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Article

Genetic structure and diversity in *Neochen jubata* (Aves: Anatidae) from the Araguaia River, GO, Brazil

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Abstract: The Orinoco Goose *(Neochen jubata)* is a few-known and endemic Anatidae to South America, inhabiting sandy beaches along medium and large rivers, with a well-developed riparian forest and in swamp savannas and large freshwater baths. Recent data indicate the presence of longitudinal migratory behavior, and despite them, there are no records on the genetic profile of this species. The Araguaia River region, in the municipality of Luiz Alves, Goiás, receives an undetermined number of ducks seasonally, and there is little information about the individuals who visit this place, constituting the ideal scenario for a study able to offer a genetic overview perspective of this species and to understand the relationship between these individuals better. For this, we genetically characterized 61 individuals sampled in three distinct years of collection using microsatellite molecular markers and mitochondrial DNA. Genetic diversity analyses revealed low levels of heterozygosity for all sampled groups. However, they are within the equilibrium proposed by Hardy-Weinberg (HWE), as inbreeding or drift are not acting in these groups. The parentage analysis supports it, showing a high number of unrelated individuals over the years. AMOVA showed a significant difference among groups. These results may reflect the structure of this migratory species in that region, with the paired differentiation test of individuals from 2013 and 2014 being more similar to each other than those from other years, indicating a possible genetic structure diagnosed by the years of capture. However, there is a high allelic sharing among the three sampled groups, suggesting that these individuals are a population that connects over time and that they have a philopatric relationship with the location. The results found in this study constitute an initial milestone for the genetic knowledge of the mallard duck that should be raised in many other genetic studies.
Keywords: *Microsatellite; mitochondrial DNA; Orinoco Goose; kinship; structure population..*

Estrutura populacional e diversidade genética de *Neochen jubata* (Aves: Anatidae) no rio Araguaia, GO, Brasil

Resumo: O ganso-do-orinoco *(Neochen jubata)* é um anatídeo endêmico da América do Sul, cujo habitat são praias arenosas ao longo de rios médios e grandes, com uma vegetação ripária bem desenvolvida e em savanas do pântano com extensos banhados de água doce. Dados recentes indicam a presença de comportamento migratório longitudinal e, apesar destes, não existem registros sobre o perfil genético dessa espécie. A região do rio Araguaia, no município de Luiz Alves, Goiás, recebe um número indeterminado de patos sazonalmente e há poucas informações sobre os indivíduos que visitam este local, constituindo o cenário ideal para um estudo capaz de oferecer uma perspectiva genética geral dessa espécie e também de entender melhor a relação de parentesco entre esses indivíduos. Para tal, caracterizamos geneticamente 60 indivíduos amostrados em três anos distintos de coleta, utilizando marcadores moleculares microssatélites e DNA mitocondrial. As análises de diversidade genética revelaram baixos níveis de heterozigosidade para todos os grupos amostrados. No entanto, eles estão dentro do Equilíbrio proposto por Hardy-

Weinberg (HWE), pois a consanguinidade ou a deriva não estão atuando nesses grupos, a análise de parentesco apoia este resultado indicando um alto número de indivíduos não relacionados ao longo dos anos. A AMOVA apresentou diferença significativa entre os grupos. Esses resultados podem refletir a estrutura dessa espécie migratória naquela região, com o teste de diferenciação pareada de indivíduos de 2013 e 2014 sendo mais semelhantes entre si do que os de outros anos, indicando uma possível estruturação genética diagnosticada pelos anos de captura. No entanto, há um alto compartilhamento alélico entre os três grupos amostrados sugerindo que esses indivíduos são uma população que se conecta ao longo do tempo e que têm uma relação filopátrica com o local. Os resultados aqui encontrados constituem um marco inicial para o conhecimento genético do ganso-do-orinoco que contribuirá para novos estudos sobre esta espécie considerada "quase ameaçada" pela IUCN.

Palavras-chave: *Microssatélite; DNA mitocondrial; ganso-do-orinoco; parentesco; estrutura populacional.*

Introduction

The Orinoco Goose (*Neochen jubata* (Spix 1825)) is one of the lesser-known species of waterfowl endemic to South America inhabiting sandy beaches, along rivers, savannas, and wetlands (Carboneras 1992, Sick 2001, Endo *et al*. 2014); in Brazil, it occupies the basis of the Amazon River (Luna *et al*. 2008, Endo *et al*. 2014) and Araguaia River (Pinheiro & Dornas 2009). It is estimated that Amazon's population is smaller and more fragmented than in the Araguaia River basin, which acts as a remnant stronghold of this species (Endo *et al*. 2014).

The existence of longitudinal migratory behavior in Orinoco Goose between southern Peru and northern Bolivia was recently described (Davenport *et al*. 2012), and there are reports of a seasonal occurrence of Orinoco Goose in the Juruá River, Amazon basis, suggesting that this population migrates to other regions during the rainy season due to restrictions imposed by the seasonal flood regime and that the dynamics of rivers determine the use of habitat in this species. (Endo *et al*. 2014). The district of Luiz Alves, Goiás, region of the Araguaia River, receives an indeterminate number of Orinoco Goose seasonally; however, little is known about these individuals who visit this area, whether they are the same population with related animals or only birds that converge annually to this region. According to BirdLife International, migratory is when "a substantial proportion of the global or regional population makes regular or seasonal cyclical movements beyond the breeding range, with predictable timing and destinations". An excellent comprehension of the migratory behavior of animals is essential to consider conservation strategies, and ecological and genetic studies from populations are necessary (Kirby *et al*. 2008).

In the IUCN, the Orinoco Goose is classified as "almost threatened," presenting a population decline rated as slow to moderate, mainly caused by anthropological activities such as hunting and habitat destruction (Endo *et al.* 2014; IUCN 2018). In the state of São Paulo, *Neochen jubata* is extinct in the wild, and the population in the Juruá River is also negatively impacted by agricultural and hydroelectric expansions due to seasonal flooding (Endo *et al*. 2014). These anthropic activities can generate great interference in the flow of the local biota because, during the flood season, most aquatic species seek refuge in the floodplains transformed into irrigated rice monocultures (Pinheiro & Dornas 2009). The increasing loss and fragmentation of natural habitats, especially in the last centuries, have contributed significantly to the decline and population isolation of wild species, causing even local extinctions, such as the Alagoas Curassow (*Mitu mitu*), Glaucous Macaw (*Anodorhynchus glaucus*) and the Brazilian Merganser (*Mergus octosetaceus*) (Leite-Pitman *et al.* 2002, Marini & Garcia 2005). Anthropic action has been responsible for the extinction of species and populations or causes a drastic decrease in their distribution and density, thus increasing their risk of extinction in the short and long term (Frankham *et al.* 2010). This decreased population can also lead to a decrease in genetic diversity, with the elimination of variants (alleles and haplotypes) of these populations. This fact could compromise the population's ability to respond to selective pressures, such as diseases and environmental changes, to guarantee their reproductive success (Frankham *et al.* 2010).

In recent decades, the evolution of molecular genetics has brought tools that make it possible to estimate genetic diversity in different species, identifying and quantifying differences between populations. Molecular markers that show variability that allows studying a biological issue have been widely used for population studies (Avise 1994). According to scientific databases, there are three studies aimed at the study of *N. jubata,* and these are focused on the description of their migratory habits and abundance estimates, with no records yet on the genetic profile of the species (Kriese 2004, Endo *et al*. 2014, Davenport *et al*. 2012). The only study carried out with *N. jubata* in Brazil was on the seasonal abundance of the population in the Juruá River, Amazonas (Endo *et al*. 2014). Thus, this work is a pioneer in investigating the distribution of genetic variability in individuals of *N. jubata*, so the objective of this study is to genetically characterize the individuals sampled in three different years using microsatellite DNA loci and mitochondrial DNA as molecular markers, therefore offering a genetic panorama that is still non-existent for *N. jubata* and better comprehension about the relationship among these individuals.

Material and Methods

1. Genetic sampling

A total of 60 blood samples from *N. jubata* were donated by Professor Karin Werther, Departamento de Patologia Veterinária – UNESP, FCAV, Jaboticabal (Table 1). The samples were harvested from free individuals living in the Araguaia River, Goiás State, Brazil, at two locals (13º30'00.0 "S 50º43'29.51" W [1] 13º17'49.0 "S 50º36'16.5" W [2] at) and (13º13'02.1 "S 50º34'37.8" W [3]) (Figure 1), was not specified the exact location of each individual, but the collection area in general. These animals were captured manually in September/October during the molting period from 2010 (samples 1-21), 2013 (samples 22-41), and 2014 (samples 42-60). All procedures for handling and capturing animals were approved by the Ethics Committee on the Use of Animals (C*omissão de Ética no Uso de Animais - CEUA*) of the Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal – UNESP on June 8, 2011 at number 012273/11. Blood samples collected

by venous puncture were preserved in alcohol. Subsequently, the sexing of these individuals was performed by applying molecular methods.

Genomic DNA was extracted using a phenol-chloroform method protocol developed by the *Laboratório de Biodiversidade e Evolução Molecular* – UFMG (LBEM 2018) and currently stored in the *Laboratório de Biologia Evolutiva* (LaBE) – UNESP, FCAV, Câmpus de Jaboticabal.

2. *Mitochondrial DNA*

The control region of the mitochondrial DNA was amplified using the primer pairs forward: L78-GTTATTTGGTTATGCATATCGTG and reverse: H774-CCATATACGCCAACCGTCTC (Sorenson & Fleischer 1996, Sorenson *et al.* 1999). Polymerase chain reaction (PCR) reactions were patterned to a final volume of 25 µl containing 12.5 µl of Green Master Mix-Promega, 1 µl of the forward and reverse primers at 2 mM, 8.5 µl of deionized water and 2.0 µl of DNA with 100 ng. PCR conditions started with an initial denaturation cycle of 94°C for 7 minutes, followed by 45 cycles of 94°C for 20 seconds, 49°C for 20 seconds, 72°C for 1 minute, and 72°C for 7 minutes. The final check of the process was performed by 1% agarose gel electrophoresis to verify the presence of the expected bands. The amplified products of mitochondrial DNA were purified with Wizard® SV Gel and PCR Clean-Up System reagent set (Promega) following the manufacturer's instructions. Sequences and specimen voucher information, including collection year and sex identification, are archived in GenBank (accession numbers MT675220–MT675248).

3. *Microsatellite DNA*

Microsatellite DNA loci were amplified using five pairs of primers developed for other species of Anatidae (Table 2) with proven applicability to members of the Tadorninae subfamily (Maak *et al.* 2003, Paulus & Tiedemann 2003).

The PCRs were performed in a final volume of 25 µl containing 12.5 µl of Green Master Mix-Promega, 1 µl of the forward and reverse primers at 2 mM, 8.5 µl of deionized water and 2.0 µl of DNA with 100 ng. The reactions were amplified under the following conditions: one cycle at 94°C for 5 min, 88°C for 1 min; three cycles 94°C for 1 min, annealing temperature specific for each locus (Table 2) for 1 min; 72°C for 1 min; 38 cycles of 94°C for 1 min, annealing temperature -3°C for 1 min, 72°C for 1 min; and final extension at 72°C for 10 min. After confirming the amplification of the microsatellite loci, the PCR product was applied in 6% polyacrylamide gel, where staining with silver was performed to visualize the microsatellite alleles. Gels for the five loci were analyzed, and each allele was characterized by its size and position on the gel relative to the molecular weight marker of 50 bp (Jena Bioscience).

Table 1. List of individuals of *N. jubata* captured in the Araguaia River, GO, voucher number of the *Laboratório de Biologia Evolutiva* - LaBE and sex determined by molecular analyzes.

	Voucher Number – LaBE	
Years - Groups	**Male**	**Female**
2010 - Group A	1, 2, 4, 5, 6, 8, 9, 10, 12, 13, 16, 19, 20	3, 7, 11, 14, 15, 17, 18, 21
2013 - Group B	23, 26, 27, 28, 29, 30, 31, 32, 33, 34, 37, 40, 41	22, 24, 25, 35, 36, 38, 39
2014 - Group C	44, 45, 48, 49, 52, 56	42, 43, 46, 47, 50, 51, 53, 54, 55, 57, 58, 59, 60

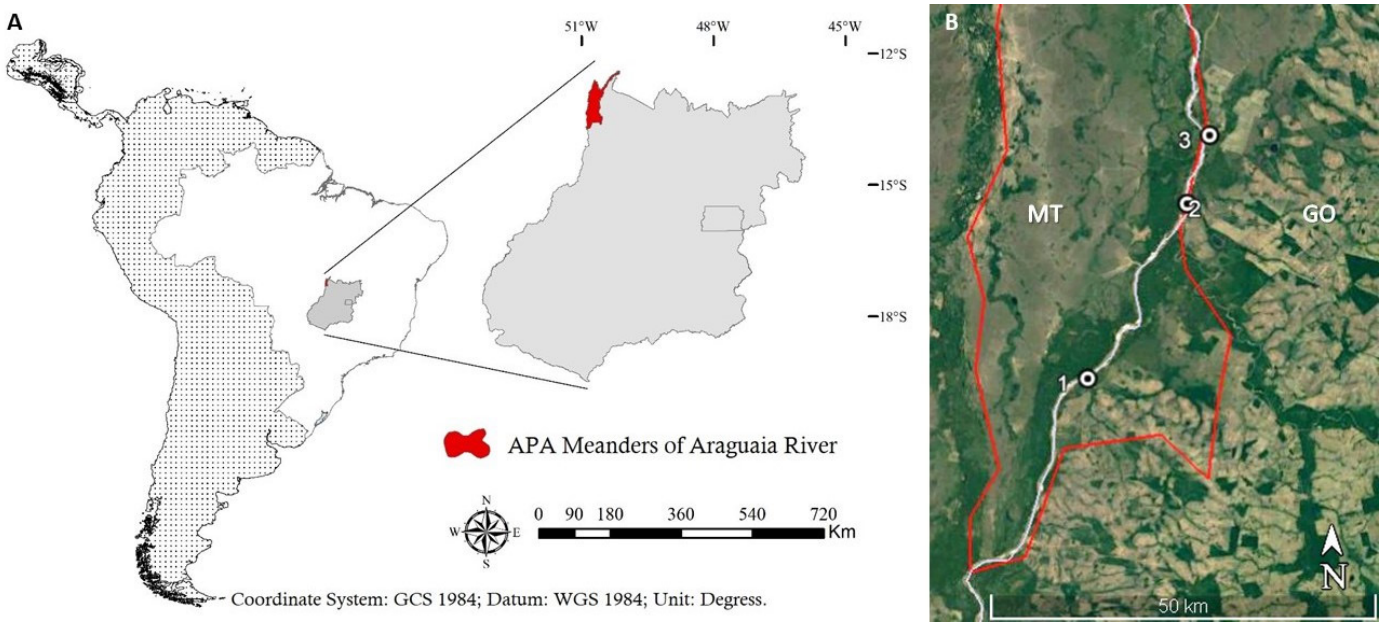

Figure 1. A) APA Meanders of Araguaia River location. Source:< https://portaldemapas.ibge.gov.br/> last access in 29/01/2018. B) Location map of the captures from individuals of *N. jubata* in 2010, 2013, and 2014, whose blood samples were used in this work. Source:< http://earth.google.com/> last access in 03/01/2021.

Table 2. Characteristics of the five pairs of microsatellite DNA primers used to amplify the genetic variability of *N. jubata* individuals from the Araguaia River, GO.

Locus	Primer Sequence	Annealing Temperature	Repeat Sequence
***Smo6**	F: GGGGTGGGAAAGAAGCAGTTTAG R: TCCTGGGACTTTGAAAGTGGCTC	65°C	(TG)18T4(TG) 2
***Smo8**	F: TGCCTTATAGGATGTCACTCTTC R: AAAATACTATGCTCGTTTCAAAA	54°C	(TG) 11
***Smo10**	F: TCCTAGCGACAGCAATTCTAATG R: CATTGTTCATTGTTTCTTCTTCA	50°C	(TG) 31
***Smo1**	F: CTTAAGGTATTGTGCTTTATA R: TGGTCCAAAGGGTGTTCTCAGAA	54°C	(GA) 17
****APH08**	F: AAA GCC CTG TGA AGC GAG CTA R: TGT GTG TGC ATC TGG GTG TGT	58°C	(CA) 12

*Paulus & Tiedemann 2003; **Maak et al. 2003

4. Genetic diversity

We amplified a total of 29 sequences with 650 bp each for the mtDNA control region gene, including samples 1-8 and 10 (Group A), 22-31 (Group B), and 42-51 (Group C). These samples were divided according to each collected the years 2010, 2013 and 2014, respectively and each specimen was genotyping (Table 1). The genetic diversity levels of the groups were estimated using ARLEQUIN software (Excoffier *et al*. 2005), considering indices of the total number of alleles (Na), observed heterozygosity (H_O) and expected heterozygosity (H_E). They were also tested for possible deviations, the Hardy-Weinberg equilibrium (HWE), and linkage equilibrium. Additionally, the same software estimated the degree of differentiation between the three sampled groups employing Wright's statistics by analyzing the percentages of F_{ST} (AMOVA) and calculated the pairwise F_{ST} indices to three groups sampled, and the test was run with 10000 permutations to evaluate the statistical significance of the calculated value ($\alpha = 0.05$). For the distribution of genetic diversity, the assurance of the best number of clusters for the genotypes sampled was calculated by STRUCTURE v.2.3.4 (Pritchard *et al*. 2000), where the models used were admixture and the frequencies of correlated alleles. Group numbers (k) were tested, fluctuation from one to seven with ten runs for each one, and the parameters placed were 50000 burn-in and 1000000 Markov Chain Monte Carlo (MCMC) replicates. Finally, the most likely number of groups was assessed with the statistic described by Evanno *et al*. (2005) and visualized in STRUCTURE HARVESTER v.0.6.92 (Earl & vonHoldt 2012).

5. Parentage analysis

The possible parentage among individuals was performed using the software CERVUS v.3.0.7 (Marshall *et al*. 1998), which realizes genetics analysis on population study doing inferences based on molecular markers such as microsatellites, generating through exclusion process locus-by-locus a likelihood score. This value corresponds to a similarity between parentages and offspring and is expressed more commonly as an LOD score.

The LOD score is obtained by the natural log (log to base e) of the overall likelihood ratio, returning positive and negative values. When the candidate's parent is more likely to be the birth parent, the LOD score value is positive; however, if the offspring are less likely to relate to their possible parentage, the LOD score sets a negative value. Lastly, an LOD score equal to zero indicates that the candidate parent has the same chances of being the birth parent as not being.

Results

1. Genetic diversity

We amplified a total of 29 sequences with 650 bp each for the mtDNA control region gene, including individuals 1-8 and 10 (group A), 22-31 (group B), and 42-51 (group C). As a result, we obtained only two haplotypes (Attached 1), and this analysis revealed low haplotype diversity (Hd) of 0.069 and only eight polymorphic sites (S). The genetic distance among the three sampled groups was 0.001. Therefore, no further analysis was performed to detect the partition of genetic variability.

For the five microsatellite loci, it performed on a total sample of 60 individuals of *N. jubata*, and all loci were polymorphic; the average number of alleles per locus was 5.4, ranging from 4 (Smo8) to 7 (APH08), resulting in 27 alleles for the individuals (Table 3).

According to the collection years, analysis of diversity patterns revealed exclusive alleles in the three sampled groups (Table 3). Each group was analyzed separately, and we observed H_O values ranging from 0.083 (B) to 0.192 (C), while H_E ranged from 0.514 (A) to 0.655 (C) (Table 4). The heterozygosity levels observed in the three groups were lower than the expected heterozygosity, but there were no deviations in the balance of HWE (Table 4). The test of linkage disequilibrium between pairs of loci has as a null hypothesis that the genotypic distribution in one locus is independent of the distribution in another. The three years sampled presented loci in this condition ($p = > 0.05$), with group A showing the highest number of loci in equilibrium, while group C had the lowest level.

2. Distribution of genetic diversity

The genetic structure used to investigate the individuals sampled in three different years resulted in three analysis groups. AMOVA was used to test the structure considering all groups as a single group, without hierarchical levels. For this grouping, the *p*-value ($p = 0.000$) was significant and showed a moderated value to F_{ST} (0.21) (Table 5), also evidencing that the highest percentage of the variation is within

Table 3. Frequency of the alleles identified in the five microsatellite loci for 60 individuals of *N. jubata* captured in the Araguaia River, GO in the years of 2010 (group A), 2013 (group B), and 2014 (group C). Exclusive alleles found indicated with an asterisk.

Loci		Years of sampling		
		2010 (A)	2013 (B)	2014 (C)
Smo1		N=16	N =17	N =16
Allele	1	0.250	0.588	0.406
	2	0.593	0.323	0.468
	3	0.093*	0.000	0.000
	4	0.062	0.058	0.093
	5	0.000	0.029	0.031
Smo6		N=20	N=19	N=18
Allele	1	0.225	0.210	0.111
	2	0.675	0.157	0.277
	3	0.000	0.631	0.611
	4	0.075*	0.000	0.000
	5	0.025*	0.000	0.000
Smo8		N=20	N=19	N=18
Allele	1	0.050	0.263	0.166
	2	0.000	0.000	0.277*
	3	0.150	0.631	0.444
	4	0.800	0.105	0.111
Smo10		N=20	N=19	N=20
Allele	1	0.250	0.105	0.166
	2	0.000	0.210*	0.000
	3	0.000	0.263*	0.000
	4	0.000	0.105	0.166
	5	0.650	0.157	0.000
	6	0.100	0.157	0.666
APH08		N=20	N=19	N=18
Allele	1	0.175	0.131	0.138
	2	0.000	0.473	0.138
	3	0.325	0.157	0.111
	4	0.250	0.105	0.277
	5	0.225	0.052	0.027
	6	0.000	0.078	0.250
	7	0.025	0.000	0.055

Table 4. Allelic diversity and Hardy-Weinberg Equilibrium calculated at five microsatellite loci for 60 individuals of *N. jubata* captured in Araguaia River, Go in the years 2010 (group A), 2013 (group B) and 2014 (group C).

			HWE			
Groups	**Loci**	**A**	**H_o**	**H_E**	***p*-value**	**s.d.**
2010 (A)	Smo1	4	0.111	0.547	0.000	0.000
	Smo6	4	0.190	0.483	0.000	0.000
	Smo8	3	0.000	0.329	0.000	0.000
	Smo10	3	0.000	0.441	0.000	0.000
	APHO08	5	0.619	0.770	0.000	0.000
	Overall	3.8	0.184	0.514		
2013 (B)	Smo1	4	0.166	0.544	0.000	0.000
	Smo6	3	0.000	0.528	0.000	0.000
	Smo8	4	0.000	0.579	0.000	0.000
	Smo10	6	0.000	0.841	0.000	0.000
	APHO08	6	0.250	0.728	0.000	0.000
	Overall	4.6	0.083	0.644		
2014 (C)	Smo1	4	0.210	0.603	0.000	0.000
	Smo6	3	0.000	0.553	0.000	0.000
	Smo8	4	0.000	0.717	0.000	0.000
	Smo10	4	0.000	0.575	0.000	0.000
	APHO08	7	0.700	0.825	0.000	0.000
	Overall	4.4	0.192	0.655		

Observed number of alleles (A), observed (H_o), and expected (H_E) heterozygosities.

Table 5. Analysis of molecular variance (AMOVA) using five microsatellite loci without hierarchical analysis, for 60 individuals of *N. jubata* captured in the Araguaia River, GO in the years 2010, 2013 and 2014.

Source of variation	d.f.	Variance components	Percentage of variation
Among groups	2	0.410	21.00
Within groups	119	1.549	79.00
Total	121	1.959	
Fixation Index		F_{ST}: 0.21	

Table 6. Pairwise F_{ST} for the three groups of *N. jubata* analyzed in the Araguaia River, GO in the years 2010 (group A), 2013 (group B) and 2014 (group C).

Groups of sampling	(A)	(B)	(C)
(A)	0.00000		
(B)	0.28292*	0.00000	
(C)	0.25148*	0.07234*	0.00000

*$p < 0.05$

the groups, with only a small part of the variation distributed between the groups. We estimated the pairwise differentiation indices with an exact comparison among the three groups (Table 6). According to the comparisons, those involving the pair of groups A and B tended to yield the highest values of differentiation index analyses, unlike the pairs of groups B and C that presented the lowest differentiation values.

Structure analysis realized by STRUCTURE was used to visualize the genetic similarity among individuals and test the structure among the three groups analyzed. The results produced using the method proposed by Evanno *et al*. (2005) revealed that the individuals sampled's genetic variation can be ideally distributed in two groups (K = 2) according to their similarity. Individuals from groups B and C showed similar proportions of colored segments representing the probability that they belong to the cluster with that color (Figure 2).

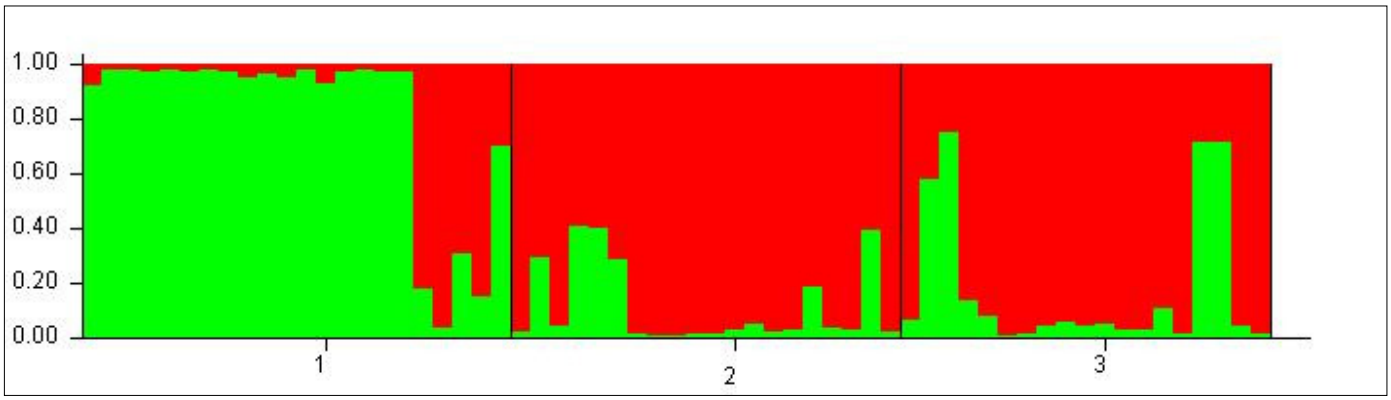

Figure 2. Graph of the Bayesian analysis with the distribution of *N. jubata* individuals captured at the Araguaia River, GO in the years 2010, 2013, and 2014. Each individual is represented by a vertical bar; each color per bar's length indicates the probability of membership in each genetic cluster.

3. Parentage analysis

We evaluated the sample genetically intended possible relationships among individuals of the groups over the years. Using CERVUS software to analyze five microsatellite loci, we compared the possible offspring with candidates' mothers and fathers. Our results show loci Smo8 and Smo10 with the highest frequency of null alleles (1.0000), and the lowest locus frequency found was APH08 (0.2462). In addition, it had a total of 13 possible kinships involving 22 individuals, in which four relationships were in strict confidence (95%) and nine relationships were in relaxed confidence (80%). Of these 13 relationships, 12 parents were individuals collected in the same year, only one pair of parents had their mother and father sampled in different years; that is, the software indicated that they were parents of the same individual, but the mother was sampled in one year and the father in another year. Furthermore, individual parentage analysis at relaxed confidence showed 3% and 4% of offspring assigned to mothers and fathers, respectively. These results could demonstrate that over the years, there was an exchange of alleles between the groups of *N. jubata*.

Discussion

Our results produced by the mitochondrial DNA gene's control region showed that the *N. jubata* groups from the Araguaia River showed very low diversity indexes and identified only two haplotypes. However, the five loci of microsatellite DNA demonstrated a moderate difference between the three groups and indicated a higher percentage of variation occurred in the same years of sampling. Although we had little mitochondrial data, this result can occur because SSR nuclear markers are higher polymorphic than mitochondrial DNA and can reveal more recent effects of reproductive isolation or low gene flow (Loxdale & Lushai 1998, Hartl & Clark 2010). Another possible explanation for these results is that low genetic variability is linked to the birds' physiological characteristics. With few exceptions, bird species often have high longevity (Holmes *et al*. 2001). As documented in other bird species, the long life cycle associated with less oxidative damage to mitochondrial DNA can result in a lesser accumulation of mutations (Barja 2004, Hickey 2008).

Due to different allelic compositions, the AMOVA showed a significant difference between the groups (F_{ST} 0.21). However, it is possible to see that the highest variation is within the groups, so the three groups share 79% of all the allelic variation detected here. These results may reflect the structure of this migratory species in that region, with the paired differentiation test of individuals 2013 and 2014 being more similar to each other than those of other years. However, it should be highlighted that the value considered moderate for AMOVA (F_{ST} 0.21) may include the geographical structure of the individuals, as although the groups in the analysis were drawn up by the years of capture, they also contain the distinct sampling locals. This indicates that this species can both present a genetic structure diagnosed by the years of capture, as well as a geographic structure, evidenced by the different locals sampled.

To assess whether differences in the temporal scale of sampling for the three groups could have influenced the observed genetic structure, we focused particular attention on STRUCTURE analysis. This result showed that the B and C groups are more genetically similar to each other, confirming the pairwise analysis data.

The biology of the Anatidae family could explain the existence of these two genetic groups in this population, one formed by the individuals captured in 2010 (A) and the other formed by the individuals captured in the years 2013 (B) and 2014 (C), once to the sexual maturation of the individuals occurring after the first or the second year of life and the formation of peers that is also slow to establish and tends to last for several years (Johnsgard 2010). Therefore, any allelic diversity introduced between groups will take at least three years to reflect the population's genetic pool. Another possible cause is the absence of samples in the intervening years between "2010" and "2013" since individuals' analysis in those years could gradually detect this homogenization.

Our results showed a different genetic pattern from that observed for other species of migratory birds *Somateria mollissima* (Linnaeus, 1758) (Aves: Anatidae) studied in northern Europe. Mitochondrial DNA showed differences between colonies as a product of the oldest history of colonization of these species in the region related to the Pleistocene refuge event and little divergence between colonies when studied by microsatellite DNA loci, probably influenced by the philopatric of females (Tiedemann *et al*. 2004). When comparing these data with ours by *N. jubata* presented here, we could infer that due to the absence of difference for mitochondrial DNA, our sampled groups could represent a single population that migrates to this region seasonally, in addition to having a high rate of gene flow that prevents diversification among individuals. Although it is not possible to affirm, it may be that *N. jubata* is philopatric by males, so that the difference verified over the

years sampled by the nuclear marker is the product of the dispersion incorporated to distinct locals of permanence promoted by the other sex capable of preventing moderately population structuring, since most of the variation is within the sampled groups.

The kinship tests demonstrated a high number of unrelated individuals over the years, agreeing with HWE results and reinforcing that there is no deviation in the sampled groups. Nevertheless, the presence of closely related individuals in all years, even though to a lesser extent reinforces, as stated above, the hypothesis that the three groups A, B and C form a single population that connects over time that returns to the Araguaia River seasonally. These findings are in accord with the premise that groups of *N. jubata* formerly considered independent are the same population occupying different reproduction areas (Endo *et al*. 2014, IUCN 2018).

The recurrence of Orinoco Goose in the Araguaia River, coinciding with its reproduction, in the dry season may also indicate some degree of philopatric individuals who tend to return to their area of origin to mate (Greenwood 1980), agreeing with our genetic data. Familiarity with a specific local brings several advantages for an animal, such as predator evasion, higher resource efficiency, reduced hostility, and possible cooperation with related individuals (McKinnon *et al*. 2006, Sonsthagen *et al*. 2009)

It is the first molecular study on the populations of *N. jubata*, and many questions still exist to be answered, but this study may already aid in the conservation policies of this species.

Supplementary Material

The following online material is available for this article:

Attached 1 - Polymorphic sites and two haplotypes for the control region of the mitochondrial DNA of *Neochen jubata*.

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Author Contributions

Maria Cecília Pereira: The author had a fundamental role in the development of this work, realized laboratory work to produce data for analysis and analyzed and interpreted the data on genetic diversity, in addition to contributing substantially to the writing and revision of the manuscript.

Thaís Coelho: The co-author analyzed and interpreted data related to kinship analysis and their relationship with genetic diversity data, in addition to contributing to the writing and revision of the manuscript.

Karin Werther: The co-author had a fundamental role in the data collection of this work, collecting biological material that was later processed and analyzed, in addition to contributing to the writing of the manuscript and its review.

Rafael Biccio Andreazzi: The co-author analyzed and interpreted data related to kinship analysis and their relationship with genetic diversity data, in addition to contributing to the writing of the manuscript.

Adriana Coletto Morales: The co-author and advisor had a fundamental role in the development of the intellectual and experimental design of this work, in addition to contributing to the writing of the manuscript, supervising the development and revising.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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