The molecular cytogenetic characterization of *Conopophaga lineata* indicates a common chromosome rearrangement in the Parvorder Furnariida (Aves, Passeriformes)

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Abstract

Cytogenetic analyses of the Suboscines species are still scarce, and so far, there is no karyotype description of any species belonging to the family Conopophagidae. Thus, the aim of this study is to describe and analyze the karyotype of *Conopophaga lineata* by chromosome painting using *Gallus gallus* (GGA) probes and to identify the location of the 18/28S rDNA cluster. Metaphases were obtained from fibroblast culture from two individuals of *C. lineata*. We observed a diploid number of 2n=78. GGA probes showed that most ancestral syntenies are conserved, except for the fission of GGA1 and GGA2, into two distinct pairs each. We identified the location of 18S rDNA genes in a pair of microchromosomes. The fission of the syntenic group corresponding to GGA2 was observed in other Furnariida, and hence may correspond to a chromosomal synapomorphy for the species of Parvorder Furnariida.

Keywords: Birds, avian chromosomal evolution, chromosomes, rDNA.

Received: January 27, 2020; Accepted: April 12, 2020.
**Gallus gallus** probes (GGA) have presented conservation of ancestral macrochromosomes, except for ancestral pair 1-which corresponds to two pairs representing a synapomorphy for Passeriformes – and pair 2, which has undergone fission in *Satrapa icterophrys* (Parvorder Tyrannida), *Synallaxis frontalis* and *Glyphorynchus spirurus* (Parvorder Furnariida) (Rodrigues et al., 2017; Kretschmer et al., 2018b; Ribas et al., 2018). Additionally, the use of 18S rDNA probes has revealed that the number and distribution of NORs varies from 1-3 pairs in Passeriformes (Kretschmer et al., 2014, 2015; Rodrigues et al., 2017).

Despite these data, information on events occurring during the karyotype evolution of Passeriformes is still fragmentary, as observed in most groups of birds. In this sense, studies involving species from basal clades are important to reconstruct the sequence of rearrangements arising during Passeriformes diversification. Considering that Conopophagidae represents one of the most basal lineages of passerines (Selvatti et al., 2015), a detailed study of one species of this family may shed some light on the chromosome evolution of Passeriformes. Hence, we describe here for the first time the rufous gnateater (*Conopophaga lineata*).

The protocols were approved by the Committee of Ethics on the use of Animals (CEUA- Universidade Federal do Pampa, 026/2012), and SISBIO (Permission Number: 101 33860-4). Skin biopsies were collected from two females of *C. lineata* in Porto Vera Cruz and São Gabriel (Rio Grande do Sul, Brazil), and used for cell culture, following Sasaki et al. (1968), with modifications. In this process, cells were dissociated with collagenase type IV (Sigma) and grown in DMEM medium supplemented with fetal bovine serum (20%). Chromosome preparations were obtained after exposure to colcemid (1 h, 37 °C), hypotonic treatment (0.075M KCl, 15 min, 37 °C) and methanol/acetic acid (3:1) fixative.

Fluorescence in situ hybridization (FISH) experiments were performed using whole chromosome probes from *Gallus gallus* (GGA 1-10), obtained by flow cytometry at the Cambridge Resource Centre for Comparative Cytogenetics, (Cambridge, UK), amplified and labeled with biotin by DOP-PCR. Hybridizations were carried out according to Oliveira et al. (2010). Detection was performed with the use of Streptavidin-CY3 (Invitrogen). 18S rDNA probe fragments were labeled with digoxigenin by Nick Translation (Nick Translation Kit, Roche) and detected with Anti-Digoxigenin-Rhodamine, following the manufacturer’s instructions, slide preparation, hybridization and washing were performed according to Daniels and Delany (2003).

Approximately 30 mitotic metaphases from each specimen were analyzed in order to determine the diploid number, chromosome morphology and confirm FISH experiments. Metaphases were analyzed in an epifluorescence light microscope (Imager Z2, Zeiss, Germany), and the images were acquired with the software Axiovision 4.8 (Zeiss, Germany).

The diploid number of *C. lineata* is 78. Pairs 1 to 7 are acrocentric, except for pair 4, which is submetacentric. The other autosomal chromosomes are telocentric, while the Z sex chromosome is submetacentric and W sex chromosome possibly is a telocentric microchromosome (Figure 1).

GGA probes 1-10 produced 13 different signals, revealing chromosome rearrangements. Most of the ancestral macrochromosomes are conserved in *C. lineata*, except for GGA1 and GGA2, which are fisioned in two pairs each. GGA 4 probe hybridized to two chromosome pairs, as in the putative bird ancestral karyotype. GGA3 and 5-10 hybridized to only a single pair each, revealing conserved syntenies. In addition, CLI 5 is the result of a fusion between a segment of GGA2 and an unidentified chromosome, possibly a microchromosome (Figures 2A,B and 3).

The diploid number observed, 2n = 78, is found in most bird species and is similar to the hypothetical bird ancestor (80 chromosomes) (Griffin et al., 2007). It was possible to observe that the first and second pairs have a similar size, differently from most of Passerines studied so far (Kretschmer et al., 2014; Santos et al., 2017), indicating an additional fission in *Conopophaga lineata*.

In fact, FISH results (Figure 2) revealed that GGA 1, 2 and 4 probes hybridized on two chromosome pairs each, whereas all other probes hybridized to only one chromosome pair each. While GGA1 fission is commonly found in Passeriformes and considered a synapomorphy for this group (Kretschmer et al., 2015; Santos et al., 2017), the hybridization of GGA4 to two chromosome pairs - CLI4 and CLI13 (Figure 2D) - is common to most birds, representing the ancestral state, and hence, in *G. gallus* this pair is the result of the fusion of two chromosomes of the putative avian ancestral karyotype (PAK), PAK4 and PAK10 (Griffin et al., 2007, Kretschmer et al., 2018a). Additionally, centric fission of GGA1 is also observed in species of the orders Strigiformes, Psittaciformes, Falconiformes, and Accipitriformes (Guttenbach et al., 2003; Oliveira et al., 2005, 2008; Nanda et al., 2006, 2007).

Interestingly, the fission of GGA2, into two chromosomes in *C. lineata* (CLI1 and CLI5q) (Figure 3), is atypical for Passeriformes; normally GGA2 is conserved and corresponds to the largest pair (Table 1) (Kretschmer et al., 2014, 2015; Santos et al., 2017). Moreover, the centric fission of GGA2 was observed in other Suboscines species, belonging to parvorder Furnariida - *Synallaxis frontalis* (Kretschmer et al., 2018b) and *Glyphorynchus spirurus* (Ribas et al., 2018).

![Figure 1 - Metaphase and partial karyotype of a female specimen of Conopophaga lineata. *It was not possible to identify the W sex chromosome.](image-url)
Molecular cytogenetic in *C. lineata*

-, and parvorder Tyrannida - *Satrapa icterophrys* (Rodrigues *et al.*, 2017), which also shows pairs 1 and 2 with similar sizes, as in *C. lineata*. Hence, this fission explains the minimum size difference between the first and second pairs in other Suboscines species in which only classical cytogenetic data (Giemsa staining and chromosome banding) are available, such as *Sittasomus griseicapillus*, *Lepidocolaptes angustirostris* (Dendrocolaptidae) and *Pyriglena leucoptera*, *Dysithamnus mentalis* (Formicariidae) – all of them are members of Parvorder Furnariida (Ledesma *et al.*, 2002; Moyle *et al.*, 2009; Barbosa *et al.*, 2013; Kretschmer *et al.*, 2018b). Consequently, GGA2 fission in species of parvorder Furnariida and in *Satrapa icterophrys* of parvorder Tyrannida may be indicative of convergent evolution (Table 1).

![Figure 2 - Representative FISH experiments with GGA1 (A), GGA2 (B), GGA3 (C), GGA4 (D), GGA5 (E) and 18S rDNA probes (F) in metaphases of Conopophaga lineata. Arrows indicate the homologous chromosomes to the probes used.](image)

![Figure 3 - Homology map of Conopophaga lineata with Gallus gallus (GGA) probes indicated by color. *Not hybridized segment with any GGA probes used.](image)
In addition to the fission of GGA2, we have identified that pair 5 of C. lineata was formed from a fusion between one of the segments originated from the GGA2 fission and a microchromosome (Figure 3).

Despite the fact that these rearrangements have been observed in a species belonging to the basal family Conopophagidae, the localization of ribosomal clusters in a pair of microchromosomes, corresponds to a plesiomorphic characteristic, usually observed in the order Passeriformes and in other avian orders, demonstrating the conservation of the ancestral state (Figure 2F) (Nishida-Umehara et al., 2007; Oliveira et al., 2017; Santos et al., 2017).

In conclusion, we demonstrate that the morphology of macrochromosomes in C. lineata is significantly different from other Passeriformes species. Furthermore, we found a fission in GGA2, which appears to be a common chromosome rearrangement in Furnariidae and possibly other Parvorder Furnariida species that have minimal size difference between the first chromosomal pairs, in addition to the fissions that are typically found in Passeriformes (GGA1). However, since passerines present a high degree of chromosomal rearrangement, subsequent mapping and sequencing studies allowing the investigation of intrachromosomal rearrangements may elucidate these events.

Acknowledgments

The authors would like to thank the Group of “Diversidade Genética Animal” and laboratory “Cultura de Tecidos e Citogenética” SAMAM of the Evandro Chagas Institute for technical and financial support. CAPES and CNPq for the scholarships.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

RJG, EHCO, TDO conceived and designed the study; TDO, RK, NAB, PCMO, ADVG performed the experiments; TDO, RK, NAB wrote the manuscript; MAFS, PCMO English and critical review. All authors read and approved the final version.

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Table 1 - Rearrangements in putative avian ancestral karyotype homologous segments (PAK1-10) in Suboscines species.

| Parvorders | Species | Rearrangements | References |
|------------|---------|----------------|------------|
| Tyrannida  | Elaenia spectabilis | fission PAK1 (ESP2 and 5) | Kretschmer et al., 2015 |
| Tyrannida  | Pitangus sulphuratus | fission PAK1 (PSU3 and 5) | Rodrigues et al., 2018 |
| Tyrannida  | Seropophaga suberistata | fission PAK1 (SSU3 and 5) | Rodrigues et al., 2018 |
| Furnariida | Synallaxis frontalis | fission PAK1 (SFR1 and 5) | Kretschmer et al., 2018b |
| Furnariida | Glyphorynchus spururus | fission PAK1 (GSP3 and 4) | Ribas et al., 2018 |
| Furnariida | Conopophaga lineata | fission PAK1 (CLI2 and 7) | Present study |

| Author contributions |

RJG, EHCO, TDO conceived and designed the study; TDO, RK, NAB, PCMO, ADVG performed the experiments; TDO, RK, NAB wrote the manuscript; MAFS, PCMO English and critical review. All authors read and approved the final version.
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Associate Editor: Marcelo Guerra

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