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Analysis of the genetic variability of *Propyrrhura maracana* (Psittaciformes, Aves) using DNA fingerprinting

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RESUMO. **Análise da variabilidade genética de *Propyrrhura maracana* (Psittaciformes, Aves) utilizando a técnica de DNA fingerprinting.** Até recentemente, acreditava-se que *Propyrrhura maracana* possuía uma vasta distribuição no território brasileiro e em algumas regiões do Paraguai e Argentina; sendo considerada abundante e sem riscos eminentes de extinção. No entanto, tais dados estão sendo revistos e se tem comprovado que a sua área de ocorrência está sofrendo uma diminuição, o que levou à recente inclusão desta espécie no livro "The World List of Threatened Birds" como vulnerável. No presente estudo, avaliamos a variabilidade genética intra-específica de 38 indivíduos de *Propyhrrura maracana* pertencentes a populações selvagens e cativas utilizando a técnica de DNA fingerprinting (identificação individual pelo DNA) com as sondas de minissatélites multilocos 33.15 e 33.6. Concluímos que a espécie apresenta coeficientes de similaridade (com as sondas 33.15 (0,2454) e 33.6 (0,2630)) próximo dos níveis obtidos em outra espécie de psitacídeo neotropical classificada como vulnerável. Além disso, a hibridação com a sonda 33.15 possibilitou a observação de polimorfismo em bandas sexo-específicas. Os dados levam a duas possíveis conclusões: ou a espécie já apresentava uma baixa variabilidade ou está sofrendo uma perda de variabilidade devido à redução da sua área de ocorrência, causada pela destruição de seu habitat e outros fatores, o que tem sido observado em diversas outras espécies de psitacídeos neotropicais.
PALAVRAS-CHAVE: Psittacidae, *Propyrrhura maracana*, variabilidade genética, marcador populacional.

ABSTRACT. Until a few years ago *Propyrrhura maracana* was believed to possess a vast distribution in Brazil and in some areas of Paraguay and Argentina, being considered abundant and without risk of extinction. However, more recent data suggest that its range is contracting, leading to its recent vulnerable status in "The World List of Threatened Birds". In the present study, we evaluated the intraspecific genetic variability of 38 individuals of *Propyhrrura maracana* from wild and captive populations. DNA fingerprinting using multilocus minisatellite probes 33.15 and 33.6 was applied. The similarity indexes obtained (with probes 33.15 (0.2454) and 33.6 (0.2630)) are close to the levels obtained in another vulnerable species of neotropical parrot. Also, hybridization with probe 33.15 revealed polymorphisms in sex-specific bands. Two possible conclusions are drawn: either the species already presented a low genetic variability or it is suffering a variability loss due to the reduction of its occurrence area, caused by the destruction of its habitat or other factors.
KEY WORDS: Psittacidae, *Propyrrhura maracana*, genetic variability, population marker.

Propyrrhura maracana is a long-tailed parrot described by Vieillot as *Macrocercus maracana* in 1816 in Nouv. Dict. d'Hist. Nat. 2ª ed. (Pinto 1938). After its description, this species was included in two genera: *Propyrrhura* and then *Ara;* but recently, Sick (1997) re-classified it as *Propyrrhura maracana.* There are indications that this species could be part of a "maracanãs" group, to which other species belong: *Propyrrhura auricollis*, *Orthopsittaca manilata*, *Diopsittaca nobilis* and possibly *Ara couloni* (Sick 1997).

The distribution of *Propyrrhura maracana* (figure 1) ranges from the east of Brazil, Pará and Maranhão to the south of Mato Grosso do Sul and Rio Grande do Sul, crossing Paraguay until Missiones in the northeast of Argentina (Forshaw 1989). But Olmos (1993) suggested some modifications due to its exceptional steep decline, explained partly by the habitat loss of gallery forests and in forest edges. In the State of Rio de Janeiro, especially in the district of Rio Paraíba do Sul and in the "mata mineira" zone, recolonization is happening and the species can be more common there than in any other place of its distribution area (Pacheco *et al. in litt.* 1994) except for its last strongholds, are the Serra Negra in Pernambuco and the Serra do Cachimbo in southern Pará (C. Yamashita 1994 in Collar *et al.* 1994).

In this sense, *Propyrrhura maracana* can be suffering the same threats of extinction just like other species whose distributions overlap with it as: *Cyanopsitta spixii, Anodorhynchus hyacinthinus, A. glaucus, A. leari, Guaruba guarouba, Aratinga auricapilla, Pyrrhura cruentata, P. hypoxantha, Touit melanonota, T. surda, Amazona brasiliensis, A. duphresniana, Salvatoria xanthops* and *Triclaria malachitacea* (Collar *et al.* 1992). These species have been suffering great pressure due to the accelerated contraction of distributions caused by the destruction of habitat, illegal captures of chicks in the nature (mainly to provision the illegal trade), hunt subsistence and competition for nestling places (generally cavities in tree logs) with other species of animals as africanized bees, opossum and others (Juniper and Yamashita 1990). They may also suffer from several other factors that may affect the variability of wild populations; these are: demographic flotations, environmental variations, diseases, catastrophes, genetic drift and inbreeding

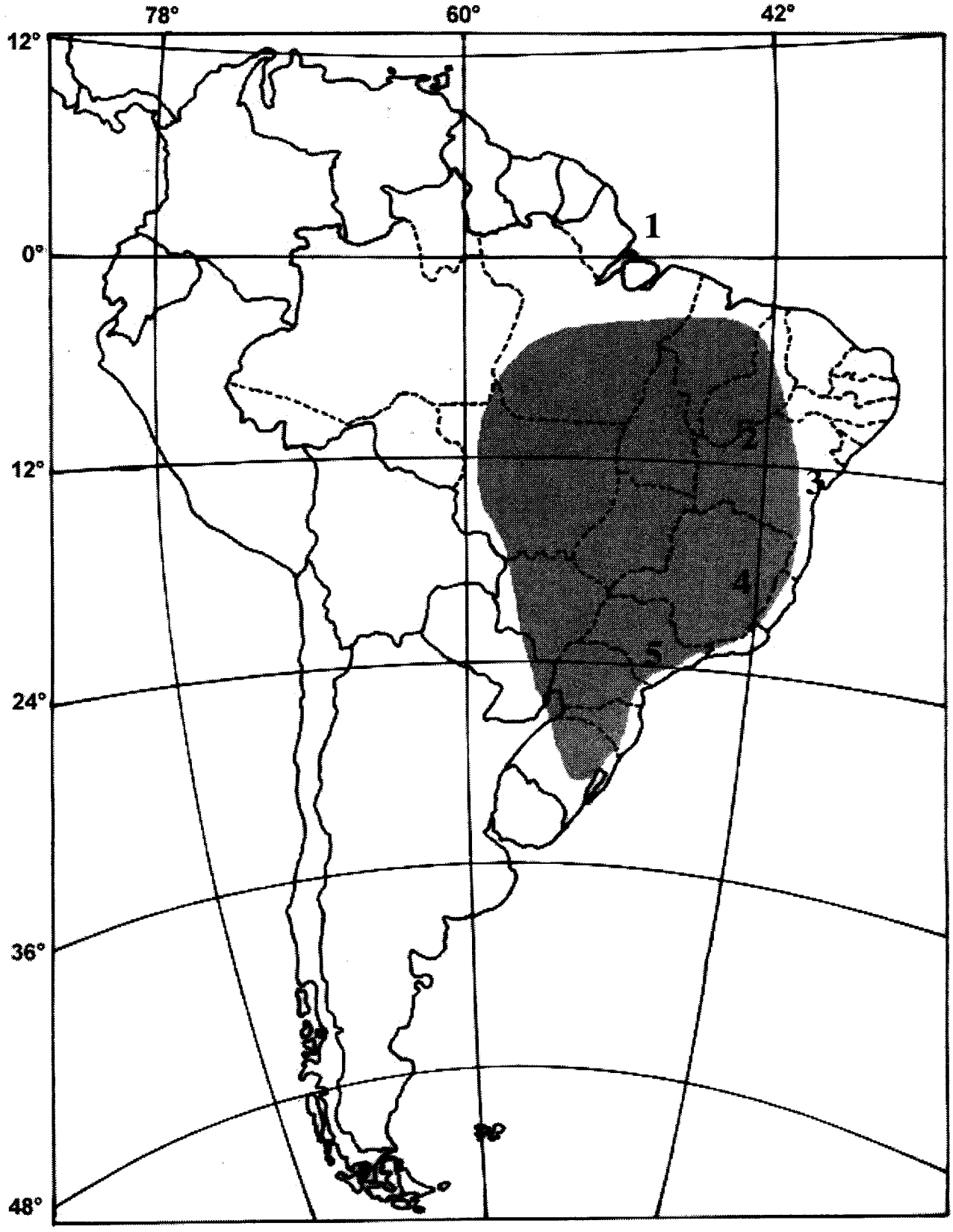

Figure 1. Distribution map of *Propyrrhura maracana* (modified from Forshaw 1989). The numbers indicate the samples collection sites in Brazil: (1) Ilha do Marajó, PA; (2) Curaçá, BA; (3) Salvador Zoo, BA; (4) Belo Horizonte Zoo, MG; (5) Sorocaba Zoo, SP.

(Mace and Lande 1991). The impact of human and natural factors may influence in the population size. The more reduced it is, the higher is the chance of extinction, in a short time span (Gilpin and Soulé 1986). Therefore, monitoring the genetic variability of captive and wild populations can be important for the conservation of several species, once there can be variation in gene frequencies.

A technique that has been presenting good results to characterize the genetic variability of populations (Miyaki *et al.* 1993) is "DNA fingerprinting" (Jeffreys *et al.* 1985). Since the markers detected are highly polymorphic, it is possible to accomplish individual identification. Besides, they possess mendelian inheritance allowing their use in parentage tests. Another application is in the study of captive and wild populations, to evaluate their genetic diversity (Rave *et al.* 1995).

In the present study, we evaluated the intraspecific genetic variability of 38 *Propyhrrura maracana* individuals from wild and captive populations using DNA fingerprinting with multilocus minisatellite probes 33.15 and 33.6.

METHODS

Among the 38 *Propyrrhura maracana* individuals we studied, 8 belong to two wild populations: Curaçá, Bahia State (6 chicks found in nests, sampled by the Projeto Ararinha-azul – IBAMA) and Ilha do Marajó, Pará State (2 individuals collected by the ornithologist Paulo Martuscelli). Regarding the captive birds, 11 belong to Brazilian zoos (Belo Horizonte, Salvador and Sorocaba) and 19 individuals belong to Loro Parque (Spain). These birds from Loro Parque were brought to Brazil for a reintroduction project in Curaçá that has already started.

Approximately 100 µl of blood from each bird were collected and stored in 500 µl of absolute ethanol at room temperature. DNA was extracted individually according to Bruford *et al.* (1992) and the concentrations were estimated in 0.8% agarose gel.

The DNA was digested with *Hae* III, purified with phenol-chloroform-isoamylic alcohol (25: 24: 1) and precipitated with ethanol. The concentrations were checked in a spectophotometer and in 0.8% agarose gel.

5 µg of each digested sample were fractionated in 1% gel agarose (20X30cm) for 40V until the 2 kb marker reached the bottom of the gel (Bruford *et al.* 1992). The DNA was transferred (Southern blot) onto a nylon membrane (Hybond Nfp, Amersham) by capillarity (Sambrook *et al.* 1989). It was then pre-hybridized in 0.263 M Na_2HPO_4, 1mM EDTA, 7% SDS and 1% BSA for 4 hours at 65°C (Westneat 1990). The human minisatellite multilocos probes 33.15 and 33.6 (Jeffreys *et al.* 1985) were labeled with (a - P^{32} dCTP) by random priming and one at a time, was added to the solution in the same temperature.

The membrane was washed with the following solutions: 0.25M Na_2HPO_4/1%SDS, 2XSSC/0.1%SDS and 1XSSC/0.1%SDS for 5 to 10 minutes at 65°C. Then, the membrane was exposed to an x-ray film.

The band sharing coefficients (BSC) were calculated between each pair of individuals according to the formula: $x = 2N_{AB}/N_A+N_B$ (Wetton *et al.* 1987); where, N_{AB} is the number of bands in common between A and B and N_A and N_B are the total number of bands in individuals A and B. This coefficient (BSC) is a similarity index and it reflects the genetic variability.

Assuming that the scored bands are independent, the mean probability of finding the same band pattern in two unrelated individuals is x^n, where x is BSC and n is the mean number of bands detected (Bruford *et al.* 1992).

The Mann-Whitney' test (confidence level of a = 0.05) was used to examine if there is statistical significant difference between captive and wild populations.

RESULTS

The female specific bands (Miyaki *et al.* 1997a), obtained through the hybridization with probe 33.15 allowed us to determine the number of males and females in each locality (table 1). These sex-specific bands presented three different patterns (figure 2).

Table 1. Number of males and females in the localities sampled determined by the female-specific bands detected by the human minisatellite probe 33.15.

Locality	Males: females
Ilha do Marajó, PA	1: 1
Curaçá, BA	1: 5
Salvador, BA	2: 0
Belo Horizonte Zoo, SP	2: 0
Sorocaba Zoo, SP	2: 5
Loro Parque, Spain	12: 7

The band sharing coefficients (similarity indexes) between individuals of three Brazilian Zoos are presented in table 2 and they vary from 0.000 to 0.0645. These indexes could be used to avoid consanguineous matings.

The individuals from zoos and Loro Parque were considered as a captive population (unknown origin in the nature). Three different couples of Loro Parque produced, respectively, 10, 5 and 4 chicks. Thus, even though minisatellite profiles were produced for all individuals, only one chick per couple was considered in the genetic similarity analyses. These captive birds probably belonged to several natural populations and possibly represent a good sample of the species (see Discussion).

Since only two individuals from the Ilha do Marajó were studied, these birds were not considered in the genetic similarity analyses. The chicks from Curaçá were found in three nests: in the first nest there were three birds, in the second one there were two birds and in the third one there was only one individual. Once the BSCs obtained between chicks of same nest were high (data not shown), they suggest that these nestlings are related (possibly siblings). Therefore, when we compared the birds from Curaçá and the captive ones, only one chick per nest was considered.

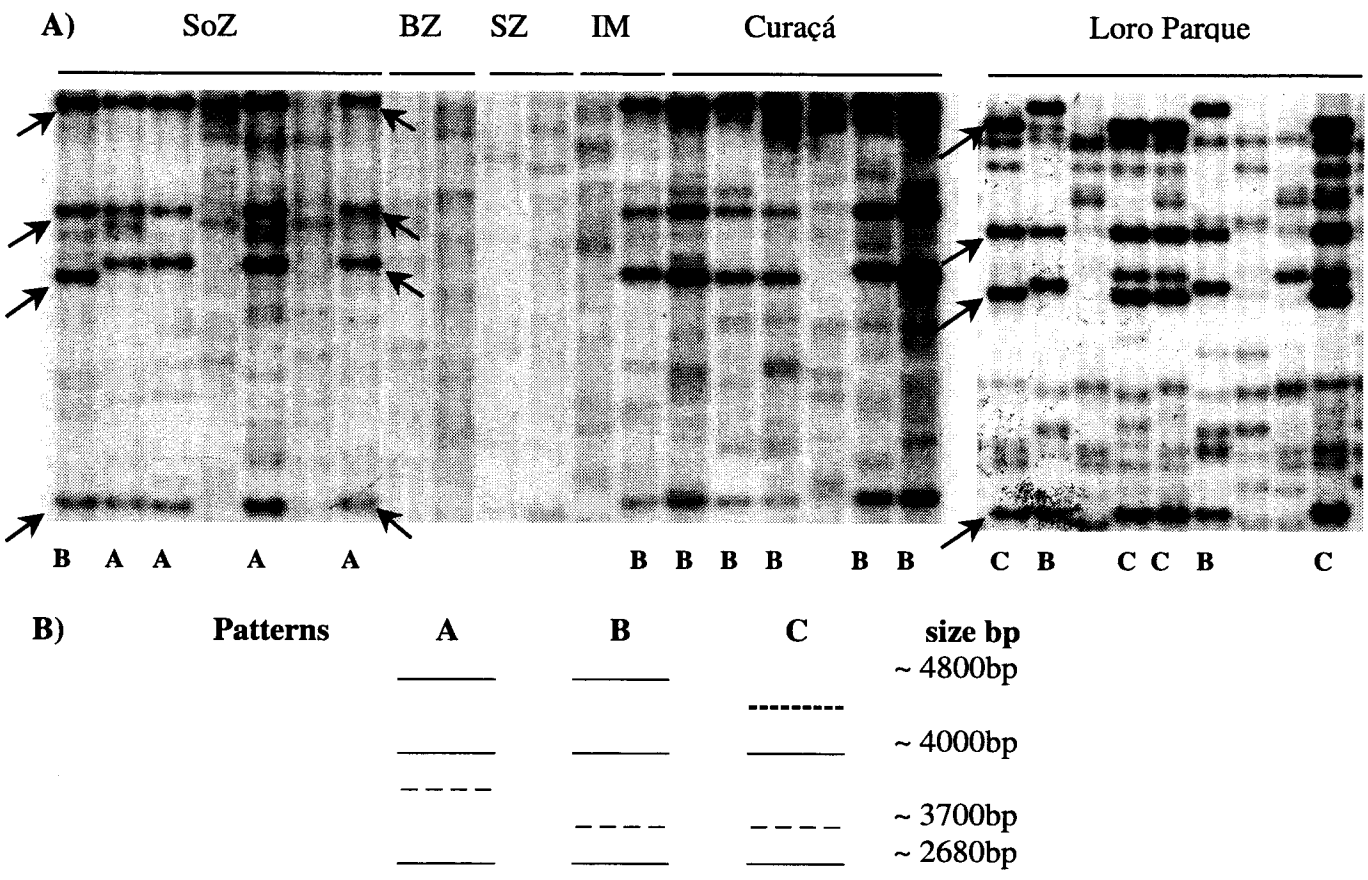

Figure 2. (A) Hybridization patterns obtained using the human minisatellite probe 33.15. SoZ: Sorocaba Zoo; BZ: Belo Horizonte Zoo; SZ: Salvador Zoo; IM: Ilha do Marajó. The arrows indicate the female-specific bands which are represented in patterns bellow (A-C). (B) Female-specific band patterns obtained (A-C), not to scale.

Table 2. Similarity indexes obtained by the hybridization with human minisatellite probes 33.15 and 33.6 (bold) in *Propyrrhura maracana* individuals from Brazilian Zoos. SoZ: Sorocaba Zoo; BZ: Belo Horizonte Zoo; SZ: Salvador Zoo. (F) Female; (M) male.

			SoZ				BZ	SZ			
	1 F	2 F	3 F	4 M	5 F	6 M	7 F	8 M	9 M	10 M	11 M
1 F		0.4211	0.4651	0.2500	0.2500	0.2941	0.4000	0.1622	0.2424	0.2353	0.0645
2 F	**0.3478**		0.4390	0.5263	0.2632	0.6250	0.2424	0.3429	0.1290	0.1875	0.3448
3 F	**0.5532**	**0.2449**		0.2791	0.4651	0.4324	0.1053	0.2500	0.2778	0.2162	0.1765
4 M	**0.2667**	**0.5532**	**0.1667**		0.3000	0.5294	0.3429	0.4865	0.1818	0.2353	0.4516
5 F	**0.4000**	**0.5385**	**0.4151**	**0.3529**		0.3529	0.2286	0.1622	0.0606	0.3529	0.2581
6 M	**0.3810**	**0.5000**	**0.2222**	**0.4651**	**0.5833**		0.1379	0.2581	0.0741	0.0714	0.3200
7 F	**0.3784**	**0.2051**	**0.3000**	**0.4211**	**0.4651**	**0.3429**		0.1875	0.2143	0.3448	0.0000
8 M	**0.1951**	**0.3721**	**0.2273**	**0.4286**	**0.2979**	**0.2051**	**0.2353**		0.4000	0.1935	0.3571
9 M	**0.3590**	**0.4878**	**0.2381**	**0.3000**	**0.3111**	**0.3243**	**0.3750**	**0.3889**		0.0000	0.1667
10 M	**0.3000**	**0.2381**	**0.2326**	**0.4878**	**0.3913**	**0.3158**	**0.5455**	**0.2162**	**0.1714**		0.0800
11 M	**0.3684**	**0.3500**	**0.2927**	**0.3077**	**0.4091**	**0.3889**	**0.4516**	**0.3429**	**0.4242**	**0.3529**	

In table 3 are the mean number of bands detected, the mean indexes of similarity between unrelated birds of the captive and of free living (Curaçá) populations and the probability of two unrelated individuals sharing the same DNA profile by chance.

DISCUSSION

Parrots are believed to be monogamous and consequently, a sex ratio of 1: 1 is the ideal situation within a population. The sex ratio of wild birds from Ilha do Marajó

was 1: 1, while most of the chicks sampled in Curaçá were females (table 1). The unfavorable sex bias found in the Curaçá sample may not reflect the true sex ratio found in the population since only six individuals were studied. If captive breeding programs of *Propyrrhura maracana* are established in the three Zoos involved in this study, it will be necessary to transfer birds from one place to another. Among the Loro Parque birds studied, there is a higher number of males. These individuals were brought to Curaçá, Bahia state, for a reintroduction program.

The similarity indexes obtained between the birds from three Zoos (table 2) could be used to establish couples with lower genetic similarity. For example, female 1 should be paired to male 4 rather than to male 6 who presents higher similarity with female 1 estimated by either probe applied. However, the choice for "the best couple" depends on many factors such as the age of the birds and their behavioral compatibility.

Polymorphisms in the sex-specific bands were observed and three different patterns were detected. Unfortunately, it was not possible to test if these patterns could be used as population markers. It would be necessary to have more samples from birds of known origins. If these bands prove to be linked to determined populations, it would be possible to know which localities should be better protected against illegal poaching.

The mean number of bands detected (table 3) is similar to those observed in other parrots (Miyaki *et al.* 1993, 1997b). The low probability of two birds presenting the same band pattern show that each profile is individual-specific (table 3).

Table 3. Hybridization results using the human minisatellite multilocus probes 33.15 and 33.6 in unrelated individuals of *Propyrrhura maracana*.

Probe	Origin*	n ± sd	X ± sd	X^n
33.15	captivity	17.20 ± 3.10	0.2454 ± 0.1105	9.15×10^{-11}
	Curaçá	16.37 ± 2.45	0.3427 ± 0.0945	2.42×10^{-8}
33.60	captivity	18.06 ± 3.37	0.2630 ± 0.1157	2.02×10^{-11}
	Curaçá	20.00 ± 3.34	0.3980 ± 0.1139	2.00×10^{-7}

* captivity: individuals from Brazilian zoos (Belo Horizonte, Salvador and Sorocaba) and Loro Parque, Spain. Curaçá: nestlings; n: mean number of bands; sd: standard deviation; X : mean band sharing coefficient; X^n: probability for two unrelated individuals sharing the same band pattern.

The significant difference found between the BSC of the captive and the Curaçá populations (P = 0.0011 for probe 33.15 and P = 0.000 for probe 33.6), reinforces the suggestion that this captive population constitutes a good sample of the species. Thus, the similarity index of the species was elevated (table 3), being comparable to indexes of another threatened parrot species: *Amazona brasiliensis* (mean BSC: 0.23 and 0.30 using probes 33.15 and 33.6, respectively; Miyaki *et al.* 1997b). This result reinforces the importance of inclusion of *Propyrrhura maracana* in the list of threatened species.

In summary, with this DNA fingerprinting study in *Propyrrhura maracana*, it was possible to evaluate the genetic variability of captive and wild populations. The data obtained corroborate other studies that lead to the inclusion of the species in "The World List of Threatened Birds" (Collar *et al.* 1994). This showed that this technique can contribute for the study of threatened species.

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