Research Article

Polymorphic heterologous microsatellite loci for population genetics studies of the white-faced ibis *Plegadis chihi* (Vieillot, 1817) (Pelecaniformes, Threskiornithidae)

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Abstract

We screened 44 heterologous microsatellites isolated in species of the families Threskiornithidae, Ciconiidae and Ardeidae for their use in a migratory waterbird, the white-faced ibis *Plegadis chihi* (Vieillot, 1817) (Threskiornithidae). Of the screened loci, 57% amplified successfully and 24% were polymorphic. In two breeding colonies from southern Brazil (N = 131) we detected 32 alleles (2-10 alleles/ locus). Average *He* over all loci and colonies was 0.55, and the combined probability of excluding false parents, 98%. There was no departure from *HWE* in any loci or population. Eru6 and Eru4 loci were in non-random association in the Alvorada colony, and NnNF5 and Eru5 in both populations. AMOVA analysis indicated that most of the genetic diversity was contained within populations. Structure analysis suggested a single population, and F_{ST} value showed weak genetic structuring (F_{ST} = 0.009, p = 0.05). The two populations are apparently connected through gene-flow. The panel of six microsatellites optimized here was sufficiently informative for characterizing the genetic diversity and structure in these natural populations of the white-faced ibis. The information generated could be useful in future studies of genetic diversity, relatedness and the mating system in *Plegadis chihi* and related species.

Introduction

The white-faced ibis *Plegadis chihi* (Vieillot, 1817) (Pelecaniformes, Threskiornithidae) is a migratory long-legged wading bird, with a long, downward-curved bill and attractive metallic bronze plumage (Dark-Smiley and Keinath, 2003). Occurring from North to South America, it primarily inhabits freshwater wetlands and marshes, and forages in flooded areas and agricultural fields (Ryder and Manry, 1994). Populations in the U.S.A. nest in marshes in the western region, and winter in large flocks in the western parts of the country and Mexico (Dark-Smiley and Keinath, 2003). In South America, it occurs in Brazil, Bolivia, Paraguay, Uruguay, Argentina and Chile. In Brazil, breeding takes place in colonies in coastal lagoons in Rio Grande do Sul State, in the southernmost part of the country (Petry and Fonseca, 2005). Although flocks engage in regional movements between the breeding and wintering periods, their specific migration routes remain unknown (Ryder and Manry, 1994).

So far, populations of the white-faced ibis have not been investigated as to genetic variability, an important property of populations. Population-genetic parameters can be determined with the aid of molecular markers, such as microsatellites, also called simple sequence repeats (SSR). DNA sequences, two to six nucleotides long, repeated in tandem and widely dispersed in all eukaryotic genomes (Tautz, 1989). These codominant and highly polymorphic markers (Zane *et al.*, 2002) have high mutation rates (10^{-2}-10^{-6} per meiotic event) (Li *et al.*, 2002), the majority being selectively neutral (Selkoe and Toonen, 2006). Accordingly, SSRs have become powerful tools for use in forensic studies, kinship investigation, gene mapping, conservation biology and population genetics (Zane *et al.*, 2002).

In spite of their utility and wide application, there is still a major drawback in the use of microsatellites, since any study aimed at employing such markers requires the isolation of new loci and the development of new primers. However, the isolation of loci for a given species, besides being exceedingly labour-intensive, requires considerable funds, time and technical experience (Mukesh *et al.*, 2011). Moreover, in avian species, the process of microsatellite isolation can pose an additional challenge, since microsatellites are less abundant in avian genomes than in any other vertebrate group (Primmer *et al.*, 1997). An alternative cost-effective approach is the use of primers developed

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for closely related species, denominated cross-species microsatellite amplification or heterologous amplification, commonly applied in bird studies (Barbará *et al.*, 2007; Mukesh *et al.*, 2011). The success of this process depends on the degree of conservation of primer annealing sites among related species. High evolutionary divergence between the species from which the microsatellite loci were originally isolated (the source species) and the species to which they will be applied (the target species) results in a low number of amplified loci (Primmer *et al.*, 2005). A necessary first step in any study involving the use of heterologous markers is the screening and evaluation of their level of polymorphism in the target species.

Microsatellite loci constitute an adequate tool for estimating such important properties of populations, as genetic diversity, population structure, effective population size and gene flow, all useful in defining units for conservation (Haig *et al.*, 2011). This lack of species-specific microsatellite markers is one of the reasons why, in populations of the white-faced ibis, nuclear neutral genetic variability remains neglected. Thus, the goal in the present study was to identify and optimize heterologous microsatellite loci for population studies in this bird-species. Further information is provided on population genetic parameters and patterns of genetic structuring in samples of two breeding colonies located in the extreme geographic distribution of this species in south Brazil.

Material and Methods

Growing feathers were plucked from 44 nestlings (one per nest) in a breeding colony in Alvorada, Rio Grande do Sul State (RS), Brazil (S 29° 58' 54", W 51° 05' 11"). Samples of growing feathers and muscle tissue were also taken from 87 nestlings (one per nest) of a breeding colony in Tapes, RS, Brazil (S 30° 41' 12", W 51° 23' 53"). Genomic DNA was extracted from the feathers and muscle tissue as per the standard phenol/chloroform method (Sambrook *et al.*, 1989). Initial screening involved a panel of 44 heterologous microsatellite primers previously isolated in species belonging to the Threskiornithidae and Ardeidae families (order Pelecaniformes), viz., *Platalea ajaja* (Sawyer, 2002, PhD Thesis, Univ. North Texas), *Eudocimus ruber* (Santos *et al.*, 2006), *Nipponia nippon* (Ji *et al.*, 2004), *Ardea herodias* (McGuire and Noor, 2002) and *Bubulcus ibis* (Campanini *et al.*, 2010); and to the Ciconiidae family (order Ciconiiformes): *Mycteria americana* (Tomasulo-Seccomandi *et al.*, 2003).

Polymerase chain reactions (PCR) were carried out in 10-12 μL reaction mixtures containing by 1 x PCR buffer (1 mM Tris-HCL, pH 8.4, 50 mM KCl), 1.5-2.5 mM MgCl$_2$, 0.2 mM dNTPs (Amersham Biosciences), 0.5 U of *Taq* DNA polymerase (Biotools B&M Labs) and 10-30 ng of template DNA. Except for loci isolated in *P. ajaja* and *M. americana* (HEX-labeled), all the primers were dynami-

cally labeled with HEX, FAM or TET, using M13 methodology (Schuelke, 2000). Reactions were carried out in an Eppendorf Mastercycler Gradient® (Eppendorf AG, Hamburg, Germany) thermal cycler. The annealing temperatures described for the source species were initially tested, and then modified accordingly, in order to increase the probability of amplifying heterologous loci (Primmer *et al.*, 2005). Successful amplification conditions are subsequently described. For Aaju3 and Aaju5 loci (HEX-labeled), the PCR cycling parameters were: 1 min of initial denaturation at 94 °C, followed by 29 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 35 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 20 min. For the loci NnNF5, NnCE11, NnDD9 and NnHB12 (M13-labeled), conditions were: 4 min of initial denaturation at 94 °C, followed by 38 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min and extension at 72 °C for 30 s, 8 cycles of 94 °C for 30 s, 45 s at 53 °C and extension at 72 °C for 45 s with a final extension at 72 °C for 20 min. Eru3, Eru4, Eru6 and Eru9 loci (M-13 labeled) were amplified with the following PCR parameters: 4 min of initial denaturation at 94 °C, followed by 35 cycles of 94 °C for 45 s, annealing at specific temperature for 35 s and extension at 72 °C for 1 min, 8 cycles of 94 °C for 30 s, 45 s at 53 °C and extension at 72 °C for 1 min with a final extension at 72 °C for 20 min. Eru2, Eru5, Eru7, Eru8 and Eru11 loci (M-13 labeled) were amplified as follows: initial denaturation at 94 °C for 3 min, followed by 19 cycles at 94 °C for 45 s, annealing for 1 min at locus-specific temperatures and 72 °C for 1 min, 8 cycles of 94 °C for 30 s, 45 s at 53 °C and extension at 72 °C for 1 min with a final extension at 72 °C for 20 min. For WS03, WS04 and WS09 loci (HEX-labeled), amplification conditions were: 5 min of initial denaturation at 94 °C, followed by 21 cycles of 94 °C for 30 s, 35 s at 55 °C and extension at 72 °C for 1 min, 8 cycles of 94 °C for 30 s, 45 s at 53 °C and extension at 72 °C for 1 min with a final extension at 72 °C for 20 min. For Bi1, Bi5, Bi7, Bi9, Bi10, Bi11 and Bi12 loci (M-13 labeled), PCR parameters were: initial denaturation at 5 min for 94 °C, followed by 20 cycles of 94 °C for 30 s, annealing for 1 min at locus-specific temperatures and extension at 72 °C for 45 s, 15 cycles of 94 °C for 30 s, annealing for 1 min at locus-specific temperatures and extension at 72 °C for 45 s, 12 cycles of 94 °C for 30 s, 45 s at 53 °C and extension at 72 °C for 45 s with a final extension at 72 °C for 20 min.

In order to detect amplification, PCR products underwent horizontal electrophoresis in 1% agarose gels stained with ethidium bromide. Genotyping was carried out in a MegaBACE 1000 (Amersham Biosciences) automatic sequencer. PCR products were diluted with Tween 0.1% and added to ET550-R Size Standard (GE Healthcare). Alleles were sized after analyzing electropherograms in MegaBace Fragment Profiler software (GE Healthcare).

The Micro-Checker (Van Oosterhout *et al.*, 2004) program was applied for assessing genotyping quality, the occurrence of null alleles, allelic dropout and stuttering. The number of alleles (*A*), expected (*He*) and observed (*Ho*) heterozygosity, exclusion probability (*PE*), identify probability (*PI*), Hardy-Weinberg equilibrium (*HWE*) per locus, and analysis of molecular variance (AMOVA) were estimated using GenAlex version 6.4 (Peakall and Smouse, 2006). Polymorphic information content (*PIC*) was computed in Cervus version 3.0 (Kalinowski *et al.*, 2007). Allelic richness (*Ra*), genic diversity (*DG*, Nei, 1987), inbreeding coefficient (F_{IS}) and population differentiation (F_{ST}) were computed using Fstat version 2.9.3.2 (Goudet, 1995). Linkage disequilibrium (*LD*) between loci was computed in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). *P*-values for all tests were corrected for multiple comparisons using the sequential Bonferroni procedure (Rice, 1989). The Genepop version 4.0.10 program (Rousset, 2008) was applied for computing a multilocus estimate of the effective number of migrants (*Nm*) (Slatkin, 1985; Slatkin and Barton, 1989). Signals of reduction in population size were assayed with the Wilcoxon test (Luikart *et al.*, 1998), as implemented in Bottleneck version 1.2.02 software (Cornuet and Luikart, 1996). Population structure for microsatellite variation was also examined using the Structure version 2.1 program (Pritchard *et al.*, 2000). By this Bayesian clustering method, estimates are made of the number of genetic clusters (subpopulations; *k*) that best explain the data, and thus, based on their genotypes and disregarding sampling locations, assign individuals to subpopulations. Ten replicates of each run for *k* = 1 and *k* = 2 were carried out using the admixture model with a burn-in period of 100,000, and 10,000 MCMC reps after burn-in.

Results and Discussion

The 44 loci were initially screened in samples of 20 individuals from each population. Of the loci tested, 19 did not amplify, even after several trials: Aaju1, Aaju2 (*P. ajaja*), Eru10 (*E. ruber*), WS8, WS13, WS14, WS18, WS23, WS24 (*M. americana*), Ah211, Ah217, Ah320, Ah414 (*A. herodias*) and Bi2, Bi6, Bi8, Bi13, Bi14, Bi15 (*B. ibis*). Among the remaining 25 that did successfully amplify, six were polymorphic in 40 individuals from the two breeding colonies (Table 1). The percentages of heterologous amplification (57%) and polymorphic loci (24%) were slightly lower than those reported in the literature for birds, *i.e.*, average success in amplification from 67% (Dawson *et al.*, 2000) to 84% (Galbusera *et al.*, 2000), and polymorphic loci from 39% (Galbusera *et al.*, 2000) to 46% (Primmer *et al.*, 1996). All the heterologous loci disclosed as polymorphic in *P. chihi* had already been originally isolated in species of the same family: Threskiornithidae. This is in accordance with the hypothesis that the proportion of polymorphic loci increases with decreasing phylogenetic

distance between the source and target species (Primmer *et al.*, 2005).

At the level of families and orders, the percentages of successful amplification and polymorphism were higher than, or of the same magnitude as, those reported in the literature for the group `birds'. Herein, when considering loci isolated in other genera of the same family as *P. chihi*, the 84% of successful heterologous amplification was of the same magnitude as that obtained by Chang *et al.* (2009) (81.8%), and higher than that by Barbará *et al.* (2007) (62%), Lopes *et al.* (2010) (62.5%), and Yeung *et al.* (2009) (17%). When considering different families of the same order, 57% success was higher than Li and Merilä (2010) (47.4%), Yeung *et al.* (2009) (9%), Barbará *et al.* (2007) (38%), and Miño (2006, Master's Thesis, Universidade Federal de São Carlos, São Carlos, Brazil) (31%). The percentage of polymorphic loci among those that successfully amplified, when considering co-familial genera, was 40%, whereas Barbará *et al.* (2007) encountered 55%. The percentage of polymorphic loci found among families of the same order (24%) was higher than those found by Li and Merilä (2010) (21.1%), Barbará *et al.* (2007) (23%) and Miño (2006, Master's Thesis, Universidade Federal de São Carlos, São Carlos, Brazil) (15.5%).

The *PIC* index, an efficient indicator of loci adequacy in population genetics studies, ranged from 0.35 to 0.76, with a mean of 0.50 in our panel of microsatellites (Table 1). It is commonly accepted that *PIC* values > 0.50 are characteristic of the most useful markers, 0.25 < *PIC* > 0.50 of those moderately informative, and *PIC* < 0.25 of the less informative (Andres and Kapkowska, 2011). Thus, the loci optimized in this study can thus be considered moderate to highly informative. The potential of our set of markers for identifying individuals within the population was quite high, *i.e.*, across loci *PI* = 0.0002 in Alvorada and *PI* = 0.0001 in Tapes. Exclusion probability (*PE*) across loci of 98% falls within the range observed in other waterbirds (Miño *et al.*, 2009). This relatively high probability of excluding a false parent indicates that this panel of markers is useful not only in the estimation of the overall level of genetic variability in white-faced ibis populations, but also in the diagnosis of relationships between individuals.

Population genetic parameters were estimated using the entire sample (N = 44 for Alvorada; N = 87 for Tapes). Analysis with the Micro-Checker program indicated null alleles in Eru6 loci due to homozygote excess in both populations, probably caused by stuttering in the electrophorograms. According to Chi-square tests, with sequential Bonferroni correction, there was no departure from *HWE* in any of the analyzed loci after multiple comparisons (Table 2). Across population samples, the mean number of alleles per locus was 4.83, average *He* 0.55 and average *Ho* 0.57. Average *Ra* per population was 4.70 in Alvorada and 4.42 in Tapes, whereas mean *DG* was 0.56 in both populations (Table 2). Inbreeding coefficients (F_{IS}) did not deviate

Table 1 - Basic information on the microsatellite markers screened in this study. Summary information on the heterologous microsatellite loci screened in a pooled sample of 20 individuals of the white-faced ibis *Plegadis chihi* from each of two Brazilian colonies. Superscripts in locus names refer to references of the original primer description and PCR cycling parameters, detailed in the table footnote. An asterisk (*) to locus names indicates polymorphism in the studied sample. AT: Annealing temperature in the original species / in *P. chihi*. *PIC:* polymorphic information content, *ML*: monomorphic locus.

Locus	Repetitive unit	AT (°C)	Reverse primer volume (μL) / concentration (μM) in PCR	Forward primer volume (μL) / concentration (μM) in PCR	*PIC*
Aaju3[1]*	$(GAT)_{13}$	55 / 53	0.6 / 2.5	0.6 / 2.5	0.76
Aaju5[1]	$(GAT)_{16}(TAT)_4$	58 / 55	0.6 / 2.6	0.6 / 2.6	ML
NnNF5[2]*	$(TAGA)_2TA(CA)_8$	57 / 57	1.0 / 0.4	2.5 / 10.5	0.35
NnCE11[2]	$(CTG)_8$	59 / 56	0.8 / 4.0	0.2 / 1.0	ML
NnDD9[2]	$(GT)_8$	59 / 54	0.8 / 4.0	0.2 / 1.0	ML
NnHB12[2]	$(CAT)_7$	59 / 56	0.8 / 4.0	0.2 / 1.0	ML
Eru2[3]*	$(GA)_7(A)_4(GA)_{10}$	53 / 53	0.8 / 4.0	0.2 / 1.0	0.38
Eru3[3]	$(AG)_{21}$	53 / 53	0.5 / 2.5	1.25 / 6.25	ML
Eru4[3]*	$(TC)_{11}(C)_3(TC)_9$	62.5 / 54	0.5 / 2.5	1.25 / 6.25	0.37
Eru5[3]*	$(CA)_{15}(AT)_2(CA)_3$	65 / 62	0.8 / 4.0	0.2 / 1.0	0.53
Eru6[3]*	$(CT)_{20}$	57 / 54	0.5 / 2.5	1.25 / 6.25	0.59
Eru7[3]	$(CT)_6AG(CT)_6TC(CT)_{10}$	62.5 / 60	0.8 / 4.0	0.2 / 1.0	ML
Eru8[3]	$(GA)_8GCAAGT(GA)_9$	63 / 63	0.8 / 4.0	0.2 / 1.0	ML
Eru9[3]	$(GA)_{24}$	62.5 / 54	0.5 / 2.5	1.25 / 6.25	ML
Eru11[3]	$(GAAGA)_{18}$	62.5 / 63	0.8 / 4.0	0.2 / 1.0	ML
WS3[4]	$(AG)_{10}$	60 / 58	0.8 / 4.0	0.8 / 4.0	ML
WS4[4]	$(GT)_{15}$	60 / 58	0.8 / 4.0	0.8 / 4.0	ML
WS9[4]	$(AAC)_7$	60 / 58	0.8 / 4.0	0.8 / 4.0	ML
Bi1[5]	$(GT)_{11}$	57 / 57	0.8 / 4.0	0.2 / 1.0	ML
Bi5[5]	$(TTTG)_4$	56 / 54	0.8 / 4.0	0.2 / 1.0	ML
Bi7[5]	$(TA)_5$	56 / 52	0.8 / 4.0	0.2 / 1.0	ML
Bi9[5]	$(CT)_4(TTC)_2(TC)_4$	58 / 58	0.8 / 4.0	0.2 / 1.0	ML
Bi10[5]	$(AC)_5AA(AC)_4$	58 / 54	0.8 / 4.0	0.2 / 1.0	ML
Bi11[5]	$(GT)_4$	60 / 55	0.8 / 4.0	0.2 / 1.0	ML
Bi12[5]	$(CA)_4(TC)_2$	59 / 59	0.8 / 4.0	0.2 / 1.0	ML

Source species: [1]*Platalea ajaja* (Sawyer, 2002, PhD Thesis, Univ. North Texas); [2]*Nipponia nippon* (Ji *et al.*, 2004); [3]*Eudocimus ruber* (Santos *et al.*, 2006); [4]*Mycteria americana* (Tomasulo-Seccomandi *et al.*, 2003); [5]*Bubulcus íbis* (Campanini *et al.*, 2010).

significantly from random mating (p = 0.004), neither per locus nor population. The exact test of *LD* indicated association (p < 0.05) between Eru6 and Eru4 loci in the Alvorada colony, and between NnNF5 and Eru5 loci in both populations. Thus, NnNF5 and Eru6 loci were excluded when subsequently estimating population genetic parameters.

Overall expected heterozygosity observed in the white-faced ibis (*He*: 0.55) was similar to that observed in 17 species of aquatic birds (*He*: 0.62 ± 0.15). This *He* value was higher than the range of most common heterozygosity values (*He*: 0.40-0.50) observed in 17 aquatic bird species (Eo *et al.*, 2011), besides being higher than that observed in the wood stork, of the same magnitude as that noted in the jabiru stork (*Jabiru mycteria*), and lower than in the roseate spoonbill and great egret (Table 3). Furthermore, on considering only the aquatic species phylogenetically more related to the white-faced ibis (the great cormorant,

Phalacrocorax carbo, and the great blue heron, *Ardea herodias*), mentioned in the Eo *et al.* (2011) study, it can be seen that in the white-faced ibis, *He* values were lower (Table 3). Moreover, the mean number of alleles per locus observed in the white-faced ibis (*A*: 4.83) was lower than the average for the 17 former mentioned species examined by Eo *et al.* (2011) (*A*: 6.59 ± 6.94).

According to the Bottleneck program, there was no evidence of recent reduction in size in either of the two reproductive colonies (Wilcoxon test, Alvorada: p = 0.43; Tapes: p = 0.10). Population differentiation analysis revealed low, but significant interpopulation genetic structuring (F_{ST} = 0.009, p < 0.05). According to AMOVA analysis, most genetic variation at the microsatellite locus level (98%) was among individuals within populations, whereas only 2% (p = 0.054) of variation was interpopulational. According to Bayesian analysis, both samples can be consi-

Table 2 - Levels of genetic diversity in *Plegadi chihi*. Population genetic parameters for two Brazilian breeding-colonies of the white-faced ibis, estimated based on six heterologous microsatellite loci. *N*: Number of samples genotyped, *Ho*: Observed heterozygosity, *He*: Expected heterozygosity, *Ra*: Allelic richness, P_{HWE}: Probability values for tests of departure from Hardy-Weinberg Equilibrium, *DG*: Gene diversity, F_{IS}: Inbreeding coefficient.

Colony	Locus	*N*	H_O	*He*	*Ra*	P_{HWE}	*DG*	F_{IS}
Alvorada	Aaju3	44	0.77	0.72	8.83	0.85	0.72	0.87
	Eru2	37	0.49	0.39	4.00	0.70	0.39	1.00
	Eru4	44	0.54	0.50	2.84	0.58	0.50	0.76
	Eru5	44	0.73	0.63	3.00	0.04[1]	0.64	0.91
	Eru6	44	0.48	0.64	7.50	0.10	0.65	0.004
	NnNF5	44	0.50	0.42	2.00	0.18	0.42	0.96
	Mean	-	0.58	0.55	4.70	0.41	0.56	0.86
Tapes	Aaju3	87	0.84	0.78	9.37	0.11	0.78	0.95
	Eru2	84	0.56	0.44	2.69	0.06	0.44	1.00
	Eru4	87	0.46	0.53	3.48	0.34	0.53	0.11
	Eru5	86	0.55	0.59	3.00	0.06	0.59	0.20
	Eru6	86	0.51	0.60	6.00	0.06	0.60	0.02
	NnNF5	84	0.44	0.40	2.00	0.39	0.40	0.86
	Mean	-	0.56	0.56	4.42	0.17	0.56	0.54
	Overall mean	-	0.57	0.55	4.56	0.29	0.56	0.60

[1]Non-significant after sequential Bonferroni correction (p > 0.05).

Table 3 - Heterozygosity in seven aquatic-bird species. Comparison of average expected heterozygosity (H_E) values observed in seven species of aquatic birds analyzed using microsatellite markers.

Species	H_E	Reference
Wood stork (*Mycteria americana*)	0.37	Miño *et al.*, 2011[1]
Wood stork (*Mycteria americana*)	0.44	Van Den Bussche *et al.*, 1999[1]
White-faced Ibis (*Plegadis chihi*)	0.55	This study
Jabiru stork (*Jabiru mycteria*)	0.55	Lopes *et al.*, 2010
Great egret (*Ardea alba*)	0.58	Miño *et al.*, 2011
Great Blue Heron (*Ardea herodias*)	0.59	Eo *et al.*, 2010[2]
Roseate spoonbill (*Platalea ajaja*)	0.61	Miño *et al.*, 2011
Great Cormorant (*Phalacrocorax carbo*)	0.73	Eo *et al.*, 2010[3]

[1]The study by Miño *et al.* (2011) refers to nine microsatellite loci, whereas that by Van Den Bussche *et al.* (1999) refers to four loci, also mentioned in Eo *et al.* (2010).
[2]Original reference: McGuire and Noor (2002).
[3]Original references: Goostrey *et al.* (1998) and Piertney *et al.* (1998).

dered as a single population cluster. This is in agreement with the estimated number of migrants per generation (*Nm* = 1.7) and indicates that gene flow may be occurring between the studied populations. In theory, the migration of one individual per generation is sufficient to homogenize the genetic pools of two different populations (Hartl and Clark, 1989). Thus, the low significant differentiation de-

tected between these two white-faced ibis colonies is probably a consequence of minor genetic divergence. It must be stressed that a study with more representative samples, collected throughout the entire species-distribution range, using additional microsatellite loci, would help to clarify this issue.

In conclusion, the panel of heterologous loci optimized in this study is suitable for future investigations on genetic structure and relatedness in both natural and captive populations of the white-faced ibis. Future studies including additional loci and populations, should be carried out in order to characterize overall genetic structuring of breeding colonies distributed throughout Brazil. We believe that the data herein would be useful for studies on important questions regarding population genetics, mating systems and kinship in the white-faced ibis and related species. More specifically, the information provided in the present study can benefit studies of population genetics in other species within the genus *Plegadis*, viz., *P. falcinellus* and *P. ridgwayi*.

Acknowledgments

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