lima)	NLP	E32991	B0408
Mata dos Primos (Nova Lima)	NLP	E32993	B0409
Mata dos Primos (Nova Lima)	NLP	E32994	B0410
Mata dos Primos (Nova Lima)	NLP	D50660	B0411
Mata dos Primos (Nova Lima)	NLP	D50642	B0425
Grande Mata (São Bartolomeu)	OP	SB-14 Preta dir	B0426

**ANEXO 2:** Tabela de haplótipos de região controle do DNA mitocondrial (1174 pb analisados) encontrados para *Conopophaga lineata* (excluindo-se os gaps de alinhamento) e número de indivíduos em cada população que os apresentam.

<b>Sítios Polimórficos</b>		
<b>Haplótipo</b>	<p style="text-align: right;">111111111111111</p> <p>11111111111222233333444445555566667777777777788888888888888888888888888889999999999000000000000000</p> <p>1355790111223347801780055667788458882383344556788901122222333446666789911255666800122223445667</p> <p>23441358151676926233062248023903120603577339272976013649356789239480237732812967126417925791459183</p>	<b>Nº de haplótipos em cada população</b>
HAP1	TACTTGCGACGCAGTTGGCCCGGTTTCTGGACCTCATGTCTGAACTGCCACTTTTTTTTGTAAATACCCCGTCTTGTTTTCTCCACTGCCCTTTATC	3SB, 1AB, 2CA, 3AJ, 1SL
HAP2	.....T.....A.....A.....T.....T.....	1SB
HAP3	.....C.....A.....	1SB
HAP4	.....A.....	1SB, 7NLP
HAP5	.CA..G.....A.....A.....C.T.....	1SB
HAP6	.....C.....	1SB
HAP7	.....A.....T.....A.....T.....	1AB
HAP8	.....T.....A.....A.....C.....A.....T.....	1AB
HAP9	.....A.....T.....T.....	1AB, 2NLP
HAP10	.....C.....T.....A.....	1AB
HAP11	.....T.....	1AB, 1BO, 1VI
HAP12	.CA.....A.....A.....	1AB
HAP13	.....C.....A.....	1AB, 2CA, 2NLP
HAP14	..A.....C.....T.....	2CA
HAP15	..A.....A.....A.....T.....	2CA, 1AB
HAP16	ACA.....A.....A.....A.....T.....T.....	1CA
HAP17	.....G.....T.....	2CA
HAP18	.....G.....CAC.....	1BO
HAP19	.....A.....TA.....CA.....C.....A.....A.....A.....T.....C.....	1BO*
HAP20	.....A.....AC.....T.....	6AJ, 1SL
HAP21	.....T.....	2AJ
HAP22	.....TC.....T.....	1AJ
HAP23	...A...A...AC...T.A.CA.AC.TA...CA.AATTGACATTG..CC.CC.....TC..T.AACT.CACC.CTCTTTT.CT.TC.CGCT	1AJ**
HAP24	.....C.....CA.....T.....	1AJ, 1SL
HAP25	.CA.....A.....A.....A.....C.....T.....	2AJ
HAP26	.....A.....	1SL
HAP27	.....C.....GCA.....T.....	1SL



**ANEXO 3:** Primers utilizados neste estudo para amplificação e seqüenciamento da região controle do DNA mitocondrial de *Conopophaga lineata* e *Conopophaga melanops*



□ Amplificação de *C. lineata*

▣ Seqüenciamento de *C. lineata*

■ Amplificação de *C. melanops*

▨ Seqüenciamento de *C. melanops*

**Seqüências dos Primers:**

L1(L16087)\*: 5' tggctctgtaaaccararactgaag 3'

H1(H16137)\*: 5' aaaatrycagctttgggagttg 3'

EC1: 5' tccacactcgacatctcatt 3'

H2(H'537)\*: 5' ctgaccgaggaaccagaggcgca 3'

LEC2: 5' tgtacctgttggtggtcggcgta 3'

H4(HFII-left)\*: 5' ttgacgaggtaaaaatgtct 3'

\*Lacerda (2004)

# Livros Grátis

( <http://www.livrosgratis.com.br> )

Milhares de Livros para Download:

[Baixar livros de Administração](#)

[Baixar livros de Agronomia](#)

[Baixar livros de Arquitetura](#)

[Baixar livros de Artes](#)

[Baixar livros de Astronomia](#)

[Baixar livros de Biologia Geral](#)

[Baixar livros de Ciência da Computação](#)

[Baixar livros de Ciência da Informação](#)

[Baixar livros de Ciência Política](#)

[Baixar livros de Ciências da Saúde](#)

[Baixar livros de Comunicação](#)

[Baixar livros do Conselho Nacional de Educação - CNE](#)

[Baixar livros de Defesa civil](#)

[Baixar livros de Direito](#)

[Baixar livros de Direitos humanos](#)

[Baixar livros de Economia](#)

[Baixar livros de Economia Doméstica](#)

[Baixar livros de Educação](#)

[Baixar livros de Educação - Trânsito](#)

[Baixar livros de Educação Física](#)

[Baixar livros de Engenharia Aeroespacial](#)

[Baixar livros de Farmácia](#)

[Baixar livros de Filosofia](#)

[Baixar livros de Física](#)

[Baixar livros de Geociências](#)

[Baixar livros de Geografia](#)

[Baixar livros de História](#)

[Baixar livros de Línguas](#)

[Baixar livros de Literatura](#)  
[Baixar livros de Literatura de Cordel](#)  
[Baixar livros de Literatura Infantil](#)  
[Baixar livros de Matemática](#)  
[Baixar livros de Medicina](#)  
[Baixar livros de Medicina Veterinária](#)  
[Baixar livros de Meio Ambiente](#)  
[Baixar livros de Meteorologia](#)  
[Baixar Monografias e TCC](#)  
[Baixar livros Multidisciplinar](#)  
[Baixar livros de Música](#)  
[Baixar livros de Psicologia](#)  
[Baixar livros de Química](#)  
[Baixar livros de Saúde Coletiva](#)  
[Baixar livros de Serviço Social](#)  
[Baixar livros de Sociologia](#)  
[Baixar livros de Teologia](#)  
[Baixar livros de Trabalho](#)  
[Baixar livros de Turismo](#)