



Karyotypic characterization of Ramphastidae (Piciformes, Aves)

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Abstract

The karyotypes of nine species of the family Ramphastidae were determined and compared with that of the toucan (*Ramphastos toco*), the only ramphastid karyotype so far reported in the literature. Differences in the morphology of the largest chromosomes allowed to identify three karyotype groups. The first group included the species *R. toco*, *Baillonius bailloni*, *Pteroglossus castanotis*, *P. aracari* and *Selenidera maculirostris*, in which only the first pair of chromosomes was metacentric. The second group included four *Ramphastos* species (*R. dicolorus*, *R. ariel*, *R. vitellinus*, *R. tucanus cuvieri*) with two pairs of metacentric macrochromosomes (the first and the seventh). The third group was represented by a single species, *A. laminirostris*, in which all the autosomal chromosomes were telocentric. All of the species had subtelocentric Z chromosomes, similar in size to homologues of the first pair. Sex chromosome W was a small chromosome. The chromosome number ranged from $2n = 62$ in *P. aracari* to 114 in *R. toco*. The cytotaxonomic relationships among toucan species are discussed, based on chromosome analysis.

Key words: karyotypes, Ramphastidae, toucans.

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Introduction

The family Ramphastidae (Piciformes - Aves) is composed of toucans, the most typical neotropical birds (Haffer, 1974), endowed with extraordinary characteristics and exuberant colors (Sick, 1997). Fifty-five species were described in this family, 27 of which are found in Brazil. The only one whose karyotype had been described so far is *Ramphastos toco*. The family Ramphastidae comprises two subfamilies: Capitonidae, with three genera and 14 species, and Ramphastinae, with six genera and 41 species (Sibley, 1996).

The chromosome number of *R. toco* is $2n = 106$ (Takagi and Sasaki, 1980). Sex chromosome Z is subtelocentric and equivalent in size to the first chromosome pair, which is metacentric. The remaining chromosomes are telocentric. Sex chromosome W is a microchromosome, not identified by these authors. Nogueira and Goldshmidt (1994) analyzed six specimens of *R. toco*, and reported $2n = 80$ chromosomes. Cytogenetic data are important for the characterization of each species, and can help to establish their karyotype evolution. In this work, we examined the karyotypes of ten Ramphastidae species, in order to

assess chromosomal evolution and cytotaxonomic relationships in this family.

Materials and Methods

Ten Ramphastidae species of the subfamily Ramphastinae (Piciformes, Aves) were cytogenetically analyzed: *Ramphastos toco* Mueller, 1776 (25 males, 26 females), *R. dicolorus* Linnaeus, 1776 (9 males, 11 females), *R. ariel* Vigors, 1826 (2 males, 7 females), *R. vitellinus* Reiser, 1905 (2 females), *R. tucanus cuvieri* Wagler, 1827 (1 male), *Baillonius bailloni* Vieillot, 1819 (2 males, 5 females), *Pteroglossus castanotis* Gould, 1834 (1 male, 3 females), *P. aracari* Linnaeus, 1758 (2 males), *Selenidera maculirostris* Lichtenstein, 1823 (4 males, 3 females), and *Andigena laminirostris* Gould, 1850 (2 males). All specimens showed adult characteristics, and were maintained by ecological parks, zoological gardens or with private bird breeders in the State of São Paulo.

The chromosome preparations were obtained using the dermal pulp tissue of growing feathers (approximately 15-30 days growth). This tissue was macerated and incubated in culture medium (RPMI) for 30 min. with 0.016% colchicine, at room temperature. The cell suspension was then centrifuged at 1000 g for 10 min, and the pellet resuspended in 10 mL of 0.075 M KCl (hypotonic solution) for 30 min, after which the cells were fixed in a 3:1 (v/v)

methanol:acetic acid solution. Following two rinses with fixative, the suspension was spotted onto slides. These steps were done in the field. The slides were subsequently stained with 10% Giemsa solution (in phosphate buffer, pH 6.8), and examined at a 100x magnification.

The sex chromosomes were determined using the best metaphase plates, and the maximum chromosome number found was considered as being the diploid number of the respective species. The description of chromosome morphology was based on that of Levan *et al.* (1964).

Results

In this study on toucan species, sex was determined by cytogenetic analysis. The female specimens of the ten species had sex chromosomes (ZW) that differed in size and/or morphology. Based on the morphology of the largest chromosomes, three groups of species were identified in this family.

Group I

Ramphastos toco, *Bailloni* *bailloni*, *Pteroglossus castanotis*, *P. aracari* and *Selenidera maculirostris*

All 51 specimens of *R. toco* had the same karyotype as described by Takagi and Sasaki (1980). The chromosomes of the first pair were the only metacentric ones, their size being twice the size of the second pair (Figure 1: A₁ and A₂ - male; A₃ and A₄ - female). The Z chromosome was submetacentric and was similar in size to the autosomes of the first pair. The remaining chromosomes were telocentric and progressively decreasing in size. It was not possible to identify the homologues, nor the w chromosome. This species had a diploid number of 114 chromosomes.

The same karyotype pattern was found in *Bailloni* *bailloni* (Figure 1F), *Pteroglossus castanotis* (Figure 1G), *P. aracari* (Figure 1H), and *Selenidera maculirostris* (Figure 2I), but they differed in their diploid chromosome numbers (2n = 92; 2n = 86; 2n = 62; and 2n = 98, respectively).

Group II

Ramphastos dicolorus, *R. ariel*, *R. vitellinus* and *R. tucanus cuvieri*

The analysis of metaphases from these species showed chromosome groups similar to those observed in *R. toco*. A pair of large metacentric chromosomes was identified as the first pair. The Z chromosome was submetacentric and equivalent in size to the first pair. Among the telocentric chromosomes, there was one metacentric pair that corresponded in size to the seventh pair of the previous group. The W chromosome was impossible to identify. The diploid chromosome numbers were: 2n = 98 for *R. dicolorus* (Figure 1B), 2n = 106 for *R. ariel* (Figure 1C),

2n = 102 for *R. vitellinus* (Figure 1D), and 2n = 88 for *R. tucanus cuvieri* (Figure 1E).

Group III

Andigena laminirostris

The only species included in this group, *Andigena laminirostris* (Figure 1J), does not inhabit Brazil, but occurs in Ecuador (Gould, 1992). Two males of this species were included in this analysis, to compare their karyotype with that of the various Brazilian species. *A. laminirostris* had 2n = 108 chromosomes, one of them being an unusual chromosome that corresponded in size and morphology to the Z chromosome described above. The second chromosome pair was 2/3 the size of the first pair. The second pair and all the other chromosomes were telocentric and gradually decreasing in size.

The two specimens of *A. laminirostris* were considered to be males, because no heteromorphic chromosome pair, corresponding to ZW, was found. They were both subsequently sexed as males by DNA analysis. The unusually large pair of chromosomes found in *A. laminirostris* was similar in size and morphology to the Z sex chromosomes of the other Ramphastidae species, and was therefore considered to represent the Z chromosomes. This will only be confirmed after karyotype analysis of females and identification of the ZW sex chromosome pair.

Discussion

Based on our chromosome analysis, we worked out the following hypothesis: *R. toco* may descend from a more ancient branch, and the metacentric seventh pair may be the result of a single translocation that occurred late in the origin of the *R. toco* branch, but early enough to be present in the ancestry of *R. dicolorus*, *R. tucanus*, *R. vitellinus* and *R. ariel*.

The metacentric chromosomes identified as the seventh pair were good marker chromosomes, because of their similar size and morphology in the various *Ramphastos* species, except for *R. toco*. Independent translocations involving different chromosomes are unusual events. The presence of these chromosomes in different species suggests that they were inherited via a common ancestor. This interpretation is even more consistent when the diploid chromosome number of different species is considered.

According to Takagi and Sasaki (1980), the ancestors of many orders of birds had karyotypes with many chromosomes, all of them telocentric, except for the first pair. This same ancestral pattern was reported by Beltermann and De Boer (1984), after their analysis of the karyotypes of various species from 15 orders, and by Rodionov (1997), after examining the published karyotypes of almost 800 avian species. *R. toco* and *A. laminirostris* have probably main-

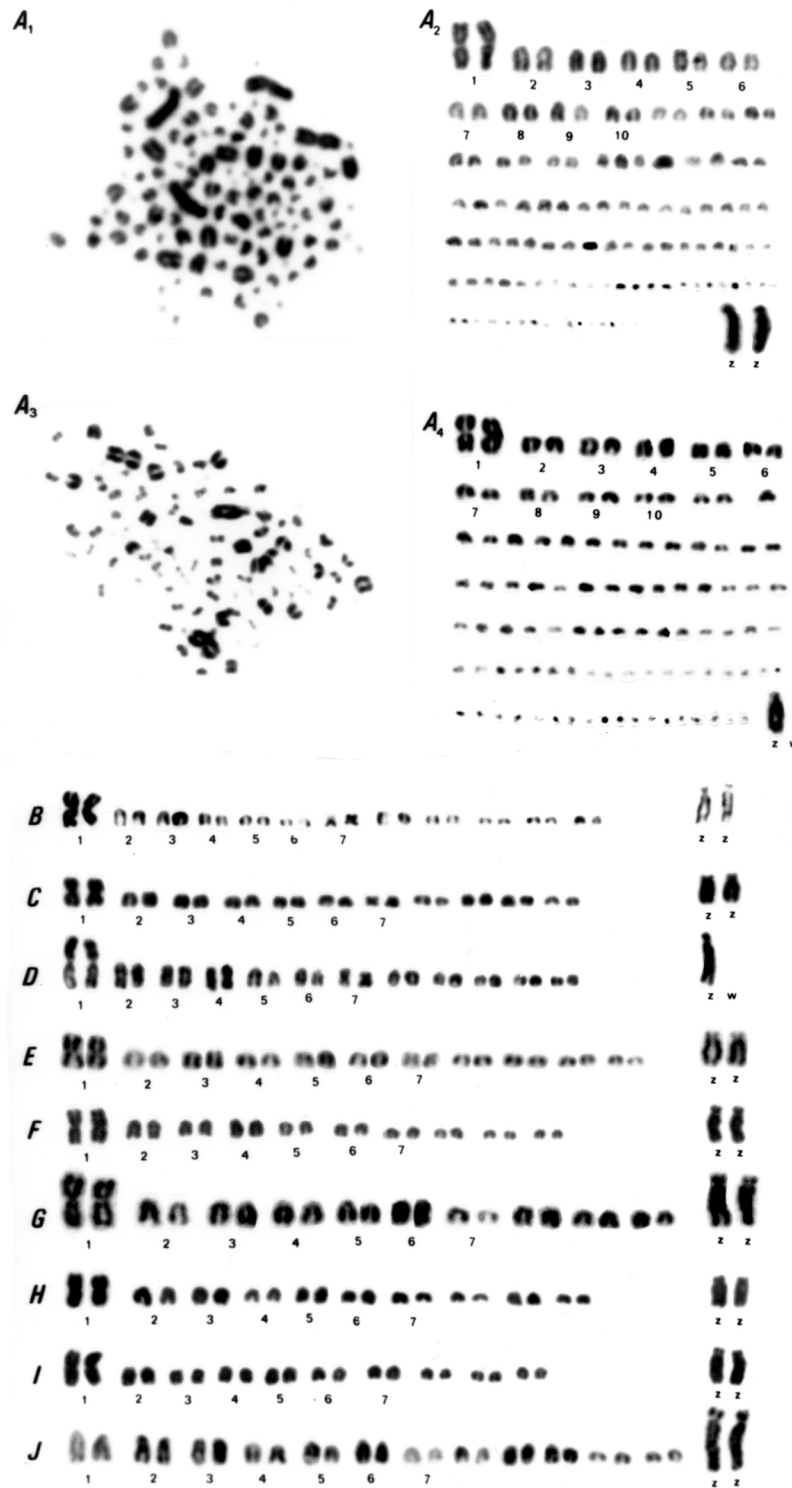


Figure 1 - A₁, Mitotic metaphase of *R. toco* (male), A₂, karyotype of *R. toco* (male), A₃, Mitotic metaphase of *R. toco* (female), A₄, Karyotype of *R. toco* (female). Partial karyotypes of B. *Ramphastos dicolorus*, C. *R. ariel*, D. *R. vitellinus*, E. *R. tucanus cuvieri*, F. *Baillonius bailloni*, G. *Pteroglossus castanotis*, H. *P. aracari*, I. *Selenidera maculirostris* and J. *Andigena laminirostris*.

tained the ancestral karyotype pattern of these genera from 800,000 years ago.

During the differentiation of more recent branches, *in-tandem* translocations may have reduced the diploid

chromosome numbers of current species. This would explain why *R. tucanus*, a representative of a more recent lineage than *R. toco*, had the lowest chromosome number (2n = 88). Centric fusion after differentiation of the *R. toco*

lineage would have given rise to the metacentric seventh chromosome pair of other *Ramphastos* species.

In-tandem translocations may have occurred after the separation of *R. toco* and *R. tucanus*, and were probably more recently intensified in *R. tucanus* ($2n = 88$), but less intensive in *R. dicolorus* ($2n = 98$), *R. ariel* ($2n = 106$) and *R. vitellinus* ($2n = 102$). In *Selenidera* and *Baillonius*, $2n$ is 98 and 92, respectively, whereas the diploid number in *P. aracari* is $2n = 62$, and in *P. castanotis*, $2n = 86$. *In-tandem* translocations could account for this reduction of the chromosome number in *Pteroglossus* species, although, as pointed out by Rodionov (1997), the rates of karyotypical change differ among phylogenetic branches of birds.

In nine of the species studied here, the first autosome pair was metacentric and was double the size of the second pair. In *A. laminirostris*, all the autosomes were telocentric and gradually decreasing in size. In the karyotypical patterns proposed by Takagi and Sasaki (1980) and Beltermann and De Boer (1984) for the ancestors of many avian orders, the first and second pairs are metacentric and submetacentric. According to Takagi and Sasaki (1980), centric fission in one of these pairs would culminate in the karyotype observed in *R. toco*. According to these authors, in certain species of some orders (Strigiformes, Gruiformes), centric fission has occurred in both chromosome pairs, resulting in telocentric chromosomes. Such a series of events (centric fission of metacentric chromosomes) could explain the karyotype of *A. laminirostris*. However, as pointed out by Beltermann and De Boer (1984), the confirmation of this hypothesis would require chromosome banding analysis, to verify the homology among the largest chromosomes of different species, families and orders.

All species examined here had submetacentric Z chromosomes, which were similar in size to the first pair. The sex chromosome W was a microchromosome not observed in females, and only C-banding patterns could identify this small chromosome in these species. This pattern was not observed in other families of the order Piciformes. Shields *et al.* (1982) reported that, in the family Picidae, the Z chromosome may be submetacentric or metacentric, whereas chromosome W can be small and telocentric (*Dendrocopos major*) or submetacentric, or one of the largest in the karyotype (*D. minor*). Similar variation in sex chromosome size and morphology occurs in *Picoides*, *Colaptes* and *Sphirapicus*. Two species of the family Megalaimidae, *Megalaima haemacephala* (Shields *et al.*, 1982) and *M. zeylanica* (Kaul and Ansari, 1981), have Z chromosomes which correspond to those seen in Ramphastidae. In contrast, the W chromosome is small and telocentric in *M. haemacephala*, and larger than the second pair of autosomes in *M. zeylanica*. Among the species of Ramphas-

tidae examined here, the size and morphology of the sex chromosomes was less variable.

A comparative chromosome analysis of a great number of Ramphastidae species and of other families of the same order would improve the interpretation of chromosomal evolution in this group. Combining cytogenetic data with biogeography, phenotypic characteristics, DNA hybridization, and other approaches should provide a better understanding of the phylogenetic relationships in the avian family Ramphastidae.

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References

- Beltermann RHR and De Boer LEM (1984) A karyological study of 55 species of birds, including karyotypes of 39 species new to cytology. *Genetica* 65:39-82.
- Gould J (1992) Family of toucans: a monograph of the Ramphastidae. Jari, Companhia Florestal Monte Dourado, Belém - PA.
- Kaul D and Ansari A (1981) Chromosomal polymorphism in a natural population of the northern green barbet, *Megalaima zeylanica caniceps* (Franklin) (Piciformes: Aves). *Genetica* 54:241-245.
- Levan A, Fredga K and Sandberg AA (1964) Nomenclature for centromeric position of chromosomes. *Hereditas* 52:201-202.
- Nogueira DM and Goldshmidt B (1995) Estudo cromossômico em *Ramphastos toco*. *Braz. J. Genet. (suppl)* 18:437. 41^o Congresso Nacional de Genética, Caxambu, MG, Brazil.
- Rodionov AV (1997) Evolution of avian chromosome and linkage groups. *Genetica* 33:725-738.
- Shields GF, Jarrel GH and Redrupp E (1982) Enlarged sex chromosome of woodpeckers (Piciformes). *The Auk* 99:767-771.
- Sibley CG (1996). *Birds of the World* (version 2.0), Thayer Birding Software.
- Sick H (1997) *Ornitologia Brasileira, uma Introdução*. Nova Fronteira, Rio de Janeiro, 827 pp.
- Takagi N and Sasaki M (1980) Unexpected karyotypical resemblance between the burmeister's seriema, *Chunga burmeisteri* (Gruiformes: Cariamidae) and the toucan, *Ramphastos toco* (Piciformes: Ramphastidae). *Chrom. Inf. Serv.* 28:10-11.