

## Article

# Genetic Variability and Kinship Analyses of Seized Red-Browed Amazon, *Amazona rhodocorytha* (Aves, Psittacidae)

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**Abstract:** Analyzing genetic variability and kinship relations is essential to guide conservation management plans for threatened species. The Red-browed Amazon (*Amazona rhodocorytha*) is one of the four Amazona parrots that are endemic to remnants of the Atlantic Forest, classified as Vulnerable (IUCN) owing to habitat loss and fragmentation and trapping for the illegal pet trade. At the end of 2021, 19 Red-browed Amazons were rescued from illegal trade in the Espírito Santo state, Brazil, including 14 nestlings reportedly captured in the Sooretama Biological Reserve, which provided the first opportunity to address the genetic parameters of a wild population of this threatened parrot. We used Single-Nucleotide Polymorphism (SNP) data to assess the genetic diversity and kinship relations between the rescued birds. We observed high heterozygosity levels and low inbreeding coefficients. Principal Component Analysis revealed the presence of at least two distinct genetic groups, suggesting past isolation followed by secondary contact. Our results suggest that the population from Sooretama is an important genetic and demographic repository of the Red-browed Amazon, and the presence of individuals from the two genetic lineages in the same area reduces concerns about potential inbreeding depression in rehabilitation and reintroduction plans and also inspires further investigations on historical and contemporary population structuring.

**Keywords:** genetic variability; kinship; population structure; single-nucleotide polymorphisms; red-browed amazon; reintroduction



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## 1. Introduction

The family Psittacidae is composed of 32 genera and 150 species of New World and African parrots [1]. Due to their coloration, loud sounds, capacity to imitate the human voice, and easy adaptability to captivity, they are among the most charismatic bird species and the most popular cage birds on Earth [2–4]. However, these characteristics incentivize poaching across many species, which, in addition to habitat destruction, has led to 28% of parrot species being classified under some level of threat [1,3,5,6]. Brazil concentrates the greatest number of Psittacidae species, holding important parrot conservation hotspots with high species richness [7]. It is also the country with the greatest number of endangered taxa [8,9], and many conservation initiatives have been started in the last few decades [3,10], including monitoring and protection of nesting sites [11–14], rehabilitation and release of seized birds [15–17], captive breeding [4,18] and reintroduction into the wild [17,19,20].

Genetic analyses are fundamental tools for the proper conservation management of endangered organisms, both in situ and exsitu [21–24]. They enable the evaluation of genetic

variability and inbreeding in populations that have experienced sharp declines [21,22,24]; guide the best pairings of captive individuals through kinship analyses to avoid inbreeding depression [21,22]; identify hybridizations (a common problem with captive populations of endangered taxa [25]); and may illuminate significant evolutionary and historical patterns (i.e., intraspecific lineages that have evolved independently, populations that have become isolated post-fragmentation or post-bottleneck, or groups with divergent ancestral units that may need to be taken into account in conservation and recovery plans) [26,27]. Genetic analyses also offer forensic insights regarding the geographic origin of individuals belonging to species with significant population structuring that may guide the return of confiscated specimens to the correct source populations [21,27,28].

In Brazil, the parrots inhabiting the Atlantic Forest are of special conservation concern. The Atlantic Forest is one of the most degraded biomes in the world. Today, it is confined to 24% of its original land cover, where only 12.4% represents native, well-preserved forest areas [29]. It is a biodiversity hotspot that holds 223 endemic bird species, of which 65 are threatened, including psittacids of the genus *Amazona* [6,9,30]. The preservation and expansion of the remaining protected areas of the Atlantic Forest are of extreme relevance for the conservation of threatened bird species [30]. The Sooretama Biological Reserve, with the adjacent Vale Reserve, in Espírito Santo state, forms the largest and most important forest block (46,050 ha) of intact, lowland Atlantic Forest, where the largest population of Red-browed Amazons is found.

The Red-browed Amazon, *Amazona rhodocorytha* (Salvadori, 1890), is one of the fourteen Atlantic Forest endemic psittacids [30]. It occupies the high forest strata, in medium-to-advanced successional stages, at altitudes below 1000 m [31]. It is currently classified as “Vulnerable” [6,9] and is experiencing population decline [6] with approximately 2500 to 10,000 mature individuals in the wild [9]. The Red-browed Amazon is found from the south of Rio de Janeiro north to Alagoas, where its disjunct, northern-most population is virtually extinct [32], and to the east of Minas Gerais [33–35]. The Sooretama Biological Reserve and Vale Reserve are the strongholds for the population of Red-browed Amazons, but recent data about the populations that inhabit these reserves are scant [36–38].

Due to policy actions, individuals of priority species such as the Red-browed Amazon that are illegally possessed are apprehended by law enforcement and sent to wildlife centers, where they join ongoing or new rehabilitation and reintroduction programs [39]. By the end of 2022, through an initiative coordinated by researchers from the Museu de Zoologia da Universidade de São Paulo, with the support of the Public Ministry, National Agencies of Protection of the Environment (ICMBio, IBAMA), Environmental Police, and private companies, 59 Red-browed Amazons were rescued from illegal captive facilities and traders [40]. Among them, 14 recently hatched parrots were rescued from a smuggler, on 11 September 2021 by a special task force of Federal and Environmental Policies and IBAMA in Vila Valério municipality, Espírito Santo state, Brazil. These chicks, with ages varying from 7 to 25 days, were thought to be taken from their nests at Sooretama Biological Reserve. A total of 5 additional subadult and adult parrots also confiscated in Espírito Santo state joined this group, totaling 19 individuals from this state. Capturing adult parrots in the wild is difficult, and confiscated individuals rarely have known origins. Our sample of 19 individuals provided the first opportunity to address the genetic profile of a contemporaneous sample of wild Red-browed Amazons.

We used a dataset of 30,071 SNPs (Single-Nucleotide Polymorphism markers) to analyze the level of genetic diversity and the kinship relations among the 14 young from Sooretama and five other subadults and adults from unknown localities (but from Espírito Santo state), by looking at parameters such as heterozygosity, nucleotide diversity, inbreeding, pairwise relatedness, and structure clustering. Based on the obtained information, we inferred the genetic implications of the potential use of these individuals in reintroduction plans.

## 2. Materials and Methods

### 2.1. Sampling and DNA Extraction

Our sample was composed of two groups: 14 young birds that were rescued together after allegedly being captured in the Sooretama Biological Reserve and 5 subadults or adults captured from unknown localities in Espírito Santo. They were sexed using standard, commercially available kits for DNA sexing.

To obtain genomic DNA from the blood samples taken, we used a PureLink® Genomic DNA Mini kit (Invitrogen Inc., Waltham, MA, USA) and followed the manufacturer's protocol to extract DNA from the blood. We measured genomic DNA concentrations using a Qubit 2.0 fluorometer with a dsDNA BR assay kit (Life Technologies Inc., Waltham, MA, USA). For sequencing, we used the sequence capture approach that targets ultra-conserved elements (UCEs) [41]. This allowed us to recover a sub-genomic marker from thousands of unlinked loci scattered throughout the genome. Genomic enrichment and Illumina sequencing were performed by Rapid Genomics LGC (Gainesville, FL, USA) using at least 1000 ng of input genomic DNA for each sample. We used standard library preparation protocols to enrich 5060 UCE loci [41], targeting a set of 5372 specific probes (MYbaits\_Tetrapods-UCE-5 K kit; Mycroarray).

### 2.2. UCE Sequence Processing, SNP Calling and Filtering

To implement quality control, assembly, and alignment of UCE sequences, we employed a Phyluce v.1.7.1 [42] pipeline. We used Illumiprocessor 2.10 [43] to remove low-quality regions, adapters, and barcode contamination. Contigs were assembled using the software SPAdes 3.14.1 [44].

SNP calling procedures were the same as described in detail by [45]. Additionally, we constructed an alternative SNP matrix by randomly selecting one SNP per locus. This approach was implemented to avoid the bias caused by linkage disequilibrium, allowing us to compare the results (Table S1, Figure S1). Binary files (BED, RAW, and BIM) were generated from PED and MAP SNP files using the following flags: `–make-bed`, `–recode A`, `–chr-set 95`, and `–allow-extra-chr` in PLINK 1.9 [46]. SNP data management and analyses were performed in R-4.2.2 (R Core Team, 2019) using functions of the R package SambaR (<https://github.com/mennodejong1986/SambaR>, accessed on 13 February 2023.) and following the directions of [47]. RAW SNP files were imported into R and stored in a genlight object using the function “`read.PLINK`” of the R package adegenet-2.1.10 [48,49].

The data was filtered using the function “`filterdata`” of the R package SambaR, with the following parameters set: `indmiss = 0.25`, `snpmis = 0.1`, `min_mac = 5`, `dohefilter = FALSE`, and `min_spacing = 0`. We used a MAC of 5 based on a strategy presented by [50] to remove low-confidence loci while retaining all individuals and further reducing the percentage of missing data.

After filtering, 19 out of 19 individuals and 8871 out of 30071 SNPs were retained. Thinning did not reduce the dataset. The filtered and thinned dataset was used for subsequent analyses. Sexing was conducted by Unigen Inc., São Paulo, Brasil.

### 2.3. Genetic Diversity and Kinship Analysis

Analyses were carried out using the functions “`calcdiversity`” and “`calckinship`” of the R package SambaR [47]. Inbreeding was estimated using the inbreeding coefficient (F), which is characterized as the probability that the two alleles at any locus of a diploid individual are identical by descent (IBD) [51]. Relatedness between sample pairs was estimated with the mean kinship coefficient [52], the KING robust score [53], and the R0, R1 statistics [52].

### 2.4. Structure Analysis

Analyses and the derivation of the maximum likelihood phylogenetic tree based on Hamming genetic distances between individuals were carried out using the function “`findstructure`” of the R package SambaR [47]. Principal Component Analysis (PCA) and

Principal Coordinate Analyses (PCoA) were performed using the function “snpgdsPCA” of the R package ape-5.7 [54] on distance matrices containing the Hamming’s genetic distance, calculated with the function “bitwise.dist” of the R package poppr-2.9.3 [55]. We verified for statistical significance between clusters of the PCA using a Tuckey HSD Test. Pairwise sequence dissimilarity (pi) was calculated with the function “calcp” of the R package SambaR [47].

### 3. Results

We analyzed 30,071 UCE SNPs of 19 Red-browed Amazons, a sample with 9% of missing data and 91.8% of all SNPs in Hardy–Weinberg Equilibrium before filtering. Since we already had less than 25% of missing data per sample, filtering was conducted to remove SNPs with more than 10% of missing data and set to a minor allele count of five per SNP. Filtering for heterozygosity excess and distance between adjacent SNPs was not conducted. This resulted in a sample size of 8871 SNPs, all with a minor allele frequency greater or equal to 0.05, with a mean spacing of 29 kb. The results obtained from the alternative SNPs matrix, which included one SNP per locus, were nearly identical, and they can be found in the Supplementary Materials. Sexing the 14 young birds revealed 8 males and 6 females, and the 5 adults/subadults revealed 2 males and 3 females.

#### 3.1. Genetic Diversity

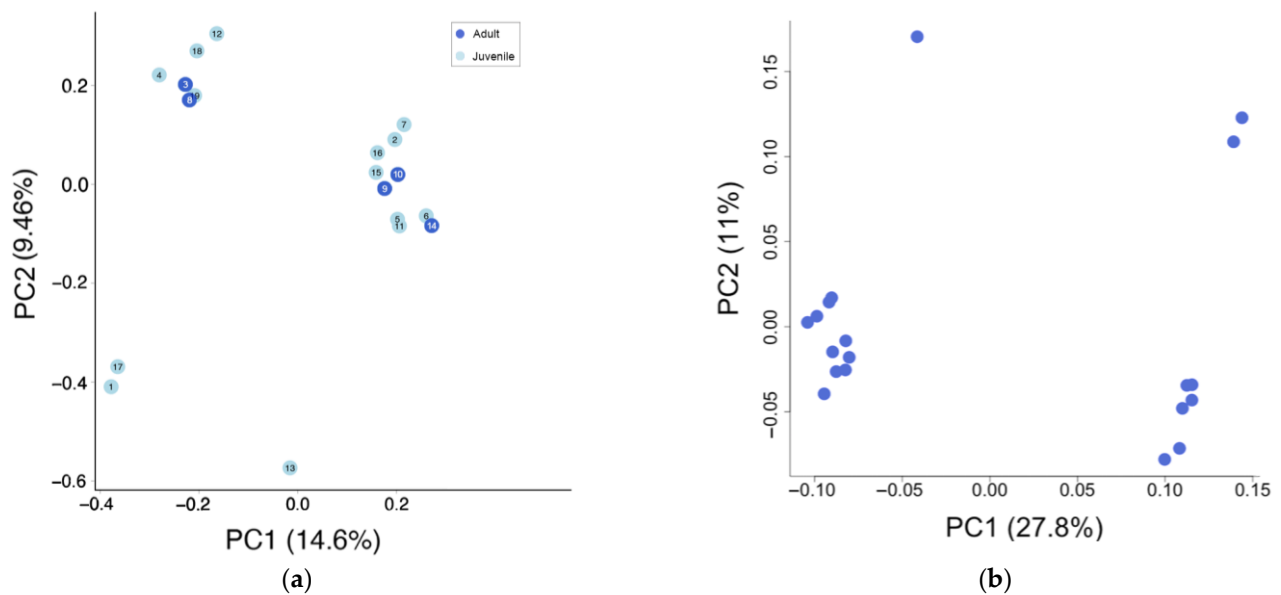
The average pairwise sequence dissimilarity (pi) was  $0.385 \pm 0.016$ . The mean observed heterozygosity ( $H_o$ ) was  $0.423 \pm 0.083$ , and the mean expected heterozygosity ( $H_e$ ) was 0.243.  $H_o$  was significantly higher than  $H_e$  ( $t = 9.502$ ,  $p \sim 0$ ). We found individual inbreeding coefficients ( $F$ ) ranging from  $-0.415$  ( $\chi^2 = 348.7$ ,  $p = 0$ ) to  $0.304$  ( $\chi^2 = 177.6$ ,  $p = 0$ ) (Table 1).

**Table 1.** Genetic diversity estimates for Red-browed Amazon (*Amazona rhodocorytha*). Individual observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, and inbreeding coefficients ( $F$ ).

| Individual | Name                    | Age      | Sex | $H_o$ | $H_e$ | $F$    | $p$ -Value |
|------------|-------------------------|----------|-----|-------|-------|--------|------------|
| 1          | 707_MZUSP_a2572_Ama_rho | Juvenile | F   | 0.363 | 0.243 | 0.037  | 0.096166   |
| 2          | 708_MZUSP_a2554_Ama_rho | Juvenile | F   | 0.533 | 0.243 | −0.416 | 0          |
| 3          | 709_MZUSP_a2563_Ama_rho | Subadult | M   | 0.337 | 0.243 | 0.106  | 0.000002   |
| 4          | 710_MZUSP_a2564_Ama_rho | Juvenile | M   | 0.262 | 0.243 | 0.304  | 0          |
| 5          | 711_MZUSP_a2556_Ama_rho | Juvenile | F   | 0.476 | 0.243 | −0.263 | 0          |
| 6          | 712_MZUSP_a2557_Ama_rho | Juvenile | F   | 0.470 | 0.243 | −0.248 | 0          |
| 7          | 713_MZUSP_a2560_Ama_rho | Juvenile | F   | 0.521 | 0.243 | −0.381 | 0          |
| 8          | 714_MZUSP_a235_Ama_rho  | Adult    | M   | 0.347 | 0.243 | 0.080  | 0.000335   |
| 9          | 715_MZUSP_a2568_Ama_rho | Subadult | F   | 0.462 | 0.243 | −0.227 | 0          |
| 10         | 716_MZUSP_a2570_Ama_rho | Adult    | F   | 0.472 | 0.243 | −0.254 | 0          |
| 11         | 717_MZUSP_a2553_Ama_rho | Juvenile | F   | 0.475 | 0.243 | −0.260 | 0          |
| 12         | 718_MZUSP_a2562_Ama_rho | Juvenile | M   | 0.333 | 0.243 | 0.115  | 0          |
| 13         | 719_MZUSP_a2575_Ama_rho | Juvenile | M   | 0.510 | 0.243 | −0.354 | 0          |
| 14         | 720_MZUSP_a2568_Ama_rho | Adult    | F   | 0.464 | 0.243 | −0.232 | 0          |
| 15         | 721_MZUSP_a2567_Ama_rho | Juvenile | M   | 0.503 | 0.243 | −0.334 | 0          |
| 16         | 722_MZUSP_a2558_Ama_rho | Juvenile | M   | 0.497 | 0.243 | −0.319 | 0          |
| 17         | 723_MZUSP_a2573_Ama_rho | Juvenile | M   | 0.344 | 0.243 | 0.088  | 0.000079   |
| 18         | 724_MZUSP_a2559_Ama_rho | Juvenile | M   | 0.337 | 0.243 | 0.107  | 0.000002   |
| 19         | 725_MZUSP_a2574_Ama_rho | Juvenile | M   | 0.350 | 0.243 | 0.070  | 0.001761   |

#### 3.2. Population Structure

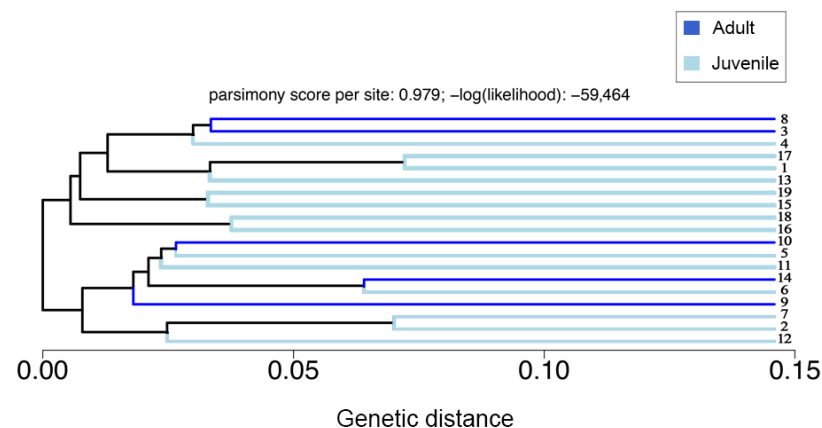
Approximately 27.8% of the variation was explained by the PC1 axis of the PCoA, and 14.6% was explained by the PC1 of the PCA. A visual assessment of the PCA (Figure 1a) and PCoA (Figure 1b) graphs showed the separation of individuals in four clouds, where juveniles were present in all clusters, and adults (individuals 3, 8, 9, 10, and 14) were split into the two largest.



**Figure 1.** Visual configuration of the individual genotypes under (a) Principal Component Analysis and (b) Principal Coordinate Analysis revealing two main clouds along the PC1 axis.

To check for the statistical significance of this primary visual split, we conducted Tukey's HSD test to search for differences between the mean values along the PC1 axis of the PCA. We confirmed that our individuals could be split into two different population clusters ( $K = 2$ ) since the mean value of PC1 was significantly different between cluster 1 (upper left, Figure 1a) and cluster 2 (upper right, Figure 1a) ( $p = 0.006$ , 95% CI = 0.096, 0.632).

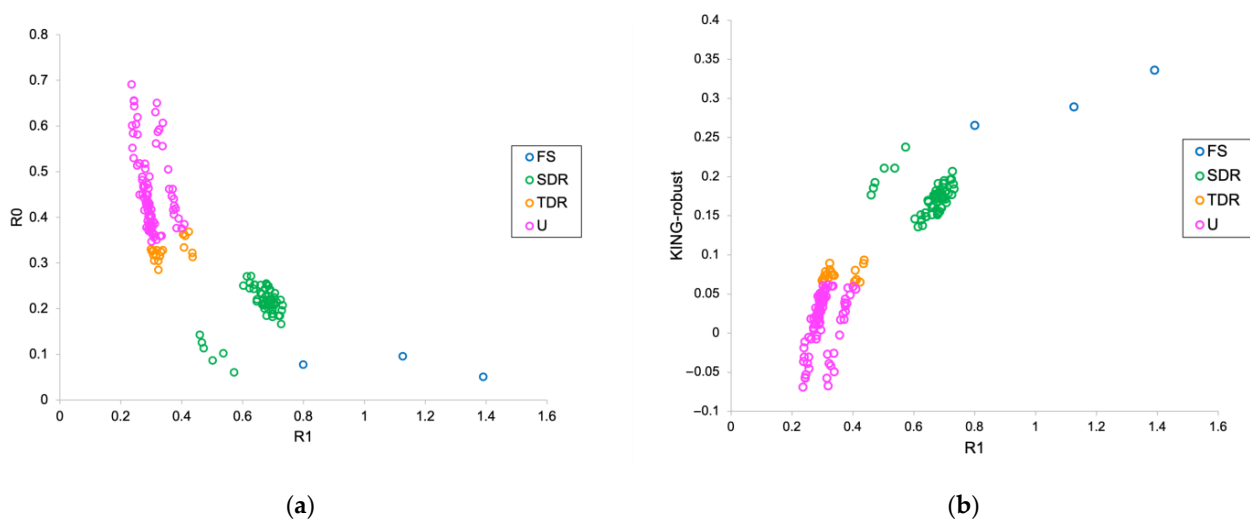
The maximum likelihood phylogenetic tree (Figure 2) based on Hamming genetic distances between individuals (parsimony score per site = 0.979, log likelihood =  $-59,464$ ) also illustrated the two main separate lineages evidenced by the PCA and PCoA.



**Figure 2.** Hamming-distance-based phylogenetic tree reveals the genetic distances between individuals with an overall parsimony score per site of 0.979 and the two main genetic clusters.

### 3.3. Pairwise Relatedness

Three individuals were revealed to be closely related, falling into the Full Sibling (FS) relationship category (Figure 3). Individuals 1 and 17 were found to be isolated together from the main clusters of PCA. Individuals 2 and 7 were young, probably from the same nest and year in Sooretama, and individuals 6 and 14, a juvenile and an adult, were siblings from different years. A total of 90 other pairs were shown to be Unrelated (U), and the remaining pairs (78) were characterized as Second- or Third-Degree Relatives (SDR or TDR).



**Figure 3.** Pairwise relatedness scatterplots where each point is a pair of individuals, and colors are assigned to points according to the four relationship category ranges they belong to Full Sibling (FS), Second-Degree Relative (SDR), Third-Degree Relative (TDR), and Unrelated (U), based on the respective pairwise KING robust score. (a) R1-R0 kinship statistics plot; (b) R1-KING robust score kinship plot.

#### 4. Discussion

Our main findings were the discovery of two distinct genetic clusters and high levels of genetic diversity among the sampled Red-browed Amazons. Furthermore, a few individuals not assigned to these two main clusters suggested that additional genetic groups that were not well represented in our samples also could exist, which was evidenced by the groupings formed across the second axis of the PCoA and PCA. Even though our study was based on 19 individuals, we analyzed more than 8000 SNPs, which is broad genome coverage capable of providing robust estimates of genetic diversity and structure.

It is worth noting that the use of family groups could confound the results because we did not know the exact origin of each of the birds, leading to artificial patterns of genetic structuring. However, we carefully addressed this issue by comparing the pairwise kinship relations among samples with the position of each individual in the PCA clusters. Our analysis revealed three sibling kin relationships, providing little support for the possibility of a cluster split due to the presence of family groups because the pairs of siblings did not influence the genetic distribution defined by the PCA and PCoA since (i) the first pair (1 and 17) were centered away from the two main clusters; (ii) the second pair (2 and 7) were depicted in the same cluster; and (iii) the third pair (6 and 14) were composed of birds that differed in age, where 6 was a young and 14 was an adult. This relationship makes it possible to infer the origin of the adult (14), which was produced by the same parents as juvenile 6, which is undisputable evidence of illegal poaching being recurrent in this area over time, where poachers are not destroying the nests, but rather are harvesting young from the same nests over successive breeding seasons. The genetic results also suggest that, at least in this particular case, the poaching is organized and structured rather than random or haphazard. Nest-site fidelity is high across all studied *Amazona* species [56]. Our findings also confirm that the Red-browed Amazon has recurring nesting locations with high geographic fidelity, such as the Blue-fronted Parrot (*Amazona aestiva*) [56], where the probability of cavity reoccupation was almost 70%. Poachers might also be capturing adult birds, but due to the feasibility of capturing young ones to be sold in the local, illegal market, we believe the first option is more valid. It is important to note that it is not possible to confidently distinguish between the relationship categories of parent-offspring (PO) and Full Siblings (FSs) only by analyzing the pairwise KING robust value. As per Waples et al. (2019) [52], we used a combination of the three statistics (R0, R1, and KING),

where, for the pair, the R1 value (1.12) allowed us to conclude that individuals 6 and 14 are in fact siblings from different years.

Apart from the neutral, highly polymorphic, and rapidly evolving microsatellite markers that can capture population structuring caused by recent population isolation events (e.g., those resulting from the dramatic Atlantic Forest deforestation and fragmentation that occurred over the last few decades [23,57]), SNPs may reflect primarily historical biogeographic events [45]. The presence of individuals from the Sooretama Biological Reserve in both genetic clusters and the observed excess of heterozygosity are consistent with a scenario in which populations of the Red-browed Amazon have been naturally fragmented in the past, accumulating significant levels of population genetic structuring. Although addressing the time of divergence and the potential phylogeographic scenarios behind these findings is beyond the scope of this work, past isolation followed by secondary contact is apparent. The past fragmentation cause is unknown, but post-decline rebound could affect genetic diversity over space and time, especially if the rebound is recent and localized, since the complex formed by the Vale Reserve and the Sooretama Biological Reserve is relatively new and surrounded by agricultural land, perhaps representing a site of rebounding for the Red-browed Amazons present in the region. For this reason, two alternative patterns of contemporary spatial genetic variability distribution are possible: (i) the current populations of the Red-browed Amazon have experienced total admixture in such a way that the genetic signatures of the past isolation no longer correspond to the geographic regions where they were originated, or (ii) the diagnosable genetic lineages are still associated with the past areas of isolation and the region of the Sooretama Biological Reserve represents a contact zone (see also [58]). *Amazona* parrots' high displacement ability, the absence of geographic barriers capable of isolating birds across the Atlantic Forest distribution, and the co-occurrence of five individuals of unknown, and likely different, origins that also had representatives in the two main genetic clusters, provide compelling evidence for widespread genetic introgression. However, the idea that the Sooretama Biological Reserve represents a contact zone warrants additional investigation involving individuals of known origin from other parts of the species' geographic range. By virtue of its size and forest integrity, Sooretama could be a genetic sink for this species and vitally important for species persistence and eventual recovery. Of note, birds from the isolated population from the Alagoas state are morphologically and genetically indistinguishable from those of Espírito Santo state, and this topic will be explored elsewhere.

Populations of the Red-browed Amazon have been extirpated throughout most of its original distribution in such a way that vast distribution gaps were artificially created between remnant populations by habitat fragmentation. The high levels of heterozygosity and low inbreeding coefficients found in the individuals from our sample suggest that genetic variability has been retained and that there is no evidence of inbreeding depression, especially in the Sooretama Biological/Vale Reserves. Therefore, individuals from these areas represent important demographic and genetic repositories for this threatened species, and these conservation units can provide a source for individuals to potentially supplement inbred populations or initiate new populations within the Red-brow's historical range.

The detection of different genetic lineages is of conservation interest because when these lineages correspond to geographic regions, they can indicate that disjoint populations could have evolved apart, or with limited genetic exchange [21,26]. Because conservation genetics is concerned with the maintenance of evolutionary processes, truly distinct lineages must be preserved as independent evolutionary entities whose genetics may represent locally coadapted gene complexes [21,26,28]. For Neotropical psittacids, divergent evolutionary units were observed, for instance, for the Blue-fronted Amazon (*Amazona aestiva*) and for the Hyacinth Macaw (*Anodorhynchus hyacinthinus*). For the Blue-fronted Amazon, two geographically divergent mtDNA lineages matched morphology and the distribution of two named subspecies, one occurring in the Brazilian states of Bahia, Minas Gerais, Tocantins, Goiás, and Distrito Federal, and another from Mato Grosso do Sul, Brazil, and Argentina [27]. Due to limited gene flow, the authors suggest that under conservation and management

perspectives, these two genetic groups should be treated as distinct evolutionary units, with the maintenance of viable populations within each one [27]. For the Hyacinth Macaw, three evolutionarily distinct management units were identified, likely reflecting local adaptations, once the structuring involved populations from Pantanal and north/northeastern Brazil. For this reason, managing them as distinct conservation genetic units was advised [28]. While the above works detected genetic structuring matching different biomes across large geographic scales, here, we observed the formation of divergent genetic clusters within the same sampling locality in such a way that the observed clusters may not be presently associated with adaptations to distinct environmental conditions. In contrast to the Blue-fronted Amazon and the Hyacinth Macaw, which are widely distributed across a variety of environments, the Red-browed Amazon is mainly restricted to the lowland Atlantic Forest, presenting a much smaller geographic distribution and higher habitat specificity [38]. Furthermore, the presence of representatives of more than two distinct genetic lineages in one of the largest remaining populations of the species suggests that outbreeding depression is less probable to occur in this species. This finding is relevant because populations of the Red-browed Amazon are continuously declining, and conservation strategies now involve captive breeding and reintroduction into the wild, as well as the rehabilitation and release of individuals of uncertain origin, derived from illegal trafficking.

Our data confirmed that the intercrossing of different genetic lineages has occurred naturally in the wild. This reduces the concern about the potential occurrence of outbreeding depression in rehabilitation and reintroduction plans, but it does not exclude the need for further studies to confirm if genetic lineages restricted to local areas could also exist. Future studies could analyze populations from different locations and compare diversity estimates and genetic structuring in light of dispersion rates between patches, which may help maintain the species' genetic diversity. In addition, the SNPs of UCEs may allow other studies to map diversity plasticity over time by comparing recent individuals with extremely valuable museum specimens, highlighting the pivotal conservation value of museum collections well beyond their traditional use in systematics and taxonomy.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15080923/s1>, Figure S1: Visual configuration of the individual genotypes under Principal Coordinate Analysis revealing two main clouds along the PC1 axis using a matrix of 1 SNP per locus. Table S1: Genetic diversity estimates for Red-browed Amazon, *Amazona rhodocorytha*: Individual Observed (Ho) and expected (He) heterozygosity, and Inbreeding Coefficients (F) using a matrix of 1 SNP per locus.

**Author Contributions:** Conceptualization, L.F.S., A.A.M. and F.B.; methodology, L.F.S., A.A.M. and F.B.; validation, all authors; formal analysis, A.A.M. and M.R.F.; investigation, L.F.S., A.A.M., M.R.F., P.R. and F.B.; resources, L.F.S.; data curation, L.F.S.; writing—original draft preparation, all authors; writing—review and editing, all authors; supervision, L.F.S.; project administration, L.F.S.; funding acquisition, L.F.S. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** UCE raw read data are available on NCBI SRR XXX-XXX (BioProject PR-JNAXXXXX). VCF files are available at <https://github.com/febocalini>, accessed on 3 February 2023.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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