birds

MDPI

Article

Genetic Monitoring of the Captive Population of the Critically Endangered Brazilian Merganser (*Mergus octosetaceus*)

Davidson P. Campos, Henry P. Granger-Neto, José E. Santos-Júnior, Renata S. O. Buzatti and Fabrício R. Santos *

Department of Genetics, Ecology and Evolution, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil; davidsonpcampos@gmail.com (D.P.C.); hp.granger98@gmail.com (H.P.G.-N.); jrsantos140782@yahoo.com.br (J.E.S.-J.)
* Correspondence: fsantos@icb.ufmg.br; Tel.: +55-3134092581

Simple Summary: We used 46 individuals of the critically endangered Brazilian Merganser, currently endemic to the Cerrado biome in Brazil, to understand genetic diversity and the genealogical relationships of the captive population. By characterizing 425 genomic variants in these captive specimens, our study unveiled a significant level of inbreeding, with approximately 70% of the captive birds tracing their lineage back to the founders from just two localities. Leveraging the identified DNA variants, we also identified less inbred individuals suitable for the potential future reintroduction into the wild.

Abstract: The Brazilian Merganser (*Mergus octosetaceus*) is one of the rarest birds in South America, and it is a critically endangered Anatidae species with an estimated population of less than 250 adult individuals in the Brazilian Cerrado. A captive population was established a few years ago at Zooparque Itatiba (São Paulo state) where 46 individuals were kept, and the founding population (progenitors derived from nature) was composed of 19 of the ex situ birds, derived from the four remaining localities with wild populations in Brazil. To characterize the genetic diversity and the genealogical relationships of the captive population, it is essential to conduct appropriate ex situ management and to assist future reintroduction projects. Thus, we have identified 425 SNPs by massively parallel sequencing of ddRAD libraries that allowed us to genotype individuals of the captive population. We observed a close relatedness between 70% of the captive population and founding individuals of Jalapão and Alto Paranaiba localities, indicating the need for supplementation with individuals from other areas of Canastra and Veadeiros. Even though many captives present a high level of inbreeding, we have identified some individuals with a high genetic value (less inbred) that can be selected for the breeding program to generate individuals for a future pilot reintroduction project.

Keywords: anseriformes; conservation genetics; captive breeding; *ex situ* management

Citation: Campos, D.P.; Granger-Neto, H.P.; Santos-Júnior, J.E.; Buzatti, R.S.O.; Santos, F.R. Genetic Monitoring of the Captive Population of the Critically Endangered Brazilian Merganser (*Mergus octosetaceus*). *Birds* **2024**, *5*, 190–201. https://doi.org/10.3390/birds5010013

Academic Editor: Jukka Jokimäki

Received: 1 December 2023
Revised: 5 March 2024
Accepted: 7 March 2024
Published: 13 March 2024

1. Introduction

The Brazilian Merganser (*Mergus octosetaceus*, Vieilot, 1817) is a critically endangered bird of South America [1–3]. In the past, the Brazilian Merganser was distributed across Brazil, Argentina, and Paraguay, mainly within the river basins of São Francisco, Paraná/Paraguay, and Tocantins. Its presence was characterized by sparse population densities occupying scattered habitats within the Cerrado and Atlantic Forest biomes [4]. However, it is likely extinct or extremely rare in the Atlantic Forest areas of Argentina, Paraguay, and Brazil [5–7]. In addition, very few studies were performed on this rare species in the wild, with many unknown aspects of its biology, reproduction, ecology, behavior, development, and genetics [3]. This is the only surviving species of the genus *Mergus* in the southern hemisphere after the extinction of the New Zealand Merganser in the twentieth century and their sister species in the northern hemisphere, like the Common

Merganser (*M. merganser*) and Red-breasted Merganser (*M. serrator*), which occur in large numbers (least concern, IUCN), although the Scaly sided Merganser (*M. squamatus*) is endangered according to IUCN [2].

The current distribution of the Brazilian Merganser appears to be restricted to central Brazil, in four disjunct areas of three states: (i) Minas Gerais, which has the largest population, 140 to 200 mature individuals that occur in the region of the Serra da Canastra National Park (Canastra) and regions outside protected areas (PAs), as in the Alto Paranaíba Region (Paranaíba) in the Paraná river basin; (ii) Goiás, in the Chapada dos Veadeiros National Park (Veadeiros), with an estimated population of less than 50 individuals; and (iii) Tocantins, in the Jalapão State Park (Jalapão), with an estimated population of eight individuals [3].

The census in central Brazil estimated the existence of less than 250 adult mergansers [3], which are constantly monitored by the Brazilian Action Plan of Conservation (PAN Pato-mergulhão-ICMBio). This National Conservation Plan coordinates several actions to protect the remaining populations of the species and its habitat, which includes the establishment of a captive population to work as a source of individuals for future reintroduction programs (PAN Pato-mergulhão-ICMBio). Furthermore, the ex situ program of the Brazilian Merganser is also being used to generate firsthand knowledge of species biology, ecology, reproduction, and captive care [2,8].

The Brazilian Merganser captive population was established in the last decade at Zooparque Itatiba in São Paulo state, Brazil, and in 2019, it was composed of 46 mergansers that included 19 founding individuals that came originally from eggs collected in nature and 27 individuals that were born from ex situ crosses. Since its start, the Brazilian merganser captive breeding program has been genetically monitored by our research group, which previously published some genetic studies of the wild merganser populations [9–13]. All these studies identified a low diversity and high inbreeding in the wild populations, although with some interpopulation diversity [13]. A current goal of genetic monitoring of captive individuals has been to promote adequate crossings of Brazilian Mergansers to allow appropriate ex situ management for future actions involving translocations into the wild.

Indeed, captive breeding programs aim to maintain a genetically "healthy" ex situ population to work as a safety measure against extinction [8,14]. Many captive individuals were reintroduced into the wild in relatively successful programs, for example, the golden lion tamarin (*Leontopithecus rosalia*) and the California Condor (*Gymnogyps californianus*), although subjected to many technical and logistical issues [15–17]. For a successful reintroduction, captive populations must be managed to avoid many genetic issues like low diversity, inbreeding depression, and adaptation to captivity. A failure in genetic management can cause individuals to perish in reintroduced areas and to present altered behaviors, such as the use of inappropriate nesting sites (for birds), risky tolerance to human presence, and improper foraging routines [14,18]. One of the most important points when establishing a captive population is the adequate number of founders derived from wild populations to maintain a representative genetic diversity ex situ since it is crucial for the success of future reintroductions [14]. Otherwise, the use of few (or non-representative) founders can compromise irreversibly the captive population management [8,19].

To maintain a genetically "healthy" captive population, many strategies are applied such as keeping the same number of breeding males and females without overlapping of different generations, equalizing reproductive success among founding individuals and promoting appropriate crossings to avoid inbreeding, and sustaining the maximum effective population number [14,20–22]. To guide these strategies, captive individuals need to be genetically monitored by different methods to characterize their inbreeding levels, pairwise kinship, and other genetic parameters. The latest technologies involve the use of genomic approaches to identify thousands of DNA variants for inter-individual comparisons, even if the genome of the managed species is still unknown. To allow the analyses of many genetic variants for species with no sequenced genomes, different genomic complexity reduction methods were developed to characterize populations of animals [23,24] and plants [25],

whether they are captive [26] or wild [13,27]. We have recently published a genetics study of in situ Brazilian Merganser individuals using SNPs generated by a Genotype by Sequencing (GBS) approach [13], which identified high inbreeding and moderate genetic structure among the four remaining wild populations.

In this work, we present the first population genomics study of captive Brazilian mergansers using SNPs generated by a ddRAD sequencing methodology. These genomic variations were used to characterize the genetic structure and diversity, individual inbreeding levels, and pairwise kinship of individuals that make up the ex situ population maintained at Zooparque Itatiba, which is part of the Brazilian Action Plan of Conservation for this critically endangered species.

2. Materials and Methods

2.1. Biological Samples

In this work, we used 46 samples of the Brazilian Merganser. Nineteen adult individuals were derived from eggs collected in the wild and hatched in captivity, comprising the founding population. These consisted of five from Paranaíba (PAR), seven from Jalapão (JAL), and seven from Canastra (CAN). Additionally, 27 individuals resulted from crosses in captivity, for which we have their pedigrees (Zooparque Itatiba and PAN Pato Mergulhão). The blood of these captive birds was previously collected and stored in absolute ethanol and later stored frozen in the Tissue Collection of the Taxonomic Collection Center of the Federal University of Minas Gerais, Brazil. The access to genetic resources of the Brazilian biodiversity was registered as SisGen/Brazil number A324339.

2.2. DNA Extraction

Genomic DNA was extracted following the phenol–chloroform protocol [28]. After extraction, we verified the integrity of the DNA samples through electrophoresis in a 2% agarose gel and the quality through the Nanodrop 2000 to obtain DNA extracts with 260/280 ratios $\geq$ 1.75 and 260/230 $\geq$ 1.8. The quantification was evaluated in the Qubit® 2.0.

2.3. ddRAD Library Construction and Sequencing

Genomic libraries were constructed (Supplementary Materials, ddRAD protocol) following an adaptation of the protocols described by Thrasher et al. [29] and Peterson et al. [30]. The ddRAD genomic libraries were submitted for Illumina HiSeq 2500 sequencing (single end with 100 bp reads) at Macrogen (Seoul, Republic of Korea).

2.4. Variant Calling and Filtering

Pipeline Stacks [31] were used to perform a de novo alignment and to identify the SNPs. Filtering was initially performed using VCFtools v.0.1.16 [32], where we filtered only SNPs (1) common to all individuals (–max-missing-count 0); (2) SNPs in which the smallest allele occurs at least twice throughout the sampling (–mac 2); (3) without the presence of indels (–remove-indels); (4) only biallelic loci (–min-alleles 2 –max-alleles 2); and (5) being in Hardy–Weinberg Equilibrium (–hwe 0.05). We have also performed another additional filtering for each SNP, where we calculated the minimum coverage depth of all individuals and retained only SNPs with depth equal to or greater than 5. To identify loci potentially under selection, we have used the BayeScan v2.01 program using default settings [33], where loci that were supposedly under selection were excluded from further analyses.

2.5. Statistical Genetics Analyses

Using VCFtools, we calculated, for each individual, the heterozygosity and the individual inbreeding coefficient, equivalent to the FIT, as it is inferred from the mean of the total population, and an inference of the degree of relationship/kinship was made using the method developed by Manichaikul et al. [34], which calculates the pairwise kinship of all individuals. Pairwise kinship values greater than 0.354 indicate that individuals may

be the same or are monozygotic twins, values between 0.177 and 0.354 suggest that they are 1st-degree relatives (full siblings or parents/children), values between 0.0884 and 0.177 indicate 2nd-degree kinship (half-siblings or grandparents/grandchildren), and values between 0.0442 and 0.0884 are considered 3rd-degree relatives. The pairwise kinship data normalized by Pearson's correlation matrix was represented in a heatmap, in which the highest relationship level was assigned the value 1 and the lowest relationship level was assigned the value −1.

Using the R hierfstat package [35], we calculated the genetic differentiation between populations through pairwise FST analysis [36]. With packages VCFR [37], poppr v.2.8.6 [38], ape [39], RcolorBrewer [40], ggrepel [41], adegenet [42], reshape2 [43], and ggplot2 [44], we performed Multivariate analyses represented in two-dimensional graphs using Principal Component Analysis (PCA). PCA was used to show inter-individual relationships through genetic distance data between individuals from different populations. All multivariate analyses were run using default procedures available in the packages.

With the NeEstimator program v.2.1 [45], we estimated the contemporary effective population size using the linkage disequilibrium method (LDNe), which is based on the pattern of linkage disequilibrium between loci [46]. To compare captive mergansers born from ex situ crossings and founding individuals (originally derived from three wild areas), we used the STRUCTURE v.2.3.4 program [47] considering the possibility of gene flow between populations (admixture model) and using 500,000 MCMC randomizations and a burn-in of 50,000. Population number parameters (K) from 1 to 5 were evaluated with 5 independent runs for each K. The choice of the appropriate K was made with Structure Harvester [48] and the final graph was generated with Clumpak [49].

3. Results

3.1. Variant Calling from Sequencing Reads

Sequencing of the ddRAD library generated 111,425,030 reads, which had a GC content of 52%, with 86.10% of the reads having a phred score of Q30 and 91% of the reads having a phred score of Q20. These raw data were initially filtered with the fastp program [50], where we selected reads with at least 30% complexity, without polyG or polyA tails, and with a phred score above Q20. After these initial filtering steps, we obtained 105,173,448 reads that were used in further steps.

The initially filtered reads were submitted to Pipeline Stacks [31] using the process_radtags algorithm that demultiplexed the individual samples and performed other cleaning steps where we detected that 27.68% of the reads did not have linkage barcodes, 0.82% were poor quality reads, and 2.02% lacked the enzyme restriction sites and were all excluded. After this cleaning process, we obtained 73,058,433 reads to use for alignment and SNP calling.

Because the Brazilian Merganser has no reference genome, we performed a *de novo* alignment [31] where we found 4530 SNPs that were subjected to another more rigorous filtering using the following parameters: minimum coverage of 5× for all loci in all individuals, minimum of two occurrences of the minor allele, all loci in Hardy Weinberg Equilibrium, and only biallelic SNPs, excluding insertions or deletions. Finally, 425 SNPs were recovered and analyzed in BayeScan v2.0 which indicated that no locus was under selection. Thus, all 425 SNPs were used in the posterior genetic analyses.

3.2. Kinship and Inbreeding Estimates

The pairwise Kinship analysis shows that some individuals (MO230, MO250, MO252) had a high degree of relationship (1st-degree–values above 0.177) with almost all other captive individuals (Table S3). In addition, the heatmap (Figure 1) shows that the vast majority of individuals have a moderate degree of relationship (colors in shades of pink and red) and a group formed by seven founding individuals from Canastra (CAN211, CAN228, CAN235, CAN237, CAN239, CAN242, and CAN243) and five captive-bred (generated

from ex situ crosses) individuals (MO238, MO241, MO246 MO256, about and MO255) have shown a larger average kinship in comparison to other captive mergansers.

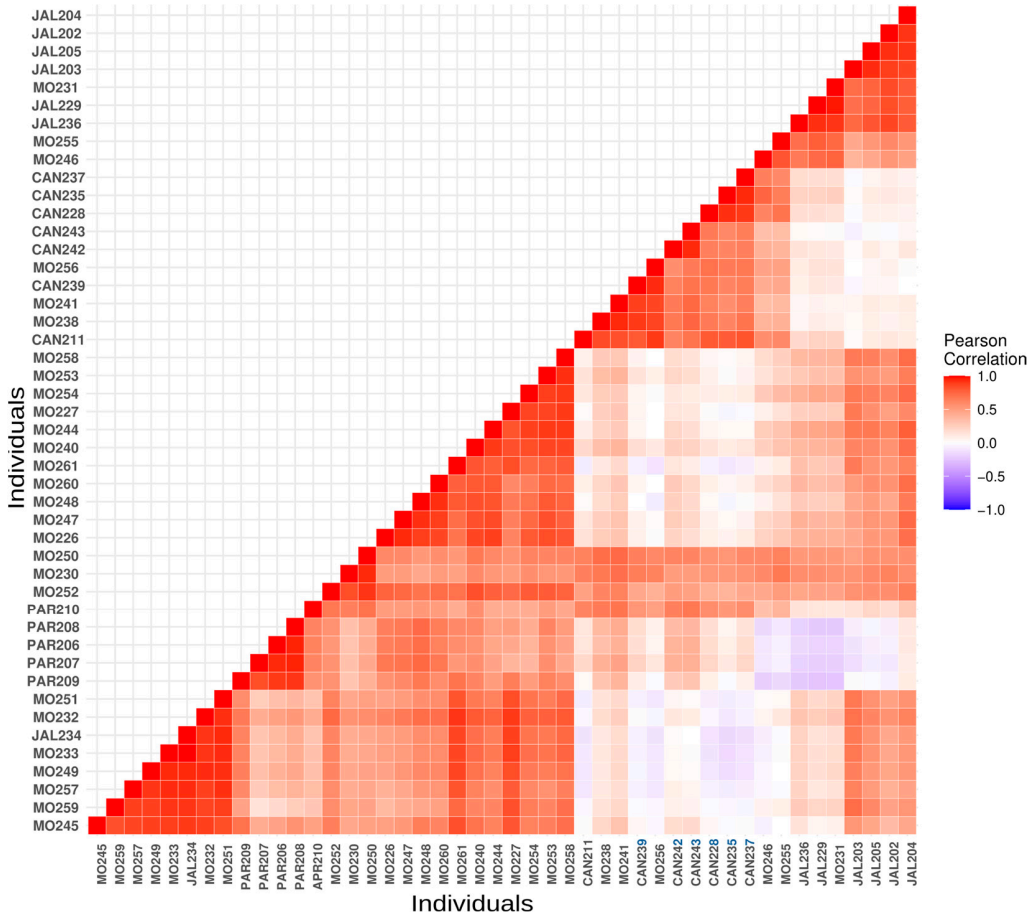

Figure 1. Heatmap plot of pairwise kinship indices corrected by Pearson's correlation matrix (values −1 to 1, see color legend) between Brazilian Merganser individuals. Sample labels of individuals are distributed in the x and y axes, corresponding four subdivisions of the *ex situ* population: Serra da Canastra National Park (Canastra—CAN); Captive-bred individuals (MO); Jalapão State Park (Jalapão—JAL); and Alto Paranaíba region (Paranaíba—PAR).

The inbreeding coefficient estimated in this work is analogous to the FIT, as it considers the average inbreeding of the population, so the composition and structure of the population will influence the individual coefficients. Thus, inbreeding estimates were made using all individuals as a single captive population (Figure 2), and we have also estimated the inbreeding coefficients by assigning the individual's origin, separating captive-bred from founders that came originally from the wild (Figure S1).

The analysis of the individual inbreeding coefficient (F), considering all captive individuals as a single population, showed us that 20 individuals (four from Paranaíba, three from Jalapão, and thirteen captive-bred) are more inbred ($F > 0$). Among the captive population, individual PAR207 presented the highest inbreeding coefficient and individual MO230 presented the lowest inbreeding (Figure 2). When we separate the founding individuals by place of origin, we find that the individuals with the lowest inbreeding coefficient are from Jalapão (Figure S1), all showing negative coefficients ($F < 0$). Otherwise, Paranaíba (Figure S1) presents the highest proportion of inbred individuals (60%). When we analyzed the mean inbreeding coefficients, we observed that the Paranaíba and captive-bred individuals showed some outliers (Figure 3).

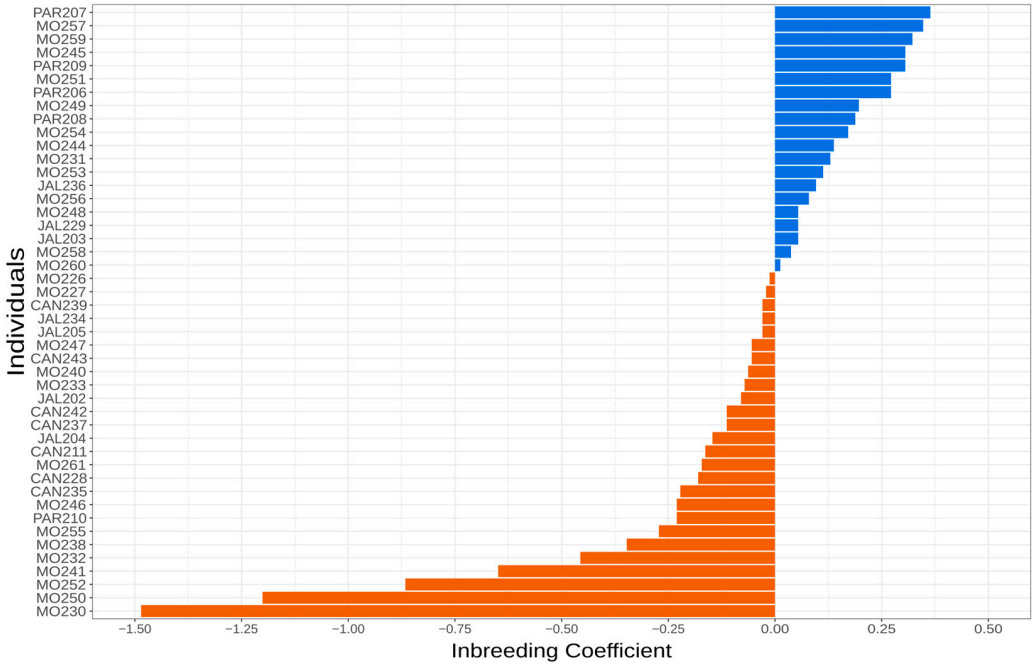

Figure 2. Inbreeding coefficients for 46 captive Brazilian Merganser individuals. Positive coefficients appear in blue and negative ones in brown. The individual samples are organized in a descending order.

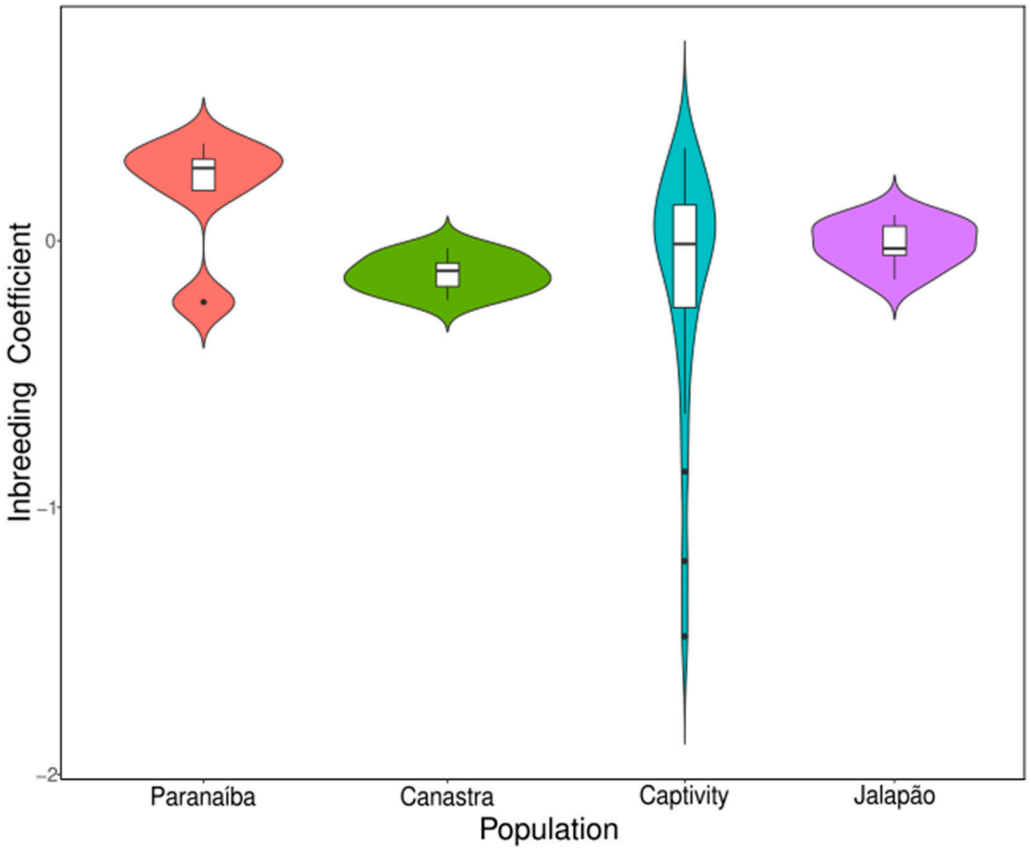

Figure 3. Violin and boxplots representing individual inbreeding coefficients (F) in y-axis per subdivision/population of Brazilian Mergansers in x-axis. Higher F values indicate that an individual is more inbred. The gray horizontal lines represent the median and the boxes are bounded by the 1st and 3rd quartiles.

The analysis of heterozygosity at the individual level showed us a wide range in the values found, such as the individual MO230 that presents the highest number of SNPs in heterozygosity (297) and the individual PAR207 that presents the smallest number of SNPs in heterozygosity (76) (Table S2).

3.3. Captive Population Structure

The PCA analysis showed us that the captive population can be separated into two groups. The first one is composed of all individuals from Paranaíba and Canastra associated with three captive-bred individuals (MO238, MO241, MO256). The second group is composed of all individuals from Jalapão associated with 24 captive-bred individuals. Moreover, three individuals MO230, MO250, and MO252 are separated from all the others, forming a more distant cluster (Figure 4).

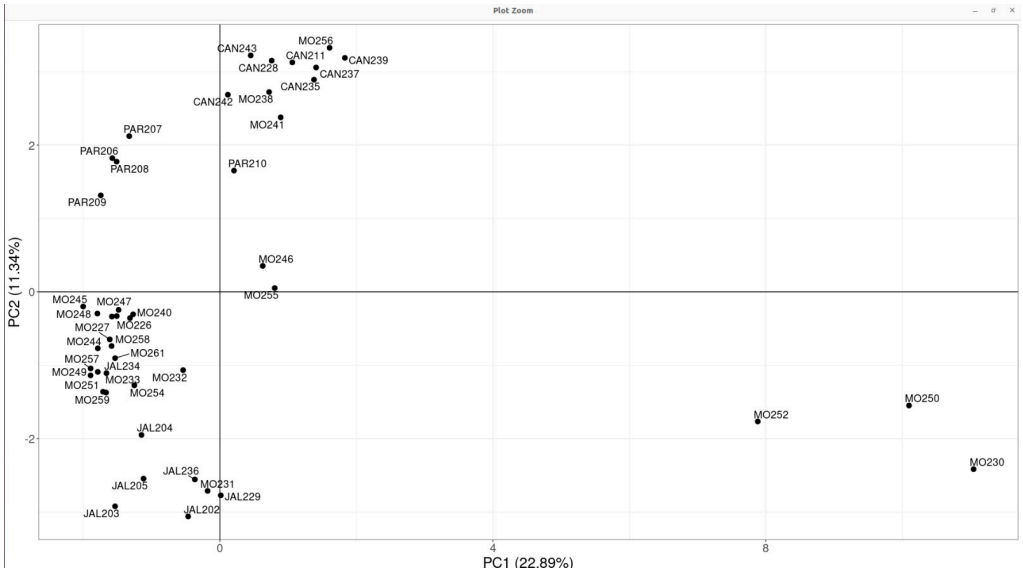

Figure 4. Bidimensional plot of PCA analysis using 425 SNPs for 46 captive individuals of Brazilian Merganser. Principal component 1 (PC1) is depicted in the x-axis, and PC2 in the y-axis, with their corresponding proportions of variance explained between parenthesis. Dots represent individuals from different sudivisions: Serra da Canastra National Park (Canastra—CAN); Captive-bred individuals (MO); Jalapão State Park (Jalapão—JAL); and Alto Paranaíba region (Paranaíba—PAR).

The pairwise FST analysis showed significant values ($p < 0.05$) for all comparisons, indicating that the captive-bred individuals are closer to the individuals from Jalapão, with a low FST (0.057). Furthermore, analyzing the founding individuals only, we observed that the Jalapão individuals are also the most differentiated from Canastra and Paranaíba, which are closely related (Table 1). This result is coherent with our recent publication comparing Brazilian Merganser wild populations [13].

Table 1. Pairwise FST analysis of Brazilian Merganser populations defined a priori based on the locality of origin. All pairwise FST values were significantly different from zero ($p < 0.05$).

	Jalapão	**Paranaíba**	**Canastra**	**Captive-Bred**
Jalapão	-	-	-	-
Paranaíba	0.2450	-	-	-
Canastra	0.1934	0.1126	-	-
Captive-bred	0.0570	0.0750	0.1018	-

Structure analysis using an uncorrelated allele frequency model found two allelic pools/genetic clusters or subpopulations (k = 2) using 425 SNPs. In general, individuals

from Paranaíba (PAR), Jalapão (JAL), and some captive-bred (MO) present a single allelic pool (green), and individuals from Canastra (CAN) and some captive-bred individuals present also a second allelic pool represented in red (Figure 5).

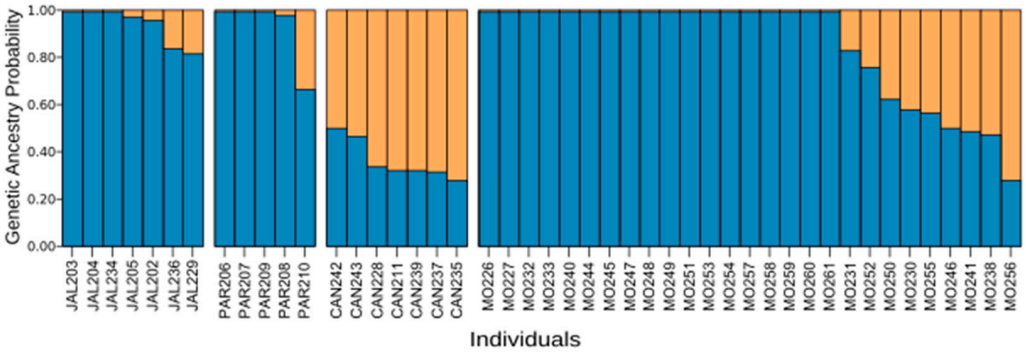

Figure 5. Structure results using the uncorrelated allele frequency model for Brazilian Mergansers. The sample numbers of mergansers are distributed on the x-axis, and each vertical bar represents the attribution of the individual genome to two different allelic pools or subpopulations as defined by Structure Harvester (k = 2). The colors blue and light brown represent the proportion of each one of the two allelic pools or subpopulations (k).

To calculate the effective population size (Ne), we considered all captive individuals as a single "population" (the captive pool), since sample sizes smaller than 30 can potentially influence the Ne estimates [51]. We found an effective population size (Ne = 4) much smaller than the estimated population census (N = 250).

4. Discussion

We have identified a high inbreeding in the captive-bred population of the Brazilian Mergansers, which was constituted by founders derived from the four remnant populations in the wild. However, some highly heterozygous individuals can be identified, and a moderate level of diversity is found between mergansers from different wild localities.

The high degree of relatedness exhibited by three of the captive-bred individuals, MO230 (F1 from Paranaíba-Canastra), MO250 (F2 from Jalapão-Paranaíba), and MO252 (F2 from Jalapão-Paranaíba) can be explained by the natural history of the species. Historically, populations of Brazilian Mergansers have always occurred in low population densities and disjointed areas [4]. Therefore, it is improbable that MO250 and MO252 individuals, who do not have Canastra individuals as their ancestors, are indeed 1st and 2nd-degree kinships with other individuals from this area, as the analyses suggest. The same fact also can be applied to the individual MO230, who, despite being the offspring of individuals from Canastra and Paranaíba, has an estimated 1st-degree kinship with individuals from Jalapão and all other localities. One fact that may explain this pattern found in the individuals MO230, MO250, and MO252 is that these individuals generated by crossings of founders present a high heterozygosity and a low coefficient of individual inbreeding (Table S2) since the pairwise kinship analyses are based on differences of shared homozygosity and heterozygosity [34].

As the captive population is formed by a few founders and only from three out of four remaining sources [13], it is expected that individuals born in captivity present a reasonable genetic similarity. The pattern of differentiation found by the seven individuals from the Canastra (CAN211, CAN228, CAN235, CAN237, CAN239, CAN242, and CAN243) and the five captive individuals (MO238, MO241, MO246, MO255, and MO256) can be explained by the fact that these individuals are from the Canastra or descendants of individuals from this area and that the captive population is composed of approximately 70% of individuals from Jalapão, Paranaíba, and their descendants, which can maximize the differences. Furthermore, of all the remaining Brazilian Merganser populations, the Canastra is the one with the greatest genetic diversity [10], which may make these individuals more

differentiated from the others, and since little is known about the mode of dispersal of the species and there is no data on dispersal or migration of individuals over long distances [52], a slight difference between populations is expected.

The results of the individual inbreeding coefficient analysis show us that despite having few founders, the captive-bred individuals show negative inbreeding coefficients, suggesting an excess of heterozygosity about the population mean [53], as expected for offspring derived from founders from different sources. Indeed, MO230, MO250, and MO252 present negative inbreeding coefficients much above the population average, a pattern that can be explained by the fact that these individuals have a high number of heterozygous SNPs, and heterozygosity influences the estimation of the FIT [34]. The individual PAR210 also showed a negative inbreeding coefficient above the average of its original population. However, this pattern can be partially explained by the fact that the other four Paranaíba individuals are full siblings (PAR206, PAR207, PAR208, and PAR209) (unpublished information from PAN Pato-mergulhão, Brazil), which present high pairwise kinship values (Table S3) that influences the mean population homozygosity, which in turn influences individual inbreeding coefficient estimates [34].

The arrangement of the two groups of individuals found in the PCA analysis corresponds to the mating pattern that was established in captivity. The group of individuals at the bottom of the figure is formed by Jalapão founder individuals and by individuals born from captive crosses (captive-bred), where at least one of the parents is a Jalapão individual (Figure S2). The closest grouping of Canastra and Paranaíba individuals has already been demonstrated in previous works [10,13], which identified greater genetic similarity between individuals from these areas. The captive-bred individuals MO238, MO241, and MO256 are offspring of Canastra and Paranaíba individuals, and consequently present a greater genetic similarity with these individuals, causing them to group in the PCA graph since its analysis uses a matrix of genetic distance between individuals [54].

The high similarity of captive-bred and founding populations (Paranaíba, Canastra, and Jalapão), demonstrated in the pairwise FST analysis was expected since it was formed by these founding populations. Canastra individuals present a relatively lower average similarity with the captive-bred individuals because only six individuals are offspring from a Canastra founder. Otherwise, couples formed by Jalapão and Paranaíba founders generated ~78% (21) of captive-bred offspring, explaining their close genetic affinity. In general, captive-bred individuals are more related to Jalapão than to Paranaíba founders, and it may be explained by genetic drift in the source Jalapão population, with a higher frequency of rare alleles in the Jalapão founders.

The genetic groupings found by Structure reaffirm the results of the PCA and pairwise FST analyses, which demonstrated a greater similarity among captive-bred Jalapão and Paranaíba individuals when compared to Canastra ones. This result is expected because Structure infers the genetic clusters based on the allelic patterns of the individuals, which are greatly shared between individuals genealogically related [47].

The Ne value found in this analysis is much lower than the population census, which is often found in other studies [55]. This low value reflects the low genetic diversity of the wild populations of the Brazilian Mergansers that also present significant inbreeding [13]. Thus, the reduction in the genetic diversity by drift and inbreeding in the captive population is a major concern for this critically endangered species [56].

However, some limitations of this study should be pointed out. These include a potential bias from the ddRAD technique, which only samples specific genome segments, and SNPs were called using a de novo approach due to the lack of a reference genome, resulting in potential loss of variants. However, this new set of ddRAD-SNPs allowed informative analyses to be performed with the captive population of Brazilian Mergansers to be used for ex situ genetic management.

5. Conclusions

The moderate degree of genetic relatedness between most captive individuals demonstrates the need to supplement the ex situ population with individuals (or eggs) from wild populations, mainly from the Canastra locality, where there is the largest remaining population and genetic diversity of mergansers [13]. In addition, Veadeiros individuals should be added to the founding population in captivity to increase the representation of all remaining areas in the ex situ genetic pool. Individuals with high inbreeding coefficients and pairwise kinships between them require greater care during the formation of couples in the reproductive period, as their intercrossing should be avoided. Even though inbreeding depression signs have not been observed in the captive population (unpublished), some females, both from the Jalapão area and captive-bred descendants, present a likely sex-linked (Z chromosome) brown phenotype [57,58].

Thus, new founders from different areas will be important to generate ex situ mergansers with low inbreeding coefficients and pairwise kinship, who are those with the highest genetic value to be used eventually for release back into the wild. In addition, appropriate genetic management of the ex situ pool will be essential to maintain the long-term viability and sustainability of the captive program [8,59] in order to allow a successful reestablishment of wild populations of Brazilian Merganser.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/birds5010013/s1, Figure S1: Inbreeding coefficients for the four subdivisions of the captive population of Brazilian Merganser; Figure S2: Pedigree of individuals from the *ex situ* population of Brazilian Merganser; Table S1: List of samples used in this work; Table S2: Number of SNPs in heterozygosity and a ddRAD library assembly Protocol. Table S3: Pairwise Kinship between sequenced individuals.

Author Contributions: Conceptualization, F.R.S. and D.P.C.; methodology, D.P.C., R.S.O.B. and J.E.S.-J.; validation, D.P.C.; formal analysis, D.P.C. and J.E.S.-J.; data curation, D.P.C. and H.P.G.-N.; writing—original draft preparation, D.P.C.; writing—review and editing, F.R.S.; funding acquisition, F.R.S. All authors have read and agreed to the published version of the manuscript.

Funding: This project received funds from Fundação o Boticário, CNPq, and FAPEMIG (Brazil).

Institutional Review Board Statement: The ethical procedures used for sampling birds were approved by CEUA-UFMG (202/2007).

Data Availability Statement: VCF raw data files for SNPs are available at https://doi.org/10.5281/zenodo.10236182 (accessed on 6 March 2024).

Acknowledgments: Davidson P. Campos thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of Brazil for his PhD fellowship in the Program of Genetics of UFMG (Belo Horizonte, Brazil). F.R.S. thanks Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq) of Brazil for his research fellowship. We thank Terra Brasilis, Funatura, Cer Vivo, Naturatins, and Zooparque Itatiba for merganser samples used in this study, which are part of the collection of the Brazilian Action Plan for Conservation of ICMBio (PAN pato mergulhão) using SisBio-ICMBio permits 17051 and 12662. Data sets were processed on the Sagarana HPC cluster CPAD-ICB-UFMG.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Collar, N.J.; Gonzaga, L.P.; Krabbe, N.; Madrono Nieto, A.; Naranjo, L.G.; Parker, T.A.; Wege, D.C. *Threatened Birds of the Americas*; IUCN and ICBP (International Council for Bird Preservation): Cambridge, UK, 1992; ISBN 978-1-56098-267-8.
2. Antas, P. The Brazilian Merganser (*Mergus octosetaceus*), the Most Threathened Duck in South America. *Gibier Faune Sauvag.* **1996**, *13*, 799–800.
3. IUCN. BirdLife International: The IUCN Red List of Threatened Species: *Mergus octosetaceus*. 2019. Available online: https://www.iucnredlist.org/species/22680482/143756439 (accessed on 7 November 2023).

4. Yamashita, C.; Valle, M.D.P. Ocorrência de duas aves raras no Brasil Central: *Mergus octosetaceus* e *Tigrisoma fasciatum*. *Ararajuba* **1990**, *1*, 107–109.
5. Benstead, P.J.; Hearn, R.D.; Nedelcoff, A.R.S. A recent sighting of Brazilian Merganser *Mergus octosetaceus* in Misiones province, Argentina. *Cotinga* **1994**, *2*, 35–36.
6. Hearn, R. The current status of the Brazilian Merganser (*Mergus octosetaceus*) in Argentina. *TWSG News* **1994**, *5*, 14–15.
7. Fragano, F.; Clay, R. Biodiversity status of the interior Atlantic forest of Paraguay. In *The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook*; Galindo-Leal, C., Câmara, I.G., Eds.; Island Press: Washington, DC, USA, 2003; p. 488, ISBN 1-55963-988-1.
8. Rose, P. Evidence for Aviculture: Identifying Research Needs to Advance the Role of Ex Situ Bird Populations in Conservation Initiatives and Collection Planning. *Birds* **2021**, *2*, 77–95. [CrossRef]
9. Vilaça, S.T.; Redondo, R.A.F.; Lins, L.V.; Santos, F.R. Remaining genetic diversity in Brazilian Merganser (*Mergus octosetaceus*). *Conserv. Genet.* **2012**, *13*, 293–298. [CrossRef]
10. Maia, T.A.; Vilaça, S.T.; Silva, L.R.D.; Santos, F.R.; Dantas, G.P.D.M. DNA sampling from eggshells and microsatellite genotyping in rare tropical birds: Case study on Brazilian Merganser. *Genet. Mol. Biol.* **2017**, *40*, 808–812. [CrossRef]
11. Faux, P.; Oliveira, J.C.P.; Campos, D.P.; Dantas, G.P.M.; Maia, T.A.; Dergan, C.G.; Cassemiro, P.M.; Hajdu, G.L.; Santos-Júnior, J.E.; Santos, F.R. Fast genomic analysis of aquatic bird populations from short single-end reads considering sex-related pitfalls. *Ecol. Inform.* **2020**, *56*, 101058. [CrossRef]
12. Maia, T.A.; Campos, D.P.; Silva, L.R.; Lins, L.V.; Ribeiro, F.; Sebaio, F.; Rodrigues, F.R.; Dantas, G.P.M. Evidence of strong population bottleneck in genetics of endangered Brazilian Merganser (*Mergus octosetaceus*). *J. Ornithol.* **2020**, *161*, 521–527. [CrossRef]
13. Campos, D.P.; Granger-Neto, H.P.; Santos-Júnior, J.E.; Faux, P.; Santos, F.R. Population genomics of the critically endangered Brazilian merganser. *Animals* **2023**, *13*, 3759. [CrossRef] [PubMed]
14. Frankham, R. Genetic adaptation to captivity in species conservation programs. *Mol. Ecol.* **2008**, *17*, 325–333. [CrossRef]
15. Toone, W.D.; Wallace, M.P. The Extinction in the Wild and Reintroduction of the California Condor (*Gymnogyps californianus*). In *Creative Conservation: Interactive Management of Wild and Captive Animals*; Olney, P.J.S., Mace, G.M., Feistner, A.T.C., Eds.; Springer: Dordrecht, The Netherlands, 1994; pp. 411–419, ISBN 978-94-011-0721-1.
16. van Dierendonck, M.C.; de Vries, M.F.W. Ungulate Reintroductions: Experiences with the Takhi or Przewalski Horse (*Equus ferus przewalskii*) in Mongolia. *Conserv. Biol.* **1996**, *10*, 728–740. [CrossRef]
17. Fischer, J.; Lindenmayer, D.B. An assessment of the published results of animal relocations. *Biol. Conserv.* **2000**, *96*, 1–11. [CrossRef]
18. Williams, S.E.; Hoffman, E.A. Minimizing genetic adaptation in captive breeding programs: A review. *Biol. Conserv.* **2009**, *142*, 2388–2400. [CrossRef]
19. Waples, R.S.; Drake, J. Risk/Benefit Considerations for Marine Stock Enhancement: A Pacific Salmon Perspective. In *Stock Enhancement and Sea Ranching*; John Wiley & Sons: Hoboken, NJ, USA, 2004; pp. 260–306, ISBN 978-0-470-75132-9.
20. Melampy, M.N.; Howe, H.F. Sex Ratio in the Tropical Tree *Triplaris americana* (Polygonaceae). *Evolution* **1977**, *31*, 867–872. [CrossRef]
21. Crow, J.F.; Denniston, C. Inbreeding and Variance Effective Population Numbers. *Evolution* **1988**, *42*, 482–495. [CrossRef]
22. Frankham, R.; Ballou, J.D.; Briscoe, D.A. *Introduction to Conservation Genetics*; Cambridge University Press: Cambridge, UK, 2002.
23. Torati, L.S.; Taggart, J.B.; Varela, E.S.; Araripe, J.; Wehner, S.; Migaud, H. Genetic diversity and structure in *Arapaima gigas* populations from Amazon and Araguaia-Tocantins river basins. *BMC Genet.* **2019**, *20*, 13. [CrossRef] [PubMed]
24. Lawson, D.M.; Williams, C.K.; Lavretsky, P.; Howell, D.L.; Fuller, J.C. Mallard–Black Duck Hybridization and Population Genetic Structure in North Carolina. *J. Wildl. Manag.* **2021**, *85*, 1243–1255. [CrossRef]
25. Diaz-Martin, Z.; Fant, J.; Havens, K.; Cinea, W.; Tucker Lima, J.M.; Griffith, M.P. Current management practices do not adequately safeguard endangered plant species in conservation collections. *Biol. Conserv.* **2023**, *280*, 109955. [CrossRef]
26. Ba, H.; Jia, B.; Wang, G.; Yang, Y.; Kedem, G.; Li, C. Genome-Wide SNP Discovery and Analysis of Genetic Diversity in Farmed Sika Deer (*Cervus nippon*) in Northeast China Using Double-Digest Restriction Site-Associated DNA Sequencing. *G3* **2017**, *7*, 3169–3176. [CrossRef] [PubMed]
27. Çilingir, F.G.; Hansen, D.; Ozgul, A.; Grossen, C. Design of SNP markers for Aldabra giant tortoises using low coverage ddRAD-Seq. *Conserv. Genet. Resour.* **2021**, *13*, 409–412. [CrossRef]
28. Sambrook, J.; Russell, D.W. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: New York, NY, USA, 2001; Volume 1.
29. Thrasher, D.J.; Butcher, B.G.; Campagna, L.; Webster, M.S.; Lovette, I.J. Double-Digest RAD sequencing outperforms microsatellite loci at assigning paternity and estimating relatedness: A proof of concept in a highly promiscuous bird. *Mol. Ecol. Resour.* **2018**, *18*, 953–965. [CrossRef]
30. Peterson, B.K.; Weber, J.N.; Kay, E.H.; Fisher, H.S.; Hoekstra, H.E. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE* **2012**, *7*, e37135. [CrossRef]
31. Catchen, J.M.; Amores, A.; Hohenlohe, P.; Cresko, W.; Postlethwait, J.H. *Stacks*: Building and Genotyping Loci *De Novo* From Short-Read Sequences. *G3* **2011**, *1*, 171–182. [CrossRef] [PubMed]
32. Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; DePristo, M.A.; Handsaker, R.E.; Lunter, G.; Marth, G.T.; Sherry, S.T.; et al. The variant call format and VCFtools. *Bioinformatics* **2011**, *27*, 2156–2158. [CrossRef]

33. Foll, M.; Gaggiotti, O. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* **2008**, *180*, 977. [CrossRef] [PubMed]
34. Manichaikul, A.; Mychaleckyj, J.C.; Rich, S.S.; Daly, K.; Sale, M.; Chen, W.-M. Robust relationship inference in genome-wide association studies. *Bioinformatics* **2010**, *26*, 2867–2873. [CrossRef] [PubMed]
35. Goudet, J. hierfstat, a package for r to compute and test hierarchical F-statistics. *Mol. Ecol. Notes* **2005**, *5*, 184–186. [CrossRef]
36. Weir, B.S.; Cockerham, C.C. Estimating F-Statistics for the Analysis of Population Structure. *Evolution* **1984**, *38*, 1358–1370. [CrossRef] [PubMed]
37. Knaus, B.J.; Grünwald, N.J. vcfr: A package to manipulate and visualize variant call format data in R. *Mol. Ecol. Resour.* **2017**, *17*, 44–53. [CrossRef]
38. Kamvar, Z.N.; Brooks, J.C.; Grünwald, N.J. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Front. Genet.* **2015**, *6*, 208. [CrossRef]
39. Paradis, E.; Schliep, K. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **2019**, *35*, 526–528. [CrossRef]
40. Neuwirth, E. RColorBrewer: ColorBrewer Palettes. Available online: https://CRAN.R-project.org/package=RColorBrewer (accessed on 7 November 2023).
41. Slowikowski, K.; Schep, A.; Hughes, S.; Dang, T.K.; Lukauskas, S.; Irisson, J.-O.; Kamvar, Z.N.; Ryan, T.; Christophe, D.; Hiroaki, Y.; et al. Ggrepel: Automatically Position Non-Overlapping Text Labels with "ggplot2". 2023. Available online: https://rdrr.io/cran/ggrepel (accessed on 12 March 2024).
42. Jombart, T.; Ahmed, I. *adegenet 1.3-1*: New tools for the analysis of genome-wide SNP data. *Bioinformatics* **2011**, *27*, 3070–3071. [CrossRef]
43. Wickham, H. Reshaping Data with the reshape Package. *J. Stat. Soft.* **2007**, *21*, 1–20. [CrossRef]
44. Wickhaml, H.; Henry, L.; Takahashi, K.; Wilke, C.; Woo, K.; Yutani, H.; Dunnington, D. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. Available online: https://ggplot2.tidyverse.org/reference/ggplot2-package.html (accessed on 18 November 2023).
45. Do, C.; Waples, R.S.; Peel, D.; Macbeth, G.M.; Tillett, B.J.; Ovenden, J.R. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* **2014**, *14*, 209–214. [CrossRef] [PubMed]
46. Waples, R.S.; Do, C. LDNE: A program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Resour.* **2008**, *8*, 753–756. [CrossRef] [PubMed]
47. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
48. Earl, D.A.; vonHoldt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
49. Kopelman, N.M.; Mayzel, J.; Jakobsson, M.; Rosenberg, N.A.; Mayrose, I. CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across *K*. *Mol. Ecol. Resour.* **2015**, *15*, 1179–1191. [CrossRef]
50. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [CrossRef]
51. Waples, R.S.; Antao, T.; Luikart, G. Effects of Overlapping Generations on Linkage Disequilibrium Estimates of Effective Population Size. *Genetics* **2014**, *197*, 769–780. [CrossRef] [PubMed]
52. Hughes, B.; Dugger, B.; Cunha, H.; Lamas, I.; Goerck, J.; Lins, L.; Silveira, L.; Andrade, R.; Bruno, S.; Rigueira, S.; et al. *Plano de Ação Para a Conservação Do Pato-Mergulhão (Mergus octosetaceus)*; IBAMA: Alta Floresta, Brazil, 2006; ISBN 978-85-7300-226-3.
53. Brandt, A.; Tran Van, P.; Bluhm, C.; Anselmetti, Y.; Dumas, Z.; Figuet, E.; François, C.M.; Galtier, N.; Heimburger, B.; Jaron, K.S.; et al. Haplotype divergence supports long-term asexuality in the oribatid mite *Oppiella nova*. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2101485118. [CrossRef] [PubMed]
54. Lever, J.; Krzywinski, M.; Altman, N. Principal component analysis. *Nat. Methods* **2017**, *14*, 641–642. [CrossRef]
55. Frankham, R. Effective population size/adult population size ratios in wildlife: A review. *Genet. Res.* **1995**, *66*, 95–107. [CrossRef]
56. Ellstrand, N.C.; Elam, D.R. Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annu. Rev. Ecol. Syst.* **1993**, *24*, 217–242. [CrossRef]
57. Nadeau, N.J.; Mundy, N.I.; Gourichon, D.; Minvielle, F. Association of a single-nucleotide substitution in TYRP1 with roux in Japanese quail (*Coturnix japonica*). *Anim. Genet.* **2007**, *38*, 609–613. [CrossRef]
58. Grouw, H. What Colour Is That Bird? The causes and recognition of common colour aberrations in birds. *Br. Birds* **2013**, *106*, 17–29.
59. Díez-del-Molino, D.; von Seth, J.; Gyllenstrand, N.; Widemo, F.; Liljebäck, N.; Svensson, M.; Sjögren-Gulve, P.; Dalén, L. Population genomics reveals lack of greater white-fronted introgression into the Swedish lesser white-fronted goose. *Sci. Rep.* **2020**, *10*, 18347. [CrossRef]