Cytogenetic and Genome Research

Original Article

Cytogenet Genome Res 2024;164:43–51
DOI: 10.1159/000538586

Received: December 26, 2023
Accepted: March 26, 2024
Published online: March 28, 2024

Comparative Cytogenetics in Tyrannidae (Aves, Passeriformes): High Genetic Diversity despite Conserved Karyotype Organization

Diego Madruga Saraiva[a] Marcelo Santos de Souza[a] Victoria Tura[a] Vitor Oliveira de Rosso[a] Edison Zefa[b] Analía del Valle Garnero[a] Ricardo José Gunski[a] Francisco de Menezes Cavalcante Sassi[c] Marcelo de Bello Cioffi[c] Rafael Kretschmer[b]

[a]Laboratório de Diversidade Genética Animal, Universidade Federal do Pampa, São Gabriel, Brazil; [b]Departamento de Ecologia, Zoologia e Genética, Universidade Federal de Pelotas, Pelotas, Brazil; [c]Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Paulo, Brazil

Keywords
Avian genome · Avian cytogenetics · Comparative cytogenetics · rDNA · Microsatellite sequences

Abstract
Introduction: Passeriformes has the greatest species diversity among Neoaves, and the Tyrannidae is the richest in this order with about 600 valid species. The diploid number of this family remains constant, ranging from $2n = 76$ to 84, but the chromosomal morphology varies, indicating the occurrence of different chromosomal rearrangements. Cytogenetic studies of the Tyrannidae remain limited, with approximately 20 species having been karyotyped thus far. This study aimed to describe the karyotypes of two species from this family, *Myiopagis viridicata* and *Sirystes sibilator*. ***Methods:*** Skin biopsies were taken from each individual to establish fibroblast cell cultures and to obtain chromosomal preparations using the standard methodology. The chromosomal distribution of constitutive heterochromatin was investigated by C-banding, while the location of simple repetitive sequences (SSRs), 18S rDNA, and telomeric sequences was found through fluorescence in situ hybridization. ***Results:*** The karyotypes of both species are composed of $2n = 80$. The 18S rDNA probes hybridized into two pairs of microchromosomes in *M. viridicata*, but only a single pair in *S. sibilator*. Only the telomeric portions of each chromosome in both species were hybridized by the telomere sequence probes. Most of the SSRs were found accumulated in the centromeric and telomeric regions of several macro- and microchromosomes in both species, which likely correspond to the heterochromatin-rich regions. ***Conclusion:*** Although both species analyzed showed a conserved karyotype organization ($2n = 80$), our study revealed significant differences in their chromosomal architecture, rDNA distribution, and SSR accumulation. These findings were discussed in the context of the evolution of Tyrannidae karyotypes.

Introduction

While bird species exhibit an extensive range of variation in behavior, morphology, and ecology [1], their chromosomal history shows an intriguing degree of evolutionary stability. About 40.6% of the species that

karger@karger.com
www.karger.com/cgr

Correspondence to:
Rafael Kretschmer, rafael.kretschmer@ufpel.edu.br

Karger

have been studied thus far have between 78 and 80 chromosomes, therefore indicating that the avian karyotypes are stable [2]. Except for a few exceptions [3], most birds have a simple ZZ/ZW sex-determination system, where the Z chromosome is relatively conserved and typically larger than the W chromosome [4, 5]. In contrast, the W chromosome shows significant variation, typically being small and heterochromatic, as observed in Passeriformes [5–8].

With over 5,700 species, representing 60% of all bird species currently described, Passeriformes is highly diverse and includes two major suborders, named Oscines and Suboscines [9]. Among Suboscines, Tyrannidae contains about 600 species [10] and represents one of the most diverse and numerous families. For the cytogenetic information, they exhibit a high degree of stability in their diploid numbers ($2n$ = 76–84). Nonetheless, they also show variations in chromosomal morphologies, suggesting the likelihood of various intrachromosomal rearrangements [11, 12]. However, only about 20 Tyrannidae species have been karyotyped thus far, implying a dearth of cytogenetic studies on this family [2].

A variety of repetitive elements can be found in eukaryotic genomes, and although bird genomes are generally rather repeat-poor, certain genomic areas (i.e., centromeres and telomeres) are almost exclusively made up of repetitive sequences [13]. Except for woodpeckers, most birds have a low number of repetitive DNAs compared to other vertebrates, as well as a compact genome that has predominantly originated from lineage-specific modification of repetitive elements, large segmental deletions, and gene loss [14]. Recent studies using microsatellite probes in some species of Columbidae, Picidae, Nyctibiidae, and Psittacidae have shown the importance of these elements in genome organization, as well as in sex chromosome differentiation [15–18]. For example, a large accumulation of microsatellite sequences on the Z chromosome has been suggested as the main cause of this chromosome's enlargement in woodpeckers and toucans [15, 17].

Nearly all the studies employing microsatellite probes on Passeriformes species were conducted on Oscines species [19, 20]. These studies evidenced an accumulation of microsatellite sequences mainly in centromeric, pericentromeric, or telomeric regions of macro- and microchromosomes. A substantial accumulation of repetitive sequences was identified along the length of the W chromosome in three species of swallows, which had a larger W chromosome than is typically found in birds [19]. On the other hand, these sequences accumulated in the short arms of the Z chromosome in the saffron finch species [17]. To date, there is only a single published study on repeated sequences in Suboscines, showing that microsatellites are scattered throughout the macro- and microchromosomes of different species of Furnariidae [21].

Other repetitive elements, such as telomeric and rDNA sequences, are also frequently mapped in bird chromosomes. Most Passeriformes species exhibit signals of 18S rDNA on a pair of microchromosomes [22], which has been postulated as the ancestral state of birds as observed in basal groups as the emu (*Dromaius novaehollandiae*) [23]. Although most Oscines species present several rDNA loci, including *Gubernatrix cristata*, *Serinus canaria*, and *Turdus albicollis* [11, 24, 25], the Suboscines demonstrate the ancestral pattern, with few exceptions as seen in *Dendrocolaptes platyrostris* (Furnariidae) chromosomes [26]. Regarding the telomeric sequences, it has been found on the telomeres of all chromosomes, with stronger signals on microchromosomes, which may contribute to the high rate of meiotic recombination observed in these elements [27, 28].

We used conventional and molecular cytogenetic techniques in two Tyrannidae species, *Sirystes sibilator* and *Myiopagis viridicata*, to gain an in-depth understanding of the chromosomal evolution in these species. The aforementioned methods were used to investigate their telomeric sequence organization, repetitive DNA distribution, and karyotypic structure.

Methods

Samples, Chromosomal Preparations, and C-Banding

Two species from the Tyrannidae, one female of *S. sibilator* and a male of *M. viridicata*, were collected in Porto Vera Cruz, State of Rio Grande do Sul, Brazil. The birds were captured in their natural habitat using mist nets. Skin biopsies were taken from each individual to establish fibroblast cell cultures, following the method described by Sasaki [29]. Chromosomal preparations were obtained using the standard methodology with colchicine (0.05%), hypotonic treatment, and cell fixation in methanol-acetic acid (3:1).

Karyotype description and diploid numbers were obtained by analyzing 50 metaphases per specimen, using conventional staining of 5% Giemsa in phosphate buffer, pH 6.8. The chromosomes were classified according to Guerra's nomenclature [30] as metacentric, submetacentric, acrocentric, and telocentric. Blocks of constitutive heterochromatin were detected through C-banding [31].

Fluorescence in situ Hybridization

For the detection of 45S DNA (rDNA), we isolated the 18S rDNA gene sequence from the *Ocyurus chrysurus* genome (Perciformes: Lutjanidae) [32] and labeled it in green using nick-translation with Atto448-dUTP, following the manufacturer's instructions (Jena Biosciences, Jena, Germany). The repetitive

sequences $(GA)_n$, $(GC)_n$, $(TA)_n$, $(CAT)_n$, $(GAC)_n$, and $(GAG)_n$ were directly labeled with Cy-3 during synthesis [33]. These repetitive sequences were chosen because it is the most abundant sequences in birds' chromosomes according to previous studies (15–21). Biotinylated telomere repeat probes (TTAGGG)n were generated by polymerase chain reaction in the absence of templates, according to Ijdo et al. [34], and detected by streptavidin Cy-3. The FISH experiments were performed following the methodology described by Kretschmer et al. [35]. The chromosomes were denatured by immersion in 70% formamide (70 mL formamide and 30 mL 2xSSC) at 72°C for 1 min 20 s, and the probes were denatured at 86°C for 10 min and then cooled to 4°C until the hybridization. The chromosomes were counterstained with VECTASHIELD with DAPI (Vector Laboratories, Burlingame, CA, USA). The FISH experiment was performed at least twice to confirm the hybridization signals, and in addition, the number of metaphases analyzed to establish the pattern obtained for each microsatellite probe ranged from 15 to 20.

Image Processing

The images were captured using an Olympus B ×53 microscope (Olympus Corporation, Ishikawa, Japan) coupled with CoolSNAP and processed using ISIS software (MetaSystems). The GNU Image Manipulation Program (GIMP) software was used to assemble the karyotypes.

Results

Karyotypic Description and C-Banding

Karyotype of *S. sibilator* is composed of $2n = 80$ (Fig. 1a), with chromosome pair 1 as submetacentric, pairs 2–7 as acrocentric, and the remaining autosomal pairs are telocentric. The Z is a medium-sized acrocentric, whereas the W is a small-sized metacentric chromosome. *M. viridicata* also present $2n = 80$ (Fig. 1b), but the pair 8 is metacentric, instated of telocentric as in *S. sibilator*. The Z is a medium-sized acrocentric. The W chromosome of *M. viridicata* could not be investigated in this study as only a male individual was obtained for analysis. C-positive heterochromatin-rich regions were detected in several microchromosome pairs and in the telomeric and centromeric regions of most macrochromosomes in both species (Fig. 2). The W chromosome of *S. sibilator* was almost entirely heterochromatic (Fig. 2a).

Chromosomal Distribution of Simple Repetitive Sequences, rDNA, and Telomeric Sequences

The simple repetitive sequences (SSRs) are primarily hybridized with both microchromosomes and macrochromosomes, while no labeling was observed in the W sex chromosomes of *S. sibilator* (Fig. 3). Table 1 provides a summary of the SSR data. Among the five sequences used, $(TA)_n$, $(CAT)_n$, and $(GAG)_n$ did not hybridize in either species; additionally, $(GC)_n$ also did not produce FISH signals in *S. sibilator* (data not shown).

The 18S rDNA probes hybridized in only one pair of microchromosomes in *S. sibilator*, while in *M. viridicata* it was found in two pairs of microchromosomes (Fig. 4). Regarding the telomeric FISH results, no interstitial telomeric sequences (ITSs) were observed in either of the two species. The probes containing telomeric sequences produced signals only in the distal region of the chromosome arms and tended to be more prominent in the microchromosomes than in the macrochromosomes (Fig. 4).

Discussion

Despite the extensive diversity of birds, there is currently a lack of available cytogenetic data. This is particularly the case for the Tyrannidae, where most of the research has relied solely on conventional methods [12, 36–38]. This study examined the chromosomal and genomic organization of two Tyrannidae species with an emphasis on their repetitive DNA content. To gain insight into their karyotypic and evolutionary aspects, we analyzed the distribution of constitutive heterochromatin regions, as well as the localization of microsatellites, 18S rDNA, and telomeric sequences.

The vast majority of cytogenetically examined Passeriformes species have a typical karyotype with around 80 chromosomes, split into macro- and microchromosomes [2, 39, 40]. Similarly, both species investigated in this study exhibited $2n = 80$, with 39 pairs of autosomes and one pair of sex chromosomes. Constitutive heterochromatin is evidenced on the q and p arms, as well as in the centromeric regions of nearly all the autosomes in both species. In addition, *M. viridicata* showed a heterochromatin block on the Z chromosome, while the W chromosome of *S. sibilator* is nearly heterochromatic. On the other hand, constitutive heterochromatin is only present in the centromeres of most macro- and microchromosomes in the Tyrannidae species *Pitangus sulphuratus*, *Serpophaga subcristata*, and *Satrapa icterophrys* [38]. Indeed, interspecific variations of heterochromatin have also been observed for other bird lineages [41]. These results imply that constitutive heterochromatin accumulation actively influences the karyotype evolution in Tyrannidae lineage.

The 18S rDNA gene was identified within a single and in two pairs of microchromosomes in *S. sibilator* and *M. viridicata,* respectively. Given that both species have the same diploid number, *M. viridicata* may have duplicated

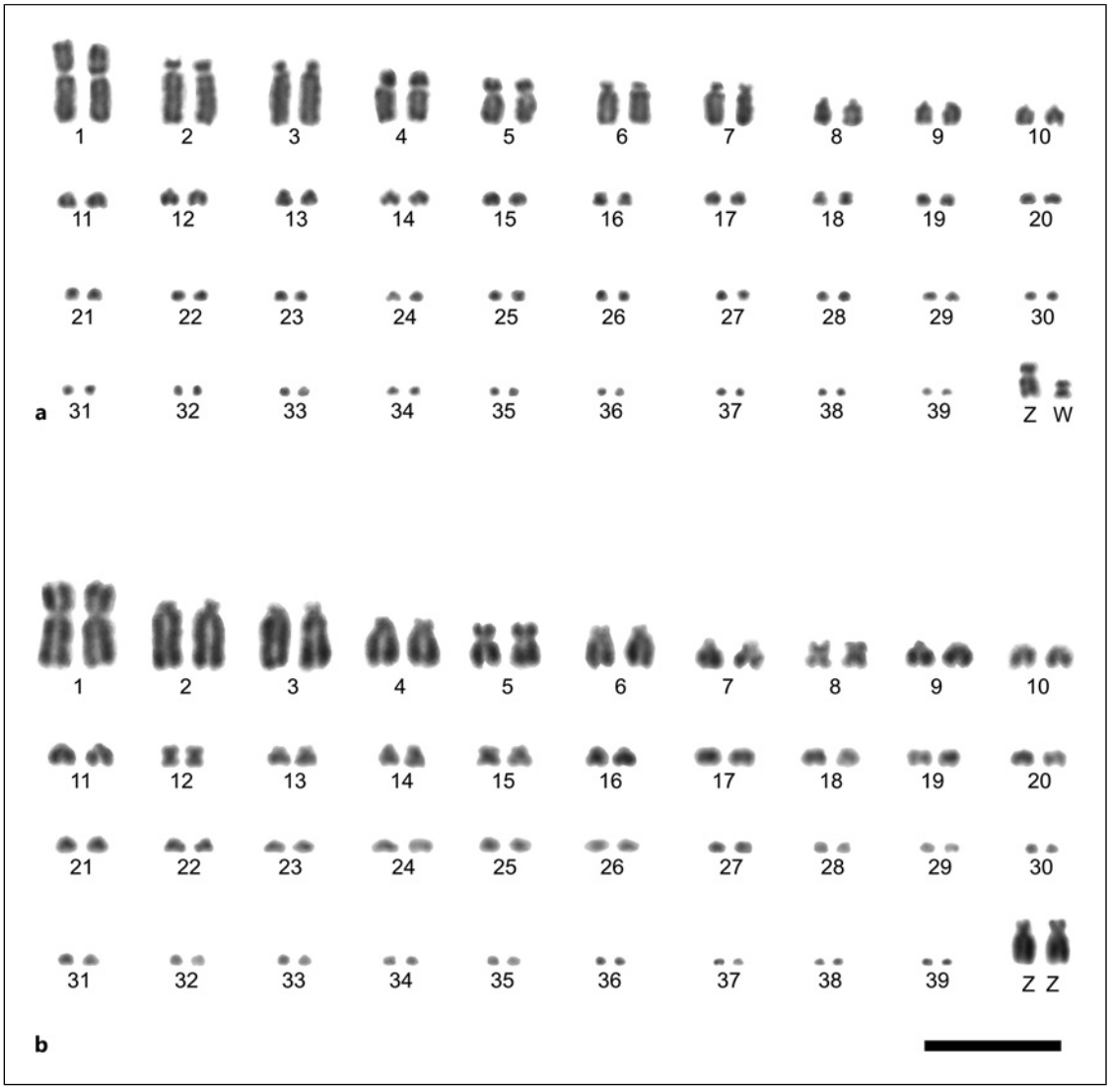

Fig. 1. Giemsa-stained complete karyotypes of female *Sirystes sibilator* (**a**) and male *Myiopagis viridicata* (**b**). Scale bar = 10 μm.

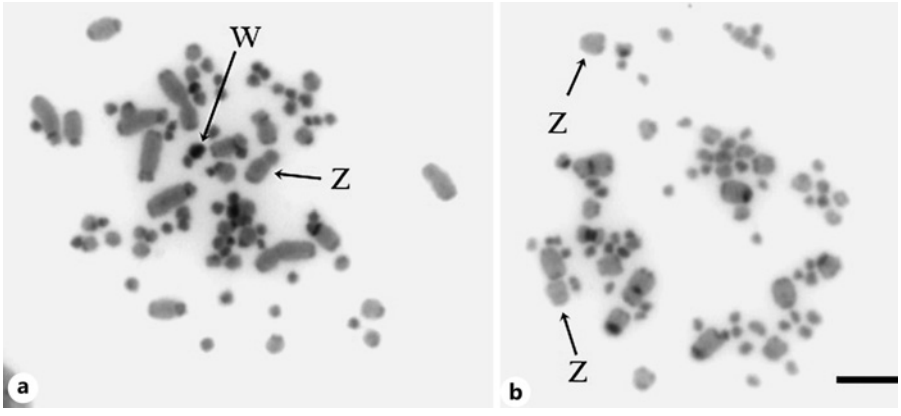

Fig. 2. C-banding patterns in *Sirystes sibilator* (**a**) and *Myiopagis viridicata* (**b**). Sex chromosomes are indicated by arrows. Scale bar = 10 μm.

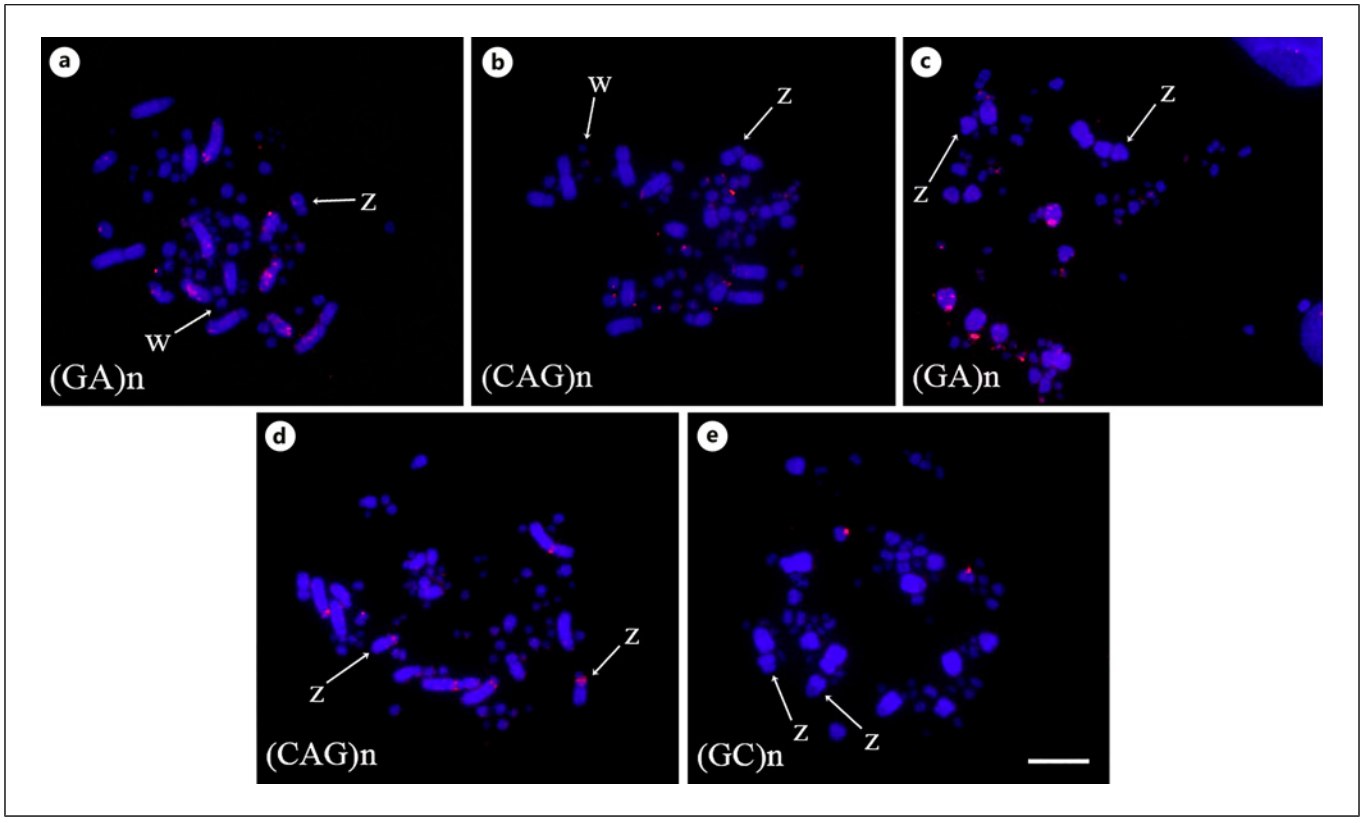

Fig. 3. Distribution of SSRs on *Sirystes sibilator* (**a**, **b**) and *Myiopagis viridicata* (**c**–**e**). Chromosomes were counterstained with DAPI (blue). Sex chromosomes are indicated by arrows. Scale bar = 10 μm.

Table 1. Distribution of SSRs on *Sirystes sibilator* and *Myiopagis viridicata*

SSRs	Species	
	S. sibilator	*M. viridicata*
$(CAG)_n$	Centromere of pairs 2, 6, and 8, centromeric region of three pairs of microchromosomes and weak signals in some additional microchromosome pairs	Centromere of pairs 1, 2, and Z chromosome
$(GA)_n$	Telomeric region pair 2, pericentromeric region pair 4, terminal region pair 5q, some microchromosome pairs	Centromere and telomere of pair 2 and some microchromosome pairs
$(GC)_n$	No signals	Centromere of a medium-size telocentric chromosome
$(CAT)_n$	No signals	No signals
$(GAG)_n$	No signals	No signals
$(TA)_n$	No signals	No signals

and translocated this sequence. It is noteworthy to point out that all species of Tyranninae and Fluvicolinae subfamilies examined, including *S. sibilator*, have consistently shown one microchromosome with 18S clusters, suggesting that the ancestral state has been conserved (Fig. 5). However, species in the subfamily Elaeniinae,

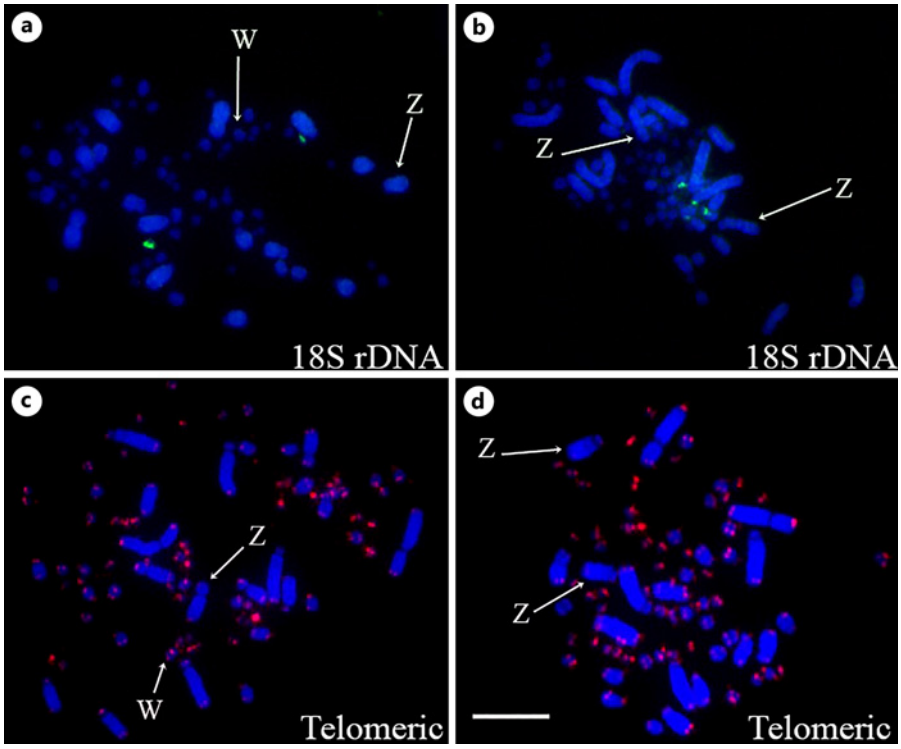

Fig. 4. FISH with 18S rDNA probe (green) in telomeric probes (red) in *Sirystes sibilator* (**a**, **c**) and *Myiopagis viridicata* (**b**, **d**). Chromosomes were counterstained with DAPI (blue). Scale bar = 10 μm.

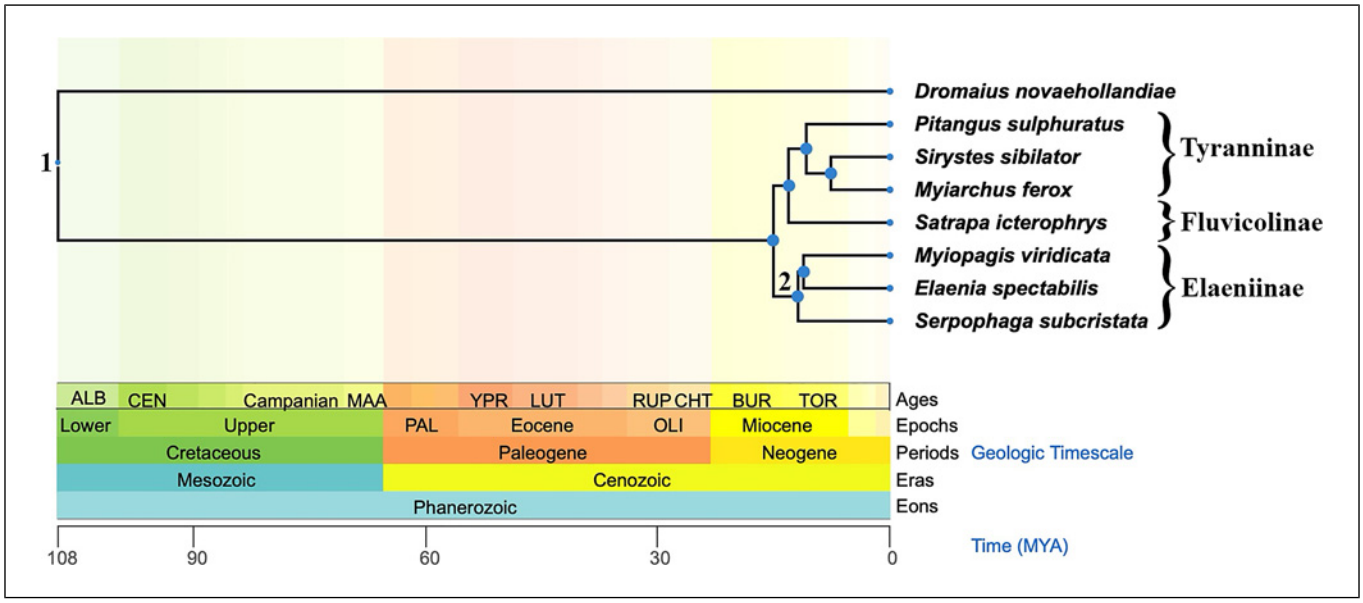

Fig. 5. Phylogenetic distribution of 18S rDNA clusters in Tyrannidae species. The ancestral state, as exemplified by *Dromaius novaehollandiae*, of a single pair of microchromosomes (identified as number 1 in the phylogeny) with 18S rDNA clusters is maintained in members of Tyranninae and Fluvicolinae subfamilies. An apomorphic state of two pairs of microchromosomes (identified as number 2 in the phylogeny) with this sequence is found in Elaeniinae subfamily. The phylogenetic tree was sourced from TimeTree databases (http://www.timetree.org, accessed on December 12, 2023) [42]. ALB, Albian; CEN, Cenomanian; MAA, Maastrichtian; YPR, Ypresian; LUT, Lutetian; RUP, Rupelian; CHT, Chattian; BUR, Burdigalian; TOR, Tortonian; PAL, Paleocene; OLI, Oligocene.

among which *M. viridicata* is a notable example, have shown a unique pattern, with two pairs of microchromosomes bearing 18S rDNA clusters, indicating an apomorphic condition (Fig. 5). Although the genomic arrangement of these sequences is dynamic, they are limited to a pair of microchromosomes in most bird species, particularly primitive ones like *D. novaehollandiae* [23]. This points to a plausible scenario where the duplication and translocation of the 18S rDNA sequence likely occurred within the last common ancestor of the Elaeniinae, considering its prevalence across all investigated species within this subgroup (Fig. 5).

While certain bird species, such as Passeriformes like *Turdus iliacus* and *Fringilla coelebs*, have ITSs [43], the two species under investigation here only displayed telomeric FISH signals in terminal regions of the chromosomes. ITS has been reported in several bird species, particularly in more basal groups like Palaeognathae, as a result of chromosomal fusions. Its gradual elimination has been noted during the divergence of Palaeognathae and Neognathae [28, 44]. Therefore, ITSs have been interpreted as an indication of the occurrence of interchromosomal rearrangements. According to chromosomal painting research, chromosome fusions did not occur in any Tyrannidae species up to now analyzed [12, 38]. Our results showing the lack of ITS further support this conclusion. However, it is noteworthy that certain species have shown no ITS in chromosomes resulting from chromosome fusions [44–46]. Likewise, in all bird species that have been investigated up to this point, we noticed strong telomeric FISH signals on most microchromosomes. This discovery implies a possible connection to the remarkably high meiotic recombination rates found in the microchromosomes of birds [28].

Our findings indicate that each Tyrannidae species exhibits a distinct pattern of SSR hybridization. Generally, these sequences were amplified in heterochromatic regions, in either pericentromeric or telomeric regions, present in both macrochromosomes and microchromosomes. Repetitive DNA is thought to be located in less active and condensed portions of the genome, which is consistent with the aforementioned pattern [47, 48]. In contrast to Suboscines species from the Furnariidae family, the W chromosome of *S. sibilator* did not accumulate SSR signals [21]. On the other hand, Oscines species displayed a large accumulation of these sequences on the W chromosome [19]. Although these chromosomes display a common origin in birds, these observations point to their different evolutionary paths, even among species that belonged to the same order (e.g., Passeriformes), highlighting the significant variability in sex chromosome differentiation within this group of birds.

To summarize, we described and characterized the karyotypes of two distinct species belonging to the Tyrannidae family: *S. sibilator* (Tyranninae) and *M. viridicata* (Elaeniinae). Both species have $2n = 80$, a diploid number that is in line with the usual range of passerines. Our research showed significant differences in their chromosomal architecture, rDNA distribution, and SSR accumulation. We examined these findings in the context of the evolution of Tyrannidae karyotypes. These results highlight the various evolutionary histories across the members of the Tyrannidae family and throw insight into unique evolutionary paths within each subfamily.

Statement of Ethics

Samples were collected according to licenses SISBIO 61047-3, 33860-2, and 81564-1, and the animal research ethics committee (CEUA 019/2020).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), finance Code 001 (D.M.S., M.S.d.S., V.T., and V.O.R.) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant No. 302928/2021-9 (M.B.C.), 407285/2021-0 and 306717/2022-0 (A.D.V.G.), and 302835/2021-0 (E.Z).

Author Contributions

D.M.S. and R.K. contributed to the study conception and design and wrote the original draft. Methodology was performed by D.M.S., M.S.S., V.T., V.O.R., F.M.C.S., and R.K. Formal analysis, data curation, and investigation were performed by D.M.S., M.S.S., V.T., V.O.R., and F.M.C.S. Visualization was performed by D.M.S., M.S.S., and R.K. Writing – review and editing – was performed by D.M.S., R.K., E.Z., A.V.G., R.J.G., and M.B.C. Funding was obtained by R.K., E.Z., A.V.G., R.J.G., and M.B.C. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article.

References

1 Moyle RG, Chesser RT, Brumfield RT, Tello JG, Marchese DJ, Cracraft J. Phylogeny and phylogenetic classification of the antbirds, ovenbirds, woodcreepers, and allies (Aves: Passeriformes: infraorder Furnariides). Cladistics. 2009;25(4):386–405. doi: 10.1111/j.1096-0031.2009.00259.x.

2 Degrandi TM, Barcellos SA, Costa AL, Garnero ADV, Hass I, Gunski RJ. Introducing the bird chromosome database: an overview of cytogenetic studies in birds. Cytogenet Genome Res. 2020;160(4):199–205. doi: 10.1159/000507768.

3 Gunski RJ, Cañedo AD, Garnero ADV, Ledesma MA, Coria N, Montalti D, et al. Multiple sex chromosome system in penguins (*Pygoscelis*, Spheniscidae). Comp Cytogenet. 2017;11(3):541–52. doi: 10.3897/CompCytogen.v11i3.13795.

4 Ellegren H. Evolution of the avian sex chromosomes and their role in sex determination. Trends Ecol Evol. 2000;15(5):188–92. doi: 10.1016/s0169-5347(00)01821-8.

5 Schartl M, Schmid M, Nanda I. Dynamics of vertebrate sex chromosome evolution: from equal size to giants and dwarfs. Chromosoma. 2016;125(3):553–71. doi: 10.1007/s00412-015-0569-y.

6 Correia VCS, Garnero ADV, Santos LP, Silva RR, Barbosa MO, Bonifácio HL, et al. Alta similaridade cariotípica na família Emberezidae (Aves: Passeriformes). Biosci J. 2009;25:99–111.

7 de Oliveira Barbosa M, da Silva RR, de Sena Correia VC, Dos Santos LP, Garnero ADV, Gunski RJ. Nucleolar organizer regions in *Sittasomus griseicapillus* and *Lepidocolaptes angustirostris* (Aves, Dendrocolaptidae): evidence of a chromosome inversion. Genet Mol Biol. 2013;36(1):70–3. doi: 10.1590/S1415-47572013000100010.

8 dos Santos MDS, Kretschmer R, Silva FAO, Ledesma MA, O'Brien PCM, Ferguson-Smith MA, et al. Intrachromosomal rearrangements in two representatives of the genus *Saltator* (Thraupidae, Passeriformes) and the occurrence of heteromorphic Z chromosomes. Genetica. 2015;143(5):535–43. doi: 10.1007/s10709-015-9851-4.

9 Ericson PG, Klopfstein S, Irestedt M, Nguyen JMT, Nylander JAA. Dating the diversification of the major lineages of Passeriformes (Aves). BMC Evol Biol. 2014;14:8–15. doi: 10.1186/1471-2148-14-8.

10 Gill F, Donsker D, Rasmussen P, editors. IOC world bird list; 2023. v13.2. doi: 10.14344/IOC.ML.13.2.

11 Kretschmer R, Gunski RJ, Garnero ADV, Furo IDO, O'Brien PCM, Ferguson-Smith MA, et al. Molecular cytogenetic characterization of multiple intrachromosomal rearrangements in two representatives of the genus *Turdus* (Turdidae, Passeriformes). PLoS One. 2014;9(7):e103338. doi: 10.1371/journal.pone.0103338.

12 Kretschmer R, de Oliveira EHC, dos Santos MS, Furo IDO, O'Brien PCM, Ferguson-Smith MA, et al. Chromosome mapping of the large elaenia (*Elaenia spectabilis*): evidence for a cytogenetic signature for passeriform birds? Biol J Linn Soc. 2015;115(2):391–8. doi: 10.1111/bij.12504.

13 Weissensteiner MH, Suh A. Repetitive DNA: the dark matter of avian genomics. Avian Genomics Ecol Evol. 2019:93–150. doi: 10.1007/978-3-030-16477-5_5.

14 Szarski H. Cell size and nuclear DNA content in vertebrates. Int Rev Cytol. 1976;44:93–111. doi: 10.1016/s0074-7696(08)61648-4.

15 de Oliveira TD, Kretschmer R, Bertocchi NA, Degrandi TM, de Oliveira EHC, Cioffi MDB, et al. Genomic organization of repetitive DNA in woodpeckers (Aves, Piciformes): implications for karyotype and ZW sex chromosome differentiation. PLoS One. 2017;12(1):e0169987. doi: 10.1371/journal.pone.0169987.

16 de Oliveira Furo I, Kretschmer R, dos Santos MS, de Lima Carvalho CA, Gunski RJ, O'Brien PCM, et al. Chromosomal mapping of repetitive DNAs in *Myiopsitta monachus* and *Amazona aestiva* (Psittaciformes, Psittacidae: psittaciformes), with emphasis on the sex chromosomes. Cytogenet Genome Res. 2017;151(3):151–60. doi: 10.1159/000464458.

17 Kretschmer R, de Oliveira TD, de Oliveira Furo I, Oliveira Silva FA, Gunski RJ, Del Valle Garnero A, et al. Repetitive DNAs and shrink genomes: a chromosomal analysis in nine Columbidae species (Aves, Columbiformes). Genet Mol Biol. 2018;41(1):98–106. doi: 10.1590/1678-4685-GMB-2017-0048.

18 de Souza MS, Kretschmer R, Barcellos SA, Costa AL, Cioffi MDB, de Oliveira EHC, et al. Repeat sequence mapping shows different W chromosome evolutionary pathways in two caprimulgiformes families. Birds. 2020;1:19–34. doi: 10.3390/birds1010004.

19 Barcellos SA, Kretschmer R, de Souza MS, Costa AL, Degrandi TM, dos Santos MS, et al. Karyotype evolution and distinct evolutionary history of the W chromosomes in swallows (Aves, Passeriformes). Cytogenet Genome Res. 2019;158(2):98–105. doi: 10.1159/000500621.

20 Kretschmer R, Rodrigues BS, Barcellos SA, Costa AL, Cioffi MDB, Garnero ADV, et al. Karyotype evolution and genomic organization of repetitive DNAs in the saffron finch, *Sicalis flaveola* (Passeriformes, Aves). Animals. 2021;11(5):1456. doi: 10.3390/ani11051456.

21 Tura V, Kretschmer R, Sassi FDMC, de Moraes RLR, Barcellos SA, de Rosso VO, et al. Chromosomal evolution of Suboscines: karyotype diversity and evolutionary trends in ovenbirds (Passeriformes, Furnariidae). Cytogenet Genome Res. 2022;162(11–12):644–56. doi: 10.1159/000530428.

22 Degrandi TM, Gunski RJ, Garnero ADV, Oliveira EHC, Kretschmer R, Souza MS, et al. The distribution of 45S rDNA sites in bird chromosomes suggests multiple evolutionary histories. Genet Mol Biol. 2020;43(2):e20180331. doi: 10.1590/1678-4685-GMB-2018-0331.

23 Nishida-Umehara C, Tsuda Y, Ishijima J, Ando J, Fujiwara A, Matsuda Y, et al. The molecular basis of chromosome orthologies and sex chromosomal differentiation in palaeognathous birds. Chromosome Res. 2007;15(6):721–34. doi: 10.1007/s10577-007-1157-7.

24 Dos Santos MDS, Kretschmer R, Frankl-Vilches C, Bakker A, Gahr M, O'Brien PCM, et al. Comparative cytogenetics between two important songbird, models: the zebra finch and the canary. PLoS One. 2017;12(1):e0170997. doi: 10.1371/journal.pone.0170997.

25 Bülau SE, Kretschmer R, Furo IDO, de Oliveira EHC, de Freitas TRO. Karyotype organization of the endangered species yellow cardinal (*Gubernatrix cristata*). DNA. 2021;1(2):77–83. doi: 10.3390/dna1020008.

26 Degrandi TM, Gunski RJ, Garnero ADV, Oliveira EHC, Kretschmer R, Souza MS, et al. The distribution of 45S rDNA sites in bird chromosomes suggests multiple evolutionary histories. Genet Mol Biol. 2020;43(2):e20180331. doi: 10.1590/1678-4685-GMB-2018-0331.

27 Delany ME, Krupkin AB, Miller MM. Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. Cytogenet Cell Genet. 2000;90(1–2):139–45. doi: 10.1159/000015649.

28 Nanda I, Schrama D, Feichtinger W, Haaf T, Schartl M, Schmid M. Distribution of telomeric (TTAGGG) n sequences in avian chromosomes. Chromosoma. 2002;111(4):215–27. doi: 10.1007/s00412-002-0206-4.

29 Sasaki M, Ikeuchi T, Maino SA. Feather pulp culture for avian chromosomes with notes on the chromosomes of the peafowl and the ostrich. Experientia. 1968;24:1923–9.

30 Guerra MS. Reviewing the chromosome nomenclature of Levan et al. Rev Bras Genet. 1986;9:741–3.

31 Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 1972;75(1):304–6. doi: 10.1016/0014-4827(72)90558-7.

32 White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315–22.

33 Kubat Z, Hobza R, Vyskot B, Kejnovsky E. Microsatellite accumulation on the Y chromosome in *Silene latifolia*. Genome. 2008;51(5):350–6. doi: 10.1139/G08-024.

34 Ijdo JW, Wells RA, Baldini A, Reeders ST. Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. Nucleic Acids Res. 1991;19(17):4780. doi: 10.1093/nar/19.17.4780.
35 Kretschmer R, Santos MS, Furo IO, Oliveira EHC, Cioffi MB. FISH–in birds. In: Liehr T, editor. Cytogenetics and molecular cytogenetics. 1st ed. Boca Raton: CRC Press; 2022. p. 263–76.
36 Shields G, Barlow JC, James RD. Karyotypes of five species of Empidonax flycathers. Wilson Bull. 1987;99:169–74.
37 Gunski RJ, Cabanne GS, Ledesma MA, Del V Garnero A. Análisis cariotípico de siete especies de Tiránidos (Tyrannidae). Hornero. 2000; 15(2):103–9. doi: 10.56178/eh.v15i2.927.
38 Rodrigues BS, Kretschmer R, Gunski RJ, Garnero ADV, O'Brien PCM, Ferguson-Smith M, et al. Chromosome painting in tyrant flycatchers confirms a set of inversions shared by Oscines and Suboscines (Aves, Passeriformes). Cytogenet Genome Res. 2017;153(4):205–12. doi: 10.1159/000486975.
39 Griffin DK, Robertson LBW, Tempest HG, Skinner BM. The evolution of the avian genome as revealed by comparative molecular cytogenetics. Cytogenet Genome Res. 2007; 117(1–4):64–77. doi: https://doi.org/10.1159/000103166.
40 Kretschmer R, Ferguson-Smith MA, de Oliveira EHC. Karyotype evolution in birds: from conventional staining to chromosome painting. Genes. 2018;9(4):181. doi: 10.3390/genes9040181.
41 Kapusta A, Suh A. Evolution of bird genomes: a transposon's-eye view. Ann N Y Acad Sci. 2017;1389(1):164–85. doi: 10.1111/nyas.13295.
42 Kumar S, Suleski M, Craig JM, Kasprowicz AE, Sanderford M, Li M, et al. TimeTree 5: an expanded resource for species divergence times. Mol Biol Evol. 2022;39(8):msac174. doi: 10.1093/molbev/msac174.
43 Derjusheva S, Kurganova A, Habermann F, Gaginskaya E. High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds. Chromosome Res. 2004;12(7):715–23. doi: 10.1023/B:CHRO.0000045779.50641.00.
44 Nishida C, Ishijima J, Kosaka A, Tanabe H, Habermann FA, Griffin DK, et al. Characterization of chromosome structures of Falconinae (Falconidae, Falconiformes, Aves) by chromosome painting and delineation of chromosome rearrangements during their differentiation. Chromosome Res. 2008; 16(1):171–81. doi: 10.1007/s10577-007-1210-6.
45 de Oliveira EHC, Habermann FA, Lacerda O, Sbalqueiro IJ, Wienberg J, Müller S. Chromosome reshuffling in birds of prey: the karyotype of the world's largest eagle (harpy eagle, *Harpia harpyja*) compared to that of the chicken (*Gallus gallus*). Chromosoma. 2005;114(5):338–43. doi: 10.1007/s00412-005-0009-5.
46 de Oliveira AM, Souza GM, Toma GA, dos Santos N, dos Santos RZ, Goes CAG, et al. Satellite DNAs, heterochromatin, and sex chromosomes of the wattled jacana (Charadriiformes; Jacanidae): a species with highly rearranged karyotype. Genome. 2024;67(4): 109–18. doi: 10.1139/gen-2023-0082.
47 Ellegren H. Microsatellites: simple sequences with complex evolution. Nat Rev Genet. 2004; 5(6):435–45. doi: 10.1038/nrg1348.
48 Pokorná M, Kratochvíl L, Kejnovský E. Microsatellite distribution on sex chromosomes at different stages of heteromorphism and heterochromatinization in two lizard species (Squamata: eublepharidae: *Coleonyx elegans* and Lacertidae: *Eremias velox*). BMC Genet. 2011;12:90. doi: 10.1186/1471-2156-12-90.