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Description of two new karyotypes and cytotaxonomic considerations on Falconiformes.

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RESUMO. **Descrição de dois novos cariótipos e considerações citotaxonômicas sobre Falconiformes.** O cariótipo de duas espécies de aves de rapina, *Spizaetus tyrannus* (2n=68) e *Coragyps atratus* (2n=80), são descritos pela primeira vez. Além disso, uma reanálise de três outras espécies é apresentada. Os dados cromossômicos foram comparados com arranjos filogenéticos propostos para Falconiformes. Os estudos citotaxonômicos evidencian uma grande diferença entre Cathartidae, que retém um cariótipo padrão das Aves, conservado, e as famílias Accipitridae e Falconidae, que apresentam cariótipos derivados. A análise citogenética também evidencia uma dicotomia entre Falconidae e Accipitridae.

Palavras-chave: Cathartidae, Falconidae, Accipitridae, citotaxonomia, *Coragyps atratus*, *Spizaetus tyrannus*.

ABSTRACT. The karyotype of two species of birds of prey, *Spizaetus tyrannus* (2n=68) and *Coragyps atratus* (2n=80) are described for the first time. Moreover, a reanalysis of three other species is presented. Chromosomal data were compared to phylogenetic arrangements proposed for Falconiformes. Cytotaxonomic studies pointed towards a great difference between Cathartidae, which retained a standard avian karyotype, and species of the families Accipitridae and Falconidae, which showed derived karyotypes. Cytogenetic analysis also revealed a dichotomy between Falconidae and Accipitridae.

Keywords: Cathartidae, Falconidae, Accipitridae, cytotaxonomy, *Coragyps atratus, Spizaetus tyrannus*.

There are no fossil records which indicate that the different families within Falconiformes share a common ancestor, allowing some authors to argue in favor of a polyphyletic origin due to evolutionary convergence (Brown and Amadon 1968, Feduccia 1980, Del Hoyo *et al*. 1994). However, some proposals based on morphological data support the monophyly of this order (Storer 1971, Stresemann and Amadon 1979, Griffiths, 1994a). Other authors, based on nucleic acid hybridization results, proposed the exclusion of Cathartidae, which seemed to be closely related to the Ciconiidae (Sibley and Ahlquist 1990).

Except for Cathartidae, which retains a conserved karyotype, with 2n=80 and only nine pairs of macrochromosomes (De Boer 1976, De Lucca 1992), the species belonging to Falconiformes, including falcons, kites, hawks and eagles, show karyotypes which differs drastically from the typical avian one. Species of this group usually have lower diploid numbers, as well as a lower number of microchromosomes (Bed'Hom 1999, Amaral and Jorge 2003).

Recent cross-species chromosome painting analysis between *Gallus gallus* (2n=78) and the Californian condor (*Gymnogyps californianus)* (2n=80) demonstrated that macrochromosomes are conserved between these species, except for chromosome GGA 4, which corresponded to two distinct pairs in *Gymnogyps* (Raudsepp *et al.* 2002).

On the other hand, de Oliveira *et al.* (2005) found a very distinct result in the karyotype of the harpy-eagle (*Harpia harpyja*). This species showed a karyotype with 2n=58, with only four pairs of very small chromosomes. Cross-species chromosome painting between *Harpia* and *Gallus* revealed the occurrence of a chromosome reshuffling in *Harpia*, and the genomic reorganization included fusions involving macro and microchromosomes, as well as fragmentation of the largest chromosomes.

Karyotypic studies in birds of prey have shown that this group has a high chromosomal diversity, which could be very informative to delineate phylogenetic relationships among the different groups within Falconiformes. Hence, in the present study we described for the first time the karyotypes of two species of Falconiformes – *Spizaetus tyrannus* and *Coragyps atratus* – and reanalyze the chromosomal complement of three other species in this group. The results were compared with previously published papers and related to different phylogenetic arrangements proposed to this order.

MATERIAL AND METHODS

Blood samples were collected from five different species belonging to three families: *Polyborus plancus* (Falconidae), *Sarcoramphus papa, Coragyps atratus* (Cathartidae) *Spyzaetus tyrannus,* and *Harpia harpyja* (Accipitridae) (Table 1). Cells were grown and harvested as described by Moorhead *et al*. (1960), with minor modifications. Metaphase preparations followed standard procedures. Chromosomes were studied by Giemsa conventional staining. After digital image acquisition of a minimum of 10 metaphases per individual, chromosomes were ordered following centromere position and chromosome size, according to the International System for Standardized Avian Karyotypes, proposed by Ladjali-Mohammedi *et al* (1999). Chromosome morphological classification followed Levan *et al*. (1964)

RESULTS

*Caracara (*Polyborus plancus*)*. This species showed a diploid number with 92 chromosomes. This number differs from previously reported results, which showed a diploid number close to 84 (De Boer 1976). Except for metacentric pair 1, all the other chromosomes were acrocentric, including the sex chromosomes. The Z chromosome was as large as pair 1, while the W chromosome corresponded to half of it (Figure 1).

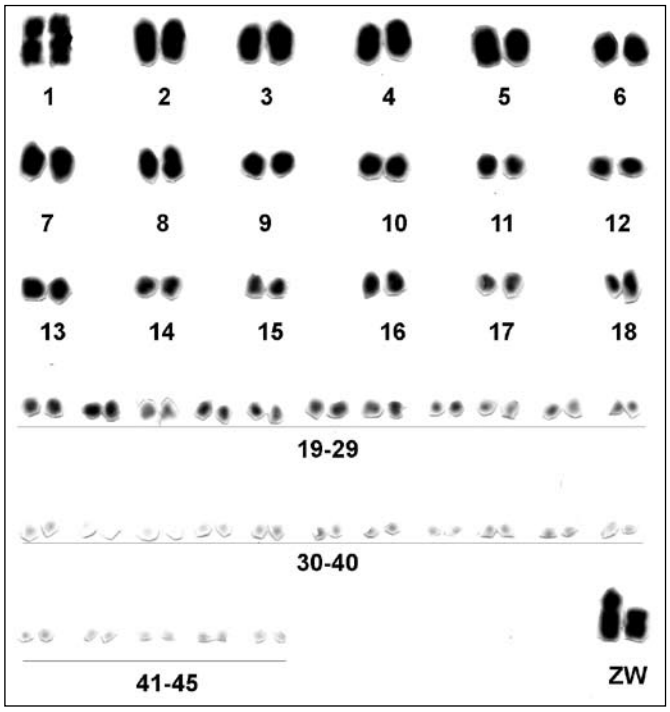

Figure 1 – The karyotype of *Polyborus plancus* (Falconidae), with 2n=92.

*Black Vulture (*Coragyps atratus*) and King Vulture (*Sarcoramphus papa*)*. These two species had similar karyotypes, with 2n=80. Pairs 1, 2, 4 and 5, as well as Z chromosome, were submetacentric, pair 9 and W chromosome metacentric and pair 3 subtelocentric. The remaining pairs were acrocentric. The Z chromosome had the size between pairs 2 and 3. The W chromosome had the size between pairs 8 and 9 (Figures 2a and 2b).

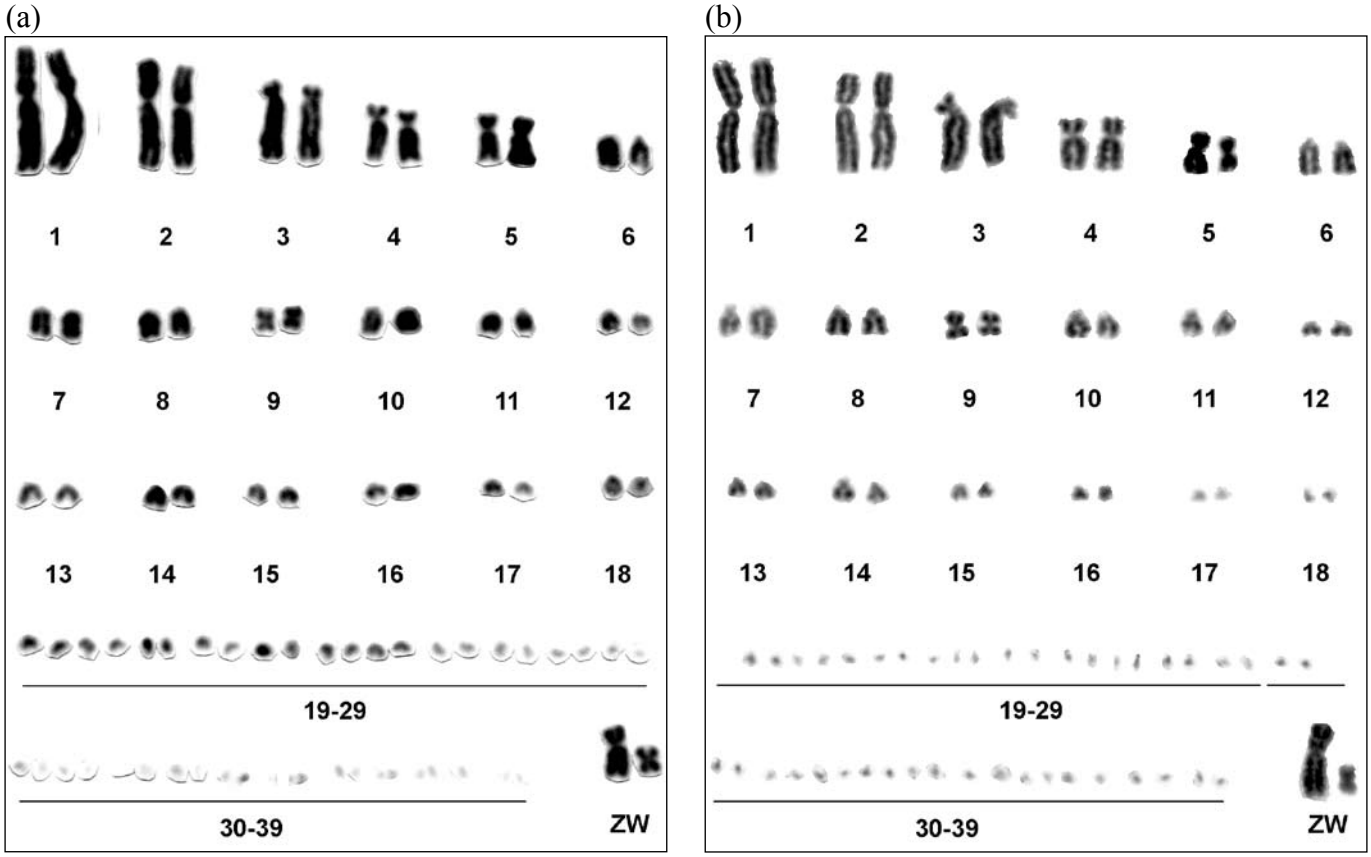

Figure 2 – The karyotype of *Coragyps atratus* (a) and *Sarcoramphus papa* (b) (Cathartidae). Both species had 2n=80.

Table 1. Specimen information and number of samples used in this study.

Species	Number of individuals	Sex	Institution
Polyborus plancus	1	FEM [1]	CGR [3]
Coragyps atratus	2	FEM MAL [2]	CGR
Sarcoramphus papa	2	FEM MAL	MPEG [4]
Spizaetus tyrannus	2	FEM	CGR
Harpia harpyja	2	FEM MAL	MPEG

[1] FEM=Female, [2] MAL=Male, [3] CGR= Criadouro Gavião Real, Capitão Poço, Pará, [4] MPEG= Museu Paraense Emílio Goeldi, Belém, Pará.

*Black Hawk Eagle (*Spizaetus tyrannus*)*. The karyotype of this species had 2n=68. Sixteen autosomic pairs were meta or submetacentric (1 to 5, 7 to 9, 11 to 13, 19 to 22, 27 and 29), as well as the sex chromosomes. Pairs 6, 8, 10, 16 and 17 were subtelocentric, while the remaining pairs were acrocentric. The Z chromosome had the size of pair 1, and the W chromosome was smaller, corresponding to one third of the size of pair 1. Four pairs of small chromosomes were considered as microchromosomes (Figure 3).

*Harpy Eagle (*Harpia harpyja*)*. This species showed a karyotype with 2n=58. Most pairs were meta or submetacentric, except for pairs 3, 8, 9, 14 and 16, which were subtelocentric,

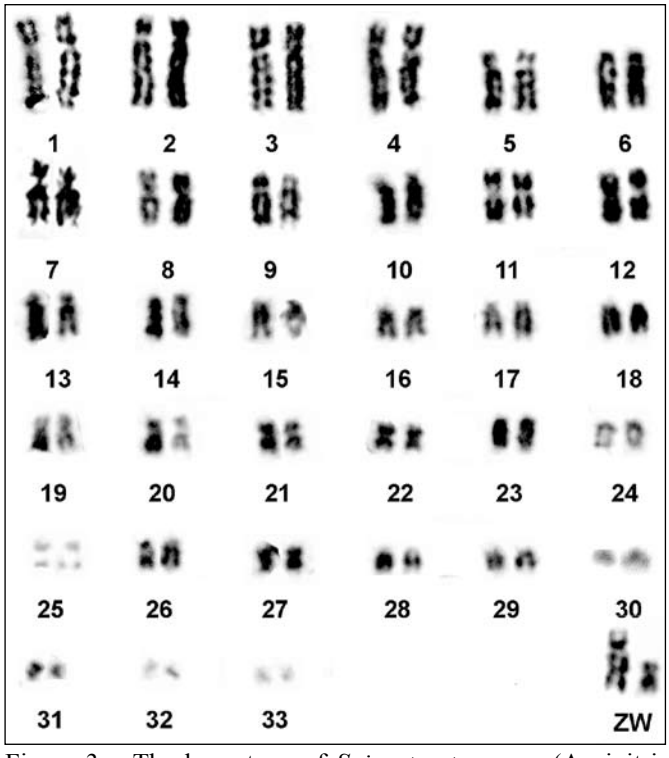

Figure 3 – The karyotype of *Spizaetus tyrannus* (Accipitridae), with 2n=68.

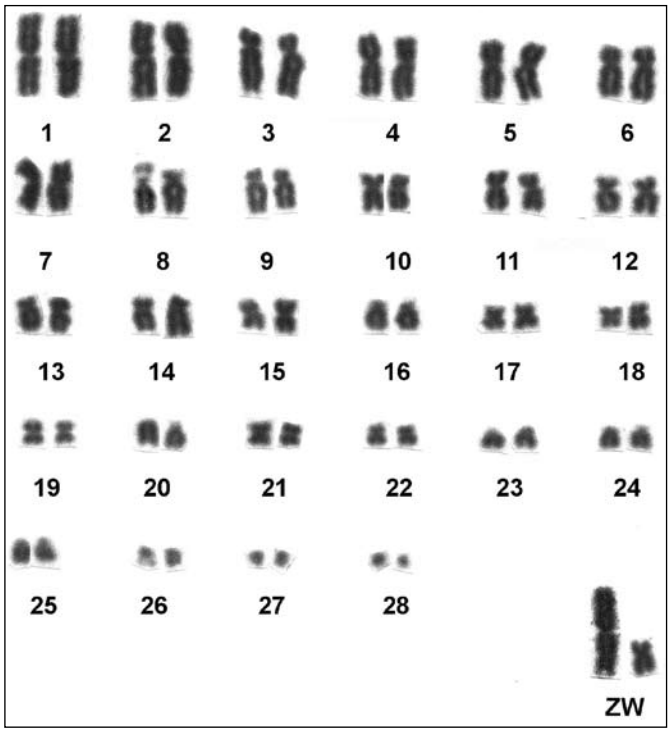

Figure 4 – The karyotype of *Harpia harpyja* (Accipitridae), with 2n=58.

and pairs 20, 25, 26, 27 and 28, which were all acrocentric. The Z chromosome was metacentric, with size between pairs 1 and 2, while the submetacentric W corresponded to approximately one third of it (Figure 4).

DISCUSSION

Compared to Mammals, birds have a small proportion of species which have been already analyzed by cytogenetic methods (de Oliveira and Jorge 2000, Amaral and Jorge 2003). Moreover, species have been analyzed randomly, which makes difficult the use of cytogenetic data in phylogenetic studies. However, cross-species chromosome painting

performed in the emu *Dromaius novaehollandiae* (Shetty *et al*. 1999), the Californian condor *Gymnogyps californianus* (Raudsepp *et al*. 2003), some Galliformes (Shibuzawa et al. 2004a, 2004b), and Passeriformes (Dejusheva *et al*. 2004) have allowed the proposition of a putative ancestral avian karyotype with 2n=80, similar to the one retained by many species belonging to different orders (Burt 2002, de Oliveira *et al*. 2005).

Birds of prey represent one of the avian groups with the highest number of species with known karyotypes. This fact is due to the peculiar characteristics of their chromosomal complement. Hence, all the families within Falconiformes have had some of their members analyzed cytogenetically at least by conventional staining. Moreover, two species belonging to distinct families have had their karyotypes analyzed by cross-species chromosome painting.

The retention of a conserved karyotype in Cathartidae was confirmed by the chromosome painting results in *Gymnogyps californianus* (Raudsepp *et al*. 2002). The other species of this family reported here - *Sarcoramphus papa* and *Coragyps atratus* – showed similar karyotypes, with 2n=80 and same chromosome morphology. It is reasonable to suppose that syntenic groups are conserved among these species. Hence, Cathartidae has a conserved karyotype, and the basal position of this group in different phylogenetic arrangements is supported by chromosomal data (e.g., (Sibley and Ahlquist, 1990). A similar karyotype is also observed in Saggitaridae: *Saggitarius serpentarius* has 2n=80, with a higher number of biarmed elements probably due to intrachromosomal rearrangements (Bed´Hom 1999). This fact tentatively places Cathartidae and Saggitaridae more basal in relation to Accipitridae and Falconidae, concerning chromosomal characteristics.

Both species of Accipitridae studied - *Spizaetus tyrannus* and *Harpia harpyja* - showed karyotypes with lower chromosome numbers than those in Cathartidae. *Harpia harpyja* had 2n=58, similar to the karyotype described by de Oliveira *et al*. (2005). The karyotype of *Spizaetus tyrannus* was described here for the first time. The chromosomal number was identical to the one observed in another species of this genus, *Spizaetus nipalensis*, with 2n=68 (Takagi and Sazaki 1974).

Lower diploid numbers in Accipitridae probably resulted of a great genomic reorganization. Although many authors have suggested that diploid numbers were reduced due to fusions involving mainly microchromosomes (De Lucca 1992, Amaral and Jorge 2003), cross-species chromosome painting had shown actually that in harpy eagles (de Oliveira et al. 2005) and Old World vultures (Nanda *et al.* 2006) this reorganization has involved not only microchromosomes, but also macrochromosomes. Hence, chromosome specific probes of *Gallus gallus* revealed that fusions involved micro and macrochromosomes. Moreover, macrochromosomes were fragmented in distinct segments. Therefore, chromosomes GGA 1 to GGA 5 corresponded each from two to six distinct chromosome pairs in these species. This fact also explains the absence of very large chromosomes in Accipitridae. A similar genomic reorganization probably occurred in the *Spizaetus* karyotype, as well as in other species of birds of prey with lower diploid numbers.

Although the lowest diploid numbers in Falconiformes were found among Falconidae, with some species of *Falco* with 2n=50 (De Lucca 1992), the caracara *Polyborus plancus* showed a very high diploid number, with 2n=92. It is important to notice that this species was previously described with 2n=84 (De Boer 1976). Our results, based on a higher number of good quality metaphases showed that this species has a higher number of microchromosome pairs. This fact is concordant with other species phylogenetically close to *Polyborus* such as *Milvago chimachima* (Belterman and De Boer, 1984) and *Phalcoboenus megalopterus* (Belterman and De Boer, 1990), which showed diploid numbers of approximately 90, exclusively made up of acrocentric chromosomes. In common with *Falco*, *Polyborus* had only one pair of biarmed chromosomes, which corresponded to the largest pair. On the other hand, *Falco* species showed much lower diploid numbers, from 2n=40 to 52 (Amaral and Jorge 2003). Hence, chromosomal data would reinforce a dichotomy between Caracarini and Falconini, as already postulated by Griffiths (1994b, 1999) based on morphological and molecular data. Unfortunately, there are no cytogenetic data concerning the other genera of Falconidae.

As it was said before, chromosomal reorganization in Accipitridae seemed to involve fissions of the largest chromosomes and further fusions of macrochromosomes segments and microchromosomes. The high number of chromosomes in *Polyborus* could be explained by the occurrence of fission events, without fusion processes which have decreased the diploid number in other species. In fact, there are no large elements in the *Polyborus* karyotype. The largest chromosomes are medium-sized, and most chromosomes are small.

The results of chromosomal complements obtained so far pointed to a closer relationship between Accipitridae and Falconidae, and a basal position for Saggitaridae and Cathartidae. These distinct families are supported by chromosomal data. However, the present data is not enough to clarify the phylogenetic relationship between Cathartidae and Ciconiiformes, since nearly identical complements were found in representatives of most avian groups (De Boer 1976, De Lucca 1992). Maybe the use of cross species chromosome painting and BACs could bring some important information to clarify the real phylogenetic position of Cathartidae, as well as the relationships among different groups of birds.

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