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Research Article

Karyotype description of two Neotropical Psittacidae species: the endangered Hyacinth Macaw, *Anodorhynchus hyacinthinus,* and the Hawk-headed Parrot, *Deroptyus accipitrinus* (Psittaciformes: Aves), and its significance for conservation plans

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Abstract

Neotropical parrots are among the most threatened groups of birds in the world, and many species are facing extinction in a near future. At the same time, the taxonomic position of many species remains unclear. Karyotype analysis has been used to elucidate the phylogenetic status of many bird groups, also providing important information for both *in situ* and *ex situ* conservation plans. The objective of the present study was to describe for the first time the karyotypes of the endangered Hyacinth Macaw, *Anodorhynchus hyacinthinus,* and of the Hawk-headed Parrot, *Deroptyus accipitrinus.* A diploid number of 2n = 70 and a karyotype similar to the main pattern previously found for the genera *Ara, Cyanopsitta, Aratinga, Propyrrhura, Pionites, Pionopsitta, Nandayus,* and *Guaruba* were found for both species. These karyotype descriptions can be a starting point for the genetic monitoring of these two declining species.

Key words: Avian karyotype, *Anodorhynchus*, *Deroptyus*, Psittaciformes, conservation biology, cytotaxonomy.

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Introduction

Neotropical parrots comprise one of the most endangered groups of birds in the world. It is estimated that about 30% of the 140 living species are facing some risk of extinction, and most of the non-endangered species are experiencing population decline (Collar and Juniper, 1992). Habitat loss and human exploitation are the major causes leading to the extinction of these birds. Brazil is the country in the world with the greatest number of Psittacidae species (Forshaw, 1989). However, 17 out of the 72 living species are cited in the Red List of Threatened Species of the IUCN (International Union for Conservation of Nature and Natural Resources), and one of them, *Anodorhynchus glaucus,* is already extinct (Sick, 1997). Large species need large areas to mantain viable demographic population (Galetti *et al.*, 2002), and are among the most threatened (Sick, 1997). If conservation actions are not implemented, many other species can disappear in a near future, just like *A. glaucus*.

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The Hyancinth Macaw, *Anodorhynchus hyacinthinus*, is the largest species within the order Psittaciformes. It inhabits a vast area of *cerrado* and gallery forests of central Brazil, from the Tapajós River eastward to the States of Maranhão and Piauí, and southward through western Bahia and Goiás to Minas Gerais and Mato Grosso, and adjacent Pantanal regions of easternmost Bolivia and northeast Paraguay (Forshaw, 1989; Collar and Juniper, 1992; Sick, 1997). Today, it is extinct in most of its original distribution sites, and the remaining populations are markedly declining, due to trading and hunting (Collar and Juniper, 1992; Sick, 1997). The total number of individuals has been estimated to be no more than 5000 (Collar and Juniper, 1992; Sick, 1997). In 1987, *A. hyacinthinus* was included in Appendix I of CITES (Convention for the International Trade of Endangered Species), and in 1989 in the list of "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais" - IBAMA (Brazilian Institute of Environment and Natural Resources) as a species at risk for extinction (Sick, 1997).

The Hawk-headed Parrot, *Deroptyus accipitrinus,* is a poorly known species that occurs north of the Amazon

Basin, from the Guyanas and the eastern part of Pará (northern Brazil), westward to southeastern Colombia, northeastern Peru and southern Venezuela. It inhabits forests along coastal sand ridges, savannas, and interior forests. Population density is known to be low, to such an extent that this species is considered naturally rare in the wild. Several local populations were exterminated by illegal pet trade (Forshaw, 1989; Strahl *et al*., 1991).

Karyotype analysis is an important tool for both *in situ* and *ex situ* conservation plans, also being able to provide important information about the phylogenetic status of species within a group (Benirschke *et al*., 1980).

In situ management strategies often include translocation of individuals among populations (Storfer, 1999). However, it has long been demonstrated by cytogenetic procedures, extensively applied to animals from different regions, that chromosomal polymorphism exists in most species (Mayr, 1977; Benirschke *et al*., 1980). If a mixture of karyotypic variants occurs, it can result in outbreeding depression (Benirschke *et al*., 1980). Various cases of chromosomal polymorphism in birds were reported (Hammar, 1970; Hammar and Herlin; 1975; Thorneycroft, 1975; Misra and Srivastava 1976; Kaul and Ansari, 1979; De Lucca and Rocha, 1985), and among Brazilian psitacids it was found in *Pyrrhura* (De Lucca *et al*., 1991) and *Forpus* (De Lucca and Marco, 1983), suggesting that karyotypic monitoring should be considered in conservation actions.

Captive propagation fulfills a short-term reprieve for endangered and rare species, saving time for preparation of reintroduction sites that may permit reestablishment of new populations and reinforcement of preexistent ones (Conway, 1980; Seal, 1988; Derrickson and Snyder, 1992). A great variety of anomaly syndromes, frequently found among species one would wish to conserve, have been linked to chromosomal errors. Furthermore, geographic chromosomal variants within species, if pooled in captivity, can lead to hybrids that will cause a deleterious impact on reproduction (Benirschke *et al*., 1980). In the case of Neotropical Psittacidae, karyotyping can also provide a safe method for sex determination of captive specimens, since most species do not present phenotypic sexual dimorphism.

Partial karyotype descriptions of both *A. hyacinthinus* and *D. accipitrinus* were previously published by Rocha *et al*. (1995) and Goldschmidt *et al*. (1996). However, for karyotype monitoring purposes, a complete characterization of the chromosome complement of each species is necessary (Benirschke *et al*., 1980). The objective of this work was to present for the first time a detailed karyotype description of *A. hyacinthinus* and *D. accipitrinus,* which can be useful for biological conservation approaches. Cytotaxonomic considerations are also discussed, since karyotype analysis has been used to elucidate relationships among Neotropical Psittacidae (De Lucca and Marco, 1993; De Lucca, 1984; Van Dongen and De Boer, 1984; De Lucca *et al*., 1991; Duarte and Giannoni, 1990, Duarte and Caparroz, 1995; Goldschmidt *et al*., 1997; Francisco and Galetti Jr., 2001; Francisco *et al*., 2001), and both *Anodorhynchus* and *Deroptyus* genera are poorly known from this point of view.

These data resulted from a long-term karyotype monitoring program of birds bred in official captivity institutions in the States of São Paulo and Rio de Janeiro, Brazil, and contributed to the Hyacinth Macaw conservation plan, the Arara-Azul Project, carried out in cooperation with "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis" (IBAMA) and the "Sociedade Paulista de Zoológicos" (São Paulo Zoological Garden Society).

Materials and Methods

Seventeen females and twenty-seven males of *Anodorhynchus hyacinthinus* and three males and two females of *Deroptyus accipitrinus* were analyzed. All specimens were maintained by ecological parks, zoological gardens or private bird breeders.

Mitotic chromosomes were obtained from cells of the pulp of growing feathers (Sandnes, 1954), with modifications suggested by Martins *et al*. (1989) and by Giannoni *et al.* (1993). Morphometric chromosome analyses were performed according to Levan *et al*. (1964).

Results

Anodorhynchus hyacinthinus showed a diploid number of 2n = 70 in 202 analyzed metaphases, comprising macro- and microchromosomes. Chromosome pairs 1, 7, and 10 were metacentric; pairs 5, 6, 8, 9, and 11 were submetacentric; and pairs 2, 3, and 4 were subtelocentric. The microchromosomes were telocentric up to the point to which their morphology could be identified. The Z-chromosome was metacentric and about the same size as chromosome 5, and the W-chromosome was submetacentric and about the same size as chromosome 9 (Figure 1).

Deroptyus accipitrinus also had a diploid number of 2n = 70, comprising macro- and microchromosomes, as observed in 80 analyzed metaphases. Chromosome pairs 1, 7, 8, and the Z-chromosome were metacentric; pair 6 was submetacentric; pairs 2, 3, 4, and 5 were subtelocentric; and pairs 9, 10, 11, and the W-chromosome were telocentric. The microchromosomes were telocentric, and the Z- and W-chromosomes were approximately the same size as chromosomes 5 and 7, respectively. The microchromosomes were telocentric whenever their morphology could be identified (Figure 2). No intraspecific karyotypic variation was detected in anyone of the two species.

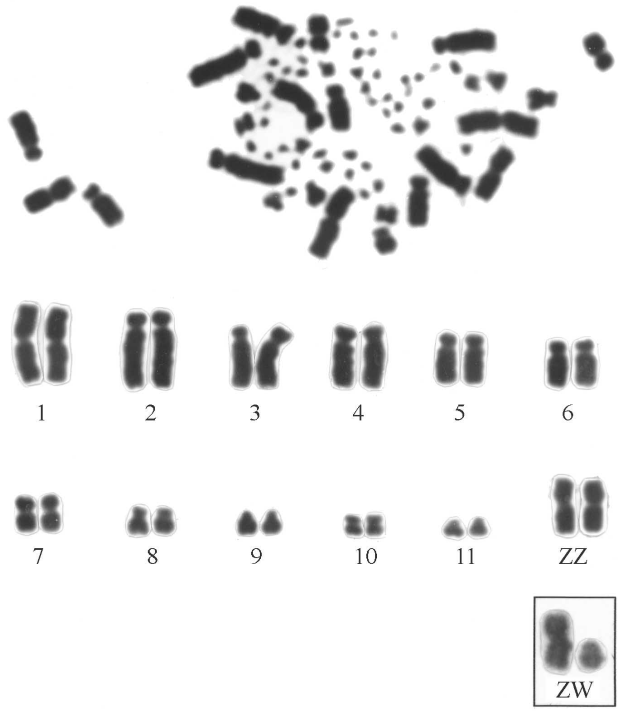

Figure 1 - Mitotic metaphase and male karyotype of *Anodorhynchus hyacinthinus*. In the inset, the ZW female sex chromosomes.

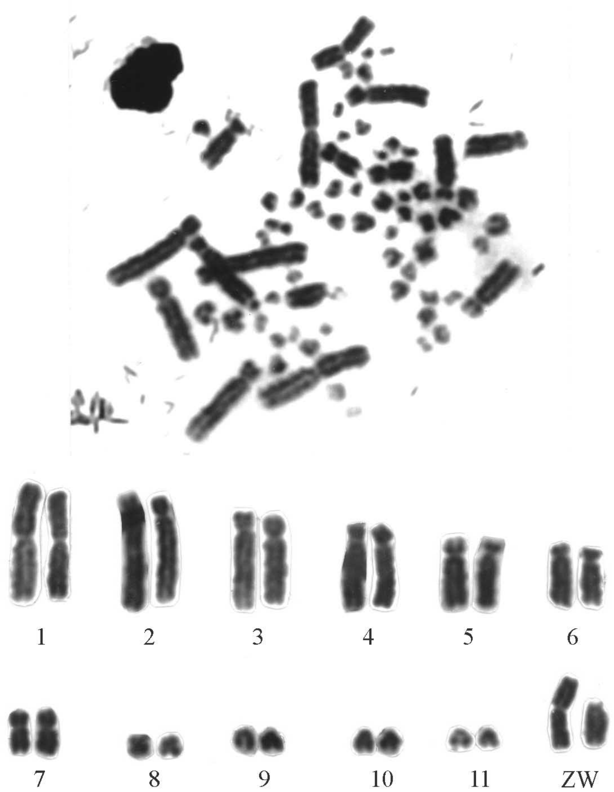

Figure 2 - Mitotic metaphase and female karyotype of *Deroptyus accipitrinus*.

Discussion

Studies on Neotropical psittacids (tribe Arine) have revealed a remarkable structural karyotype dichotomy, which supports the existence of two distinct monophyletic groups within this tribe (Francisco and Galetti Jr., 2001; Francisco *et al*., 2001), as previously suggested by mtDNA analyses (Miyaki *et al*., 1998).

A first karyotype pattern can be observed among genera *Ara, Cyanopsitta*, *Propyrrhura, Aratinga, Pionites, Pionopsitta, Nandayus* and *Guaruba,* mainly characterized by a conservative metacentric pair 1, pairs 2, 3, 4, 5, and 6 varying from submetacentric to subtelocentric, pairs 7 and 8 varying from metacentric to submetacentric, and pairs 9, 10, and 11 varying from metacentric to telocentric (De Lucca, 1984; Van Dongen and De Boer, 1984; De Lucca *et al*., 1991; Duarte and Giannoni, 1990; Goldschmidt *et al*., 1997; Francisco *et al*., 2001; Francisco and Galetti Jr., 2001). The karyotypes of *Anodorhynchus hyacinthinus* and *Deroptyus accipitrinus* described here also presented this main pattern.

A second karyotype pattern is observed in the species of genera *Amazona* and *Brotogeris,* mainly characterized by a significant number of telocentric macrochromosomes (Aquino and Ferrari, 1990; De Lucca *et al*., 1991; Duarte and Caparroz, 1995). The karyotypes of *Pyrrhura*, *Pionus* and *Forpus* seem to be intermediary (De Lucca and Marco, 1983; De Lucca *et al*., 1991).

Although in external appearance *Deroptyus* can resemble a parrot of genus *Amazona* (similar in size and not presenting long central rectrizes), our karyotypic data corroborate previous mtDNA findings (Miyaki *et al*., 1998), indicating that *Deroptyus* could be more related to species of the genera *Anodorhynchus, Ara, Cyanopsitta*, *Propyrrhura, Aratinga, Pionites, Pionopsitta, Nandayus* and *Guaruba*.

Vocalization has been considered a reliable character to distinguish monophyletic genera of Neotropical Psittacidae (Sick, 1990). Based on this character, it has been suggested that *Ara macao*, *A. ararauna*, *A. glaucogularis, A. militaris, A. ambigua, A. chloroptera*, *A. rubrogenys* and *A. severa* were related to the blue macaws (*Anodorhynchus hyacinthinus, A. glaucus* and *A. leari*), composing the group of true macaws. Other species previously assigned to genus *Ara,* such as *maracana, auricollis, manilata* and *nobilis*, were assembled in the maracanãs group, because their behavior resembles that of the small parakeets of genus *Aratinga.* Early classifications have probably overestimated body size and facial bare characters (Sick, 1990). Karyotype data of the species analyzed thus far (see Aquino and Ferrari, 1990; Francisco and Galetti Jr., 2001), obtained by conventional Giemsa staining, have not been able to support this subdivision, because a single general karyotype pattern has been observed in both the true macaws and the maracanãs. Further studies including chromosome banding could be helpful to elucidate species relationships.

The maintenance of a common diploid number of 2n = 70 in *A. hyacinthinus* and *D. accipitrinus*, as well as in most Neotropical Psittacidae species, supports the hypothesis that the main evolutionary mechanisms leading to the karyotypic diversification within the group have been pericentric inversions and/or translocations (De Lucca *et al*., 1991; Francisco *et al*., 2001, Francisco and Galetti Jr., 2001), which do not produce numerical changes in the karyotypes.

While molecular genetic analyses have been widely applied in both *in situ* and *ex situ* management plans (Miyaki *et al*., 1993; Nader *et al*., 1999; Negro and Torres, 1999; Hudson *et al*., 2000; Caparroz *et al*., 2001; Bouzat, 2001; Miyaki and Eberhard, 2002), karyotype analyses have rarely been considered. Cytogenetic studies in Neotropical psittacids have been restricted to the karyotype description of species easily found in captivity, but the origin of the specimens was usually unknown. Although the detection of chromosome variants is not less important than DNA polymorphism to conserve the genetic diversity and evolutionary potential of the species, karyotype studies in wild populations are scarce. Karyotype characterization of *Anodorhynchus hyacinthinus* and *Deroptyus accipitrinus* can be a starting point for genetic monitoring of these two declining species.

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