

October 2026, Volume 8, Number 10

Conservation Science and Practice

Open Access

A journal of the Society for Conservation Biology

Editor-in-Chief: Mark A. Burgman

Senior Associate Editors: Carly Cook, Toni Lyn Morelli, W. Maartin Strauss, Mark Schwartz,
Brady Mattsson and Christian Kiffner



WILEY

CONTRIBUTED PAPER

Importance of using genetic data to inform conservation captive breeding: A tale of two species of threatened macaws (*Ara* spp.)

George Olah^{1,2}  | Jeff A. Johnson^{3,4}  | Jeremy S. Johnson⁵  |
Donald J. Brightsmith⁶  | Janice D. Boyd^{6†}

¹Fenner School of Environment and Society, The Australian National University, Canberra, Australia

²Wildlife Messengers, Richmond, Virginia, USA

³Department of Biological Sciences, University of North Texas, Denton, Texas, USA

⁴The Peregrine Fund, Boise, Idaho, USA

⁵Department of Forestry, Michigan State University, East Lansing, Michigan, USA

⁶Schubot Center for Avian Health, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Science, Texas A&M University, College Station, Texas, USA

Correspondence

George Olah, 141 Linnaeus Way, Fenner School of Environment & Society, The Australian National University, 2600 Canberra, Australia.
Email: george.olah@anu.edu.au

Funding information

Amigos de las Aves, USA; Schubot Center for Avian Health, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University; Loro Parque Fundación, Grant/Award Number: PP-250-2024; Australian Research Council, Grant/Award Number: DE230100085

Abstract

Captive breeding in private collections is a crucial but often undocumented component of conservation for many threatened species, which can inadvertently pose risks to genetic diversity and species integrity. We used genotyping-by-sequencing (GBS) to conduct a genetic audit of one of the largest private collections of two threatened parrot species: the Great Green Macaw (*Ara ambiguus*) and the Military Macaw (*Ara militaris*). Among 45 individuals, our analysis uncovered critical, undocumented historical complexities. We identified seven interspecific hybrids and one morphologically misidentified purebred individual. We also detected pervasive high relatedness, with mean pairwise relatedness values for the Military Macaw ($r = 0.33$) and Great Green Macaw ($r = 0.27$) approaching the theoretical expectation for half-siblings, largely driven by the discovery of highly interconnected family networks. Furthermore, we revealed cryptic substructure within the Military Macaws, tracing lineages to distinct Mexican and South American (Bolivian) origins. Together, these findings highlight the unintended consequences of historical breeding without access to genetic data, which can compromise a collection's conservation value if left unresolved. Our results demonstrate that genomic screening is an essential tool for transforming historically undocumented collections from

[†]Died October 2022.

uncharacterized genetic reservoirs into valuable conservation assets. We provide a framework for conservation practitioners to use genomic data to correct pedigrees, guide pairing decisions to minimize inbreeding, and prevent hybridization. We argue that such genomic oversight should be standard policy and practice across all ex situ conservation programs to ensure meaningful contributions to global species recovery efforts.

KEYWORDS

aviculture, captive breeding, conservation genetics, conservation genomics, ex situ conservation, genetic management, hybridization, inbreeding, kinship, parrots

1 | INTRODUCTION

Parrots (Psittaciformes) are enigmatic and highly diverse birds but possess several traits associated with higher extinction risk (Olah et al., 2016). Nearly a third of the ca. 400 parrot species are facing extinction (BirdLife International, 2025) with 56% of all parrot species experiencing declining population trends in the wild (Berkunsky et al., 2017; McClure & Rolek, 2020; Olah et al., 2016). Compared to other bird groups, parrots represent a disproportionately large share of threatened evolutionary history. Although parrots comprise only about 4% of all bird species, they account for roughly 8% of designated Evolutionarily Distinct and Globally Endangered (EDGE) species—defined as those possessing above-median Evolutionary Distinctiveness alongside a threatened status on the IUCN Red List (McClure et al., 2023). In the escalating global crisis of parrot conservation, Collier and Butchart (2014) advocated for the captive breeding of 58 parrot species for conservation purposes as insurance policies against extinction or serious wild population declines. They recommended including efforts by non-institutional breeders (private aviculturists) because insufficient resources likely exist at zoos and game parks to address the large numbers of parrot species needing conservation action.

The importance of having ex situ insurance populations of threatened parrot species has become readily apparent over the past few decades, and currently 49 parrot species require ex situ conservation management action (IUCN, 2025). The Spix's Macaw (*Cyanopsitta spixii*) was officially classified in 2019 as Extinct in the Wild (BirdLife International, 2019) and is known to suffer from low genetic variability and high relatedness resulting from a severe historical population bottleneck (Caparroz et al., 2001). Despite this, it was saved from extinction by several private aviculturists, and their captive birds have now become part of an international effort to re-establish protected populations in their previous

range in Brazil (Purchase et al., 2024). Similarly, captive populations of the Orange-bellied Parrot (*Neophema chrysogaster*) and Puerto Rican Amazon (*Amazona vittata*)—both Critically Endangered species—are used to supplement their respective wild populations to avoid extinction (Faust et al., 2025; Stojanovic & Heinsohn, 2023). On the island of Hispaniola, only two captive individuals of the Critically Endangered Imperial Amazon (*Amazona imperialis*) are known to exist after the wild population was reduced from between 250 to 350 to around 10 individuals following hurricane Maria in 2017 (Palmer et al., 2018). Furthermore, species at high risk from human-exacerbated natural hazards (particularly tropical island endemics) could significantly benefit from ex situ conservation measures (Gonçalves et al., 2024).

Although many zoological parks and related public facilities (“zoos”) take seriously their responsibility of helping to preserve world biodiversity (i.e., the “ark” concept; Soulé et al., 1986), the magnitude of the problem outstrips their capability to respond. Limited space, specialized expertise requirements, and financial pressures to display charismatic megafauna constrain the number and types of species that zoos can maintain (Frynta et al., 2010; Moss & Esson, 2010; Ward et al., 1998). Consequently, if private collections are to fill this gap and meaningfully contribute to species conservation, a rigorous, science-based management approach is essential (Putnam et al., 2023). Specifically, incorporating genetic data guides management efforts to successfully maintain genomic diversity and minimize inbreeding (Witzenberger & Hochkirch, 2011). Historically, the adoption of genetic methods by psittacine breeders in the United States has been limited, largely due to the prohibitive expense of testing, a lack of accessible commercial tools, and the fact that raw genomic data require expert interpretation.

Fortunately, DNA extraction and sequencing costs are declining rapidly, and generating genetic data for parrot breeding collections is now conceivably within the budget of many individual aviculturists or members of

breeding consortiums. Next-generation sequencing techniques, such as genotyping by sequencing (GBS), allow the massively parallel identification of hundreds of thousands of single nucleotide polymorphisms (SNPs) from a small volume of avian blood (Olah et al., 2021). These tools, already widely applied in agricultural breeding and human medicine, offer unprecedented precision for animal management. One of the recommended ways of maintaining captive population diversity, minimizing inbreeding, and minimizing adaptation to captivity is to have the captive population fragmented into multiple semi-isolated populations that periodically exchange individuals with each other (Frankham et al., 2010). In this way, private aviculturists could contribute to the maintenance of smaller captive parrot populations through collaboration with zoos and other facilities breeding parrots for conservation-related activities.

Our focal taxa for this study, the Great Green Macaw (*Ara ambiguus* Bechstein, 1811) and the Military Macaw (*Ara militaris* Linnaeus, 1766), perfectly exemplify this conservation challenge. Both species face severe threats in the wild from habitat destruction and extensive poaching for the domestic and international illegal pet trade (BirdLife International, 2025). Consequently, the Great Green Macaw is currently listed as Critically Endangered by the IUCN, with global wild populations estimated at fewer than 1000 mature individuals (BirdLife International, 2025). Similarly, the Military Macaw is classified as Vulnerable, with an estimated declining population of 2000–7000 mature individuals (BirdLife International, 2025). In the United States, captive populations of these threatened macaws are largely divided between accredited zoological institutions and private aviculture. Within the Association of Zoos and Aquariums (AZA), captive management is coordinated through formal Species Survival Plans (SSPs), which rely on historical pedigree data in the Species360 database (ZIMS) to calculate mean kinship and make genetically informed breeding recommendations (Conde et al., 2019). An accurate pedigree is necessary to not only minimize mean kinship and avoid inbreeding among captive individuals but also maintain equal representation of founder lineages to avoid loss of genomic diversity (Galla et al., 2022).

Currently, ZIMS global records indicate that 202 Great Green (male = 87, female = 96, unknown = 19) and 791 Military Macaws (male = 293, female = 321, unknown = 177) are housed in 63 and 190 accredited institutions, respectively (Species360, 2026). However, SSP populations are often demographically constrained by limited holding space (Lees & Wilcken, 2009). As a result, a vastly larger, undocumented population exists within the private sector. While there is no centralized

demographic registry, an online survey in 2012 provided a basic nationwide headcount, estimating that at least 138 Great Green and 435 Military Macaws were kept privately in the USA (S. Clubb, pers. comm.), stemming from a largely unknown founder base (Boyd, 2015). Operating outside the SSP framework, private collections typically lack the multi-generational pedigree data required to minimize mean kinship and maintain demographic stability. Consequently, molecular tools are essential to evaluate their genetic health and determine their potential value for broader conservation efforts. The necessity of these tools is particularly urgent in the United States, where the 2015 listing of several macaw species under the Endangered Species Act (ESA) prohibited interstate commerce, effectively disincentivizing breeding, stranding private flocks and preventing nationwide genetic exchange.

The Great Green and Military Macaws provide an ideal model system for evaluating the genetic risks and conservation opportunities associated with private aviculture. Their close phylogenetic relationship, phenotypic similarity, and historical treatment as conspecifics make them particularly susceptible to unintentional hybridization, a primary threat to ex situ conservation integrity (Frankham et al., 2010). Despite the substantial number of both macaw species maintained in the private sector, their true genetic viability, pedigree history, and taxonomic integrity remain largely unknown. As a result, even well-intentioned breeding efforts face unavoidable challenges associated with potential cryptic hybridization, pervasive inbreeding, and the artificial mixing of distinct geographic lineages, all of which must be resolved to maximize their conservation value.

Here we use nuclear and mitochondrial genetic data generated from double digest restriction-site DNA sequencing (ddRAD-seq; Peterson et al., 2012) and targeted DNA sequencing, respectively, to evaluate one of the largest private breeding collections of these two macaw species in the United States, assembled before they were added to the ESA. This collection offers an ideal proof-of-concept, as its size captures many of the genetic complexities that characterize private aviculture, including undocumented relatedness, cryptic population structure, and uncertain founder origins. Specifically, we assess whether genomic screening can (1) detect hidden interspecific introgression, (2) quantify cryptic relatedness to reconstruct missing pedigrees, and (3) resolve geographic substructure using mitochondrial ND2 variation (Eberhard et al., 2015). By addressing these challenges, our study demonstrates how genomic tools can convert undocumented, private ex situ populations from uncharacterized genetic reservoirs into rigorously evaluated, actively managed assets for species recovery.

2 | MATERIALS AND METHODS

2.1 | Study species

The Great Green Macaw (“GG” hereafter) is a Neotropical parrot species—also called the Buffon’s Macaw in aviculture (Müller, 2000)—of average generation length of 11.2 years (BirdLife International, 2025). Like most large macaws, they are long-lived (maximum lifespan of 34 years in captivity; Young et al., 2012), socially monogamous, and typically lay clutches of 2–3 eggs per breeding season (Forshaw, 2006). It typically inhabits lowland tropical forests, and has two recognized subspecies (*Ara ambiguus* and *A. a. guayaquilensis*) with a patchy distribution ranging from Honduras and Nicaragua into Colombia and Ecuador (Boyd, 2015; Figure 1; Juniper & Parr, 1998). The Military Macaw (“MM” hereafter) is closely related to GG but is found in dryer deciduous forests (but see Fjeldså et al., 1987) and generally in more montane areas. It has a longer generation length of 15.2 years (BirdLife International, 2025), a longer lifespan in captivity (54 years maximum; Young et al., 2012), and monogamous reproductive strategy with 2–3 eggs per clutch (Forshaw, 2006). Its distribution is larger than GG, but much more disjunct, ranging from Mexico to northern Argentina (Figure 1), largely corresponding with three recognized subspecies (*Ara militaris*, *mexicanus*, and *bolivianus*; Abramson et al., 1995). Both macaw species are threatened with extinction, with GG identified as Critically Endangered and MM as Vulnerable according to the IUCN Red List (BirdLife International, 2025). Both are listed as CITES Appendix I species (CITES, 2024), a designation that prohibits the international commercial trade of wild specimens. Both species were added to the US ESA as “Endangered” in 2015, a federal designation that strictly regulates and effectively bans their commercial movement across state lines within the United States.

The GG and MM are similar in appearance (see Figure 1) and have previously been considered conspecific (Fjeldså et al., 1987). However, mitochondrial sequence data clearly distinguish them as separate evolutionary lineages (Eberhard et al., 2015), with fossil calibrated phylogenomic analyses estimating their divergence at approximately 0.7–2.7 million years ago (Smith et al., 2024). Subtle but consistent differences include smaller body size in MM relative to GG, a completely black bill and darker plumage in MM, and different vocalization (Eberhard et al., 2015; Müller, 2000). In addition, the iris of GG adults is composed of two concentric colored rings (an inner grayish circle embedded in a generally golden outer circle), whereas the iris of MM is uniformly golden. Despite these differences, the two species can interbreed and produce fertile offsprings with intermediate phenotypes (J. Boyd, personal experience).

GG is rare in American aviculture, while MM is more common. The total number of GG founders in the United States has been estimated at 26–49, most of them from Nicaragua (Boyd, 2015). A considerably larger number of MM founders were imported from Mexico and Bolivia (R. Jordan, pers. comm.) but no search of government records has been done to quantify the numbers of legally imported birds. An online survey conducted in 2012 identified 138 GG and 435 MM individuals kept as pets in the USA among 933 responses (S. Clubb, DVM, pers. comm.). For comparison, 648 Hyacinth Macaws (*Anodorhynchus hyacinthinus*) and 719 Scarlet Macaws (*Ara macao*) were also reported in the same survey.

2.2 | Study population

The captive population evaluated in this study was purposefully assembled at the Buffon’s Macaw Conservation Center (Hill Country Aviaries, Texas, USA) between 2014 and 2015. This targeted consolidation effort was initiated in direct response to the impending ESA listing (finalized in October 2015). Recognizing that the new “Endangered” designation would subsequently ban interstate commerce and restrict the movement of these birds, the founding objective was to secure and centralize as many individuals as possible prior to the regulatory deadline. Historical records from the breeding facility indicate that the founder flock was not acquired individually but rather purchased in distinct regional cohorts from dispersed private aviculturists across the United States (e.g., California, Texas, Florida).

Because of this rapid, multi-state consolidation effort, comprehensive individual-level demographic records (including exact ages, individual reproductive histories, and definitive origins) for the specific private collection analyzed in this study were mostly unavailable. It is unknown what proportion of the study population represents original wild-caught founders versus captive-bred descendants. However, following the implementation of the 1992 US Wild Bird Conservation Act, any legally imported wild-caught individuals would have entered the country over three decades ago. This lack of basic demographic and pedigree information is characteristic of many private collections and historically maintained zoo populations (Galla et al., 2022).

2.3 | Sample collection and DNA extraction

Blood samples were obtained from 30 GG, 15 MM, and one Grey Parrot (*Psittacus erithacus*) as an outgroup,

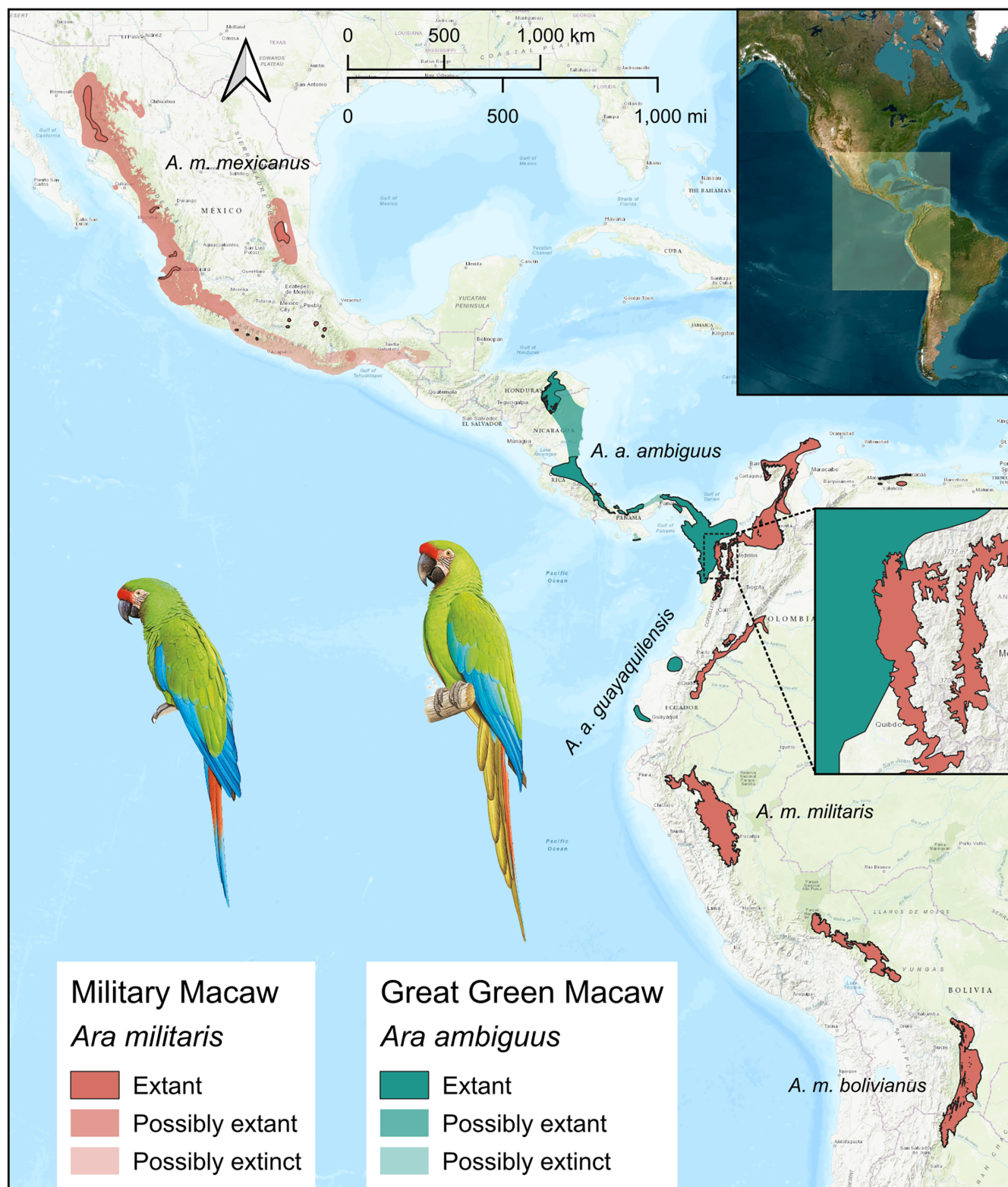


FIGURE 1 Map of distribution of the two study species, the Great Green Macaw *Ara ambiguus* (green) and the Military Macaw *Ara militaris* (red), at subspecies level. Illustrations [by Lluís Sanz] are courtesy of Cornell Lab of Ornithology, from Birds of the World (<https://doi.org/10.2173/bow>), and scaled relative to each other. Distribution polygons are sourced from BirdLife International and HBW (2022). Map was created using QGIS v3.3 with basemaps of ESRI World Topo (sources: Esri, DeLorme, HERE, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, and the GIS User Community) and ESRI Satellite (sources: Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community).

representing the entire captive GG and MM population at the facility. Blood collection was performed under the supervision of a certified avian veterinarian, following procedures approved by the Animal Welfare Office of the Texas A&M University (Animal Use Permit No. IACUC 2021-0241 CA). To minimize handling time, birds were physically restrained without sedation or anesthesia. The mean body mass of GG is 1300 g and of MM is 1134 g (Dunning, 2008). For each bird, approximately 1 mL of whole blood was drawn with jugular venipuncture, a volume that easily satisfies both the traditional field metric of 1% of body mass and the more conservative threshold of less than 10% of total blood volume of avian species (Fair et al., 2023). The blood was stored in lithium-heparin microtainers at 4°C until DNA extraction. DNA was extracted from the blood samples within 7 days using the MasterPure Complete DNA and RNA Purification Kit (Epicentre, now Lucigen).

2.4 | DNA sequencing and genome-wide marker determination

Genomic sequencing was conducted by the Texas A&M Agrilife Genomics and Bioinformatics Services (<https://www.txgen.tamu.edu>). Following Peterson et al. (2012), ddRAD-seq was employed with minor modifications. Briefly, the DNA was cut with the enzyme pair SphI and MluCI and ligated to compatible barcoded adaptors. Samples were pooled, precipitated, and subjected to Pippin DNA size-selection (Sage BioSciences, Boston, MA) targeting inserts between 260 and 400 base pairs (bp). Following low-cycle polymerase chain reaction (PCR), final libraries were purified, quantified, and sequenced in a single lane on a HiSeq 2500 (Illumina, San Diego, CA) running the 125 PE protocol with version 4 chemistry.

During bioinformatics analyses, we checked sequence read quality, de-multiplexed samples, trimmed reads, aligned reads de novo into contigs, and identified SNP variants using the dDocent pipeline (Puritz et al., 2014). Freebayes (Garrison & Marth, 2012) was used to produce the initial set of SNP sites using default settings. We used the “vcfR” package in R (Knaus & Grünwald, 2017) for filtering and removed monomorphic sites while retaining those SNPs that were biallelic, without missing data, that had a minimum quality score of 30, an average depth between 5 and 100, and a minimum minor allele frequency of 5%.

2.5 | Mitochondrial DNA sequencing

Mitochondrial DNA sequencing was conducted at the University of North Texas. Briefly, we amplified all

samples for the NADH dehydrogenase subunit 2 (ND2) gene (1041 bp) using PCR with primers L5216 and H6313 (Sorenson et al., 1999). We then sequenced the amplified products in both directions using BigDye Terminator v.3.1 on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Finally, we aligned and verified the sequences to possess no indels or premature stop codons using Sequencher (v.4.10.1; GeneCodes Corp., Ann Arbor, MI).

We compared the generated sequences from the captive birds with those collected from the wild with known geographic origin (Eberhard et al., 2015). Previously, Eberhard et al. (2015) identified reciprocal monophyly for GG and MM based on ND2, including geographic differentiation based on maximum nodal support values within MM separating individuals sampled in Eastern and Western Mexico from Bolivia. GG sampled in Ecuador and Costa Rica were polyphyletic (Eberhard et al., 2015).

2.6 | Population genetic structure analysis

We used three approaches to investigate population genetic structure. First, we employed standard principal component analysis and second, a discriminant analysis of principal components (DAPC) (Jombart et al., 2010), both with the R package “adeigenet” v. 2.0.1 (Jombart, 2008; Jombart & Ahmed, 2011). Third, we used sparse non-negative matrix factorization (SNMF) algorithms implemented in the “LEA” package in R (Frichot & François, 2015). The “snmf” function computes ancestries of each individual in chains of given clusters ($K = 1-10$ with 100 repetitions each). It estimates an entropy criterion that evaluates the quality of fit of the statistical model to the original data using a cross-validation technique where we blinded 10% of the data. Low cross-entropy values reflect the number of most likely ancestral populations that best explains the genotypic data. We kept the alpha parameter at the default setting.

2.7 | Phylogenetic relationships

Using the genome-wide SNP dataset including the Grey Parrot sample as an outgroup, we calculated the Nei's distance in 1000 permutations with the R package “poppr” (Kamvar et al., 2014) and constructed a neighbor joining tree with 1000 bootstrap permutations using the R package “ape” (Paradis & Schliep, 2019). We plotted the phylogenetic tree with bootstrap values and rooted with the Grey Parrot sample with the R package “ggtree” (Yu et al., 2017). We also generated a minimum spanning

network with “poppr” (Kamvar et al., 2014) based on Prevosti’s pairwise genetic distance (Prevosti et al., 1975) to visualize individual sample dissimilarity.

Similar to Eberhard et al. (2015), we constructed a 50% majority consensus tree from Bayesian inference using Markov chain Monte Carlo (MCMC) sampling approach to reconstruct the ND2 gene tree phylogeny with MrBayes v. 3.2.5 (Ronquist et al., 2012; Ronquist & Huelsenbeck, 2003). The GTR + I model of sequence evolution with no partition for codon positions was used for the ND2 dataset based on results from jModelTest (Darriba et al., 2012). The Bayesian analysis included two parallel runs with four chains, one cold and three heated, for 100 million generations with sampling every 1000 generations and a burn-in of 25%. The log-likelihood values and posterior probabilities were checked to ascertain that the chains had reached the posterior distribution. We also ensured that the potential scale reduction factor approached 1.0 for all parameters and the average standard deviation of split frequencies converged toward zero. We used Tracer v.1.6.0 (Drummond & Rambaut, 2007) to ensure that our sampling of the posterior distribution had reached a sufficient effective sample size (ESS >200).

2.8 | Genetic diversity

We calculated general genetic diversity measures in GenAlEx v. 6.5 (Peakall & Smouse, 2006, 2012), including the percent of polymorphic loci, observed (H_O) and expected heterozygosity (H_E), along with their standard errors (SE) for each cohort. We also computed pairwise F_{ST} values between the two species and significance levels based on 999 random permutations with G-statistics.

We calculated individual inbreeding coefficients (F_{ind}) from the mean probability of identity-by-state across loci with the “rrBLUP” package in R (Endelman, 2011), and plotted the results by each cohort. For each cohort, we calculated mean F with their SE. Conceptually, this F_{ind} coefficient measures the correlation of alleles within an individual relative to the allele frequencies of the entire study population. Unlike pedigree-based inbreeding, this genomic metric quantifies the actual proportion of homozygous loci in an individual compared to the proportion expected under random mating.

2.9 | Kinship

Since the birds in this study were from multiple independent breeders without prior information concerning geographic origin, date of birth, and familial relationships,

we used genetics-based methods for assessing pairwise relatedness among all samples. Our primary method for pedigree reconstruction was the “Sequoia” package in R (Huisman, 2017), which uses a global likelihood framework to assign relationships without relying on pre-defined relatedness value cutoffs.

To distinguish between relationship types with similar expected relatedness values, such as parent-offspring (PO) and full-siblings (FS), Sequoia evaluates the likelihood of each potential relationship across all individuals simultaneously. A key advantage of this method is its ability to use Mendelian inheritance patterns to differentiate pairs (Huisman, 2017). For example, the presence of opposing homozygotes at even one locus makes a PO relationship highly unlikely, while it is permissible for FS pairs. Another pedigree was inferred based on log-likelihood ratios, where relationships are assigned only when there is high statistical confidence. The function “GetMaybeRel” was subsequently used to identify plausible relationships (e.g., further siblings) conditional on the high-confidence pedigree.

As a complementary, quantitative analysis, we estimated pairwise relatedness using the R package “related” (Pew et al., 2015). We first compared seven estimators using the package’s internal simulation function and selected the TrioML estimator (Wang, 2007) as it showed high correlation with true simulated relatedness values (Data S1). The TrioML values were therefore used to quantify and visualize the continuous distribution of relatedness for all pairs, corroborating the discrete relationships assigned by Sequoia. We visualized pairwise TrioML relatedness values among all samples with the “heatmap” function in R, and integrated with the Sequoia results using the R package “corrplot” (Wei & Simko, 2021). We calculated the average relatedness of individuals within each cohort.

3 | RESULTS

The reduced representation sampling resulted in a total of 207,767,356 unique sequence reads in 46 samples. Freebayes produced an initial set of 31,357 SNP sites, and after filtering, we retained 4181 biallelic SNPs among all samples including the outgroup, the Grey Parrot. After excluding the outgroup sample, a total of 4143 SNPs were retained for subsequent analysis.

3.1 | Population genetic structure

With the Grey Parrot sample included in the analysis with GG and MM, the cluster analysis showed three

unambiguous groupings (Figure S1A,B) largely corresponding to the three species on the DAPC plot (Figure S2A). One GG individual (#41) clustered unambiguously with the other MM samples with and without the Grey Parrot sample included in the analysis (Figure S2A,B). After this sample was reclassified as MM, the cluster analysis identified three groups (Figure S1C,D), one with seven individuals as possible hybrids (see below) between GG and MM (Figure 2a). Based on this same dataset, the admixture SNMF results suggested that $K = 2-4$ had the lowest cross-entropy values (Figure S3), indicating $K = 3$ with the highest support. Both species were grouped accordingly in the analyses (Figure S3), with seven individuals originally classified as GG (#2, 3, 4, 6, 7, 8, 9) identified as admixed individuals, which are likely GG and MM hybrids (Figure 2b). This was also confirmed based on the mtDNA ND2 analysis (see below).

Excluding these hybrid individuals (“HH” hereafter) revealed a consistent optimal clustering of three distinct genetic groups across all three methods (Figures S1E–F, S2C, S4). The GG remained a single, cohesive genetic cluster. In contrast, the MM clearly partitioned into two distinct groups. One of these clusters corresponds to samples putatively assigned to the Bolivian subspecies (Figure S2C), a division that aligns with the geographic differentiation as determined using ND2 sequence data (Figure 3; see details below).

Genetic analysis separately on the two groups—GG ($n = 23$) and MM ($n = 15$)—revealed further patterns of population structure. The MM group exhibited clear genetic subdivision into two distinct clusters (optimal $K = 2$). This analysis separated individuals #12, 13, 14, 15, and 21 from the other MM samples (Figure S5). This genetic differentiation within the MM group was robust, as it was supported by both additional nuclear DNA markers (Figures S7–8) and mitochondrial DNA (ND2) analysis (Figure 3).

In stark contrast, the individuals of the GG group largely showed a homogeneous genetic background (indicated by the lowest cross-entropy at $K = 1$ in SNMF analysis). However, there was a subtle hint of structuring, as individuals #33, 38, and 42 clustered together in ancestry plots (Figure S6). However, inferences about geographic origin could not be confidently drawn. The mitochondrial DNA (ND2) data for the GG birds lacked sufficient resolution (due to low posterior probability estimates for the ND2 clade) to determine the potential ancestral wild locations of these captive individuals.

3.2 | Phylogenetic relationships

The phylogenetic analyses using the generated SNPs generally agreed with results of the population genetics

structure analyses. Both the neighbor joining tree using Nei's genetic distance (Figure S7) and the minimum spanning network using Prevosti's genetic distance (Figure S8) clearly differentiated the two species, with the misidentified GG sample (#41) clustering with other MM samples and identified the same set of previously identified hybrids. The maximum Prevosti's genetic distance between all study individuals was relatively small (10%, Figure S8). Notably, within this network, full-sibling groups within the MM cohort clustered tightly, reflecting the higher background relatedness of that group (mean $r = 0.33$; see below) and likely lower parental heterozygosity. In contrast, some GG full-sibling sets displayed greater dispersion, a pattern expected from more heterozygous parents.

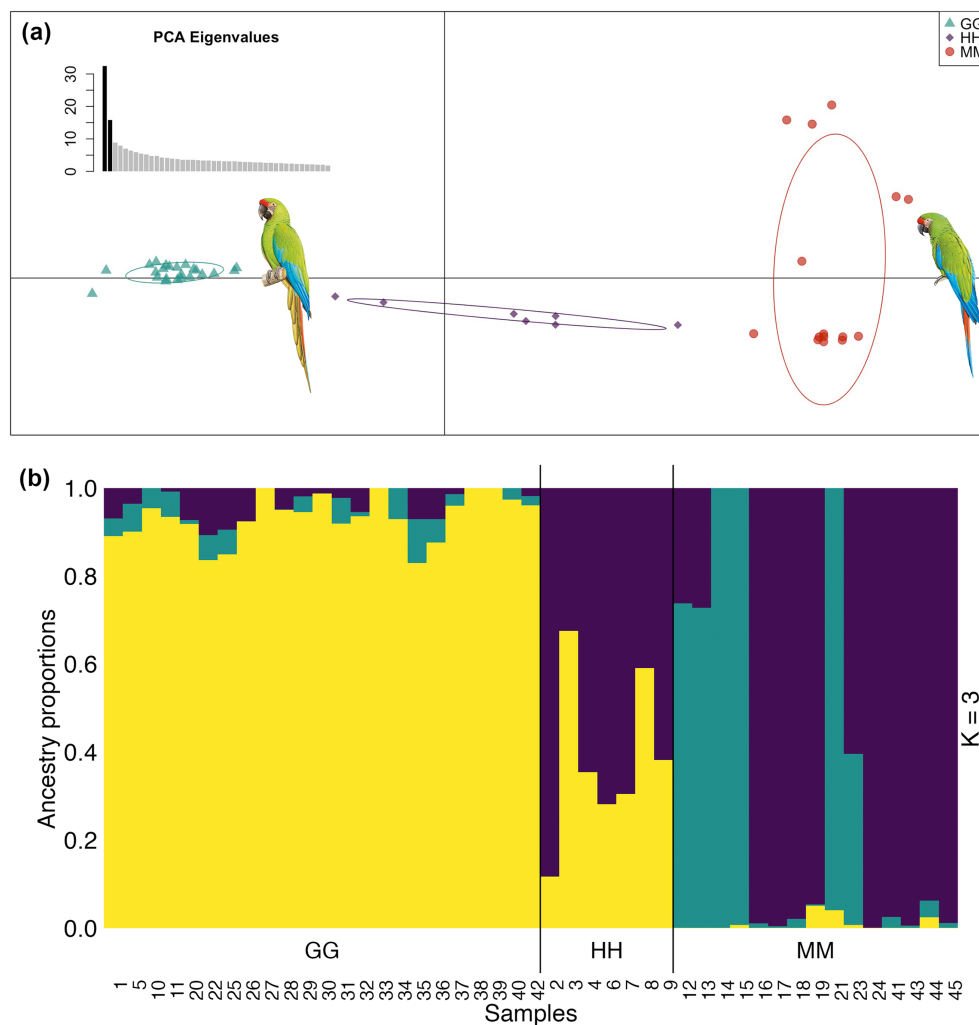
We were also able to assign some of the captive birds to broad geographic regions, based on the Bayesian ND2 tree; however, several samples clustered into clades lacking reference sequences of known origin (Figure 3). Within the GG cohort, two subgroupings were observed, but because the published reference sequences from Costa Rica and Ecuador exhibited no genetic differentiation at this locus, it was not possible to assign the captive GG individuals to a specific geographic origin (Figure 3). Within the MM cohort, five subgroupings were observed, corresponding broadly to Central and South American lineages. While some geographic differentiation was apparent, precise spatial assignment was precluded by the absence of reference sequences from intermediate South American ranges, like Peru, Colombia, Ecuador, and Venezuela (Figure 3).

3.3 | Genetic diversity

Of the total 4143 biallelic SNPs, 0.7% of the variants were fixed for all individuals of either one of the two ingroup species, 40.8% shared between both species, and 35.9% were private or not shared with the other species. The GG cohort contained the highest percentage of polymorphic SNPs (91%), followed by MM (88%), and the hybrids (82%). The observed heterozygosity estimates of GG and MM were comparable after excluding admixed samples, while the potential hybrid samples showed higher values (Table 1). The fixation index averaged over all loci was negative in all three cohorts (Table 1). Pairwise F_{ST} values showed moderate genetic differentiation between GG and MM ($F_{ST} = 0.055$, $p = .001$) which further supported separate species designation. As expected, the pairwise F_{ST} values between the potential hybrid cohort and GG ($F_{ST} = 0.035$, $p = .001$) and MM ($F_{ST} = 0.033$, $p = .002$) were lower.

The median of individual inbreeding coefficients within each cohort was lowest among the hybrids,

FIGURE 2 Population genetic structure analyses using 4143 SNPs from Great Green Macaws (*Ara ambiguus*; GG, $n = 23$), Military Macaws (*Ara militaris*; MM, $n = 15$), and identified hybrids (HH, $n = 7$). (a) Grouping of samples by discriminant analysis of principal components (DAPC) that identified three clusters by lowest BIC. Illustrations are courtesy of Cornell Lab of Ornithology, from Birds of the World (<https://doi.org/10.2173/bow>). (b) Sparse non-negative matrix factorization (SNMF) that also identified three cohorts by cross-entropy. Individual ancestry coefficients are shown for $K = 3$ clusters.



followed by the GG and MM cohorts (Figure 4). We identified some outliers from the expected variations (the bird #14 of MM, and #38, 42, 33, 39, and 27 of GG). One GG individual (#38) had a positive F value ($F_{\text{ind}} = 0.037$), representing an inbred individual.

3.4 | Kinship

Pairwise comparisons from the primary pedigree reconstruction using Sequoia resulted in 15 full sibling pairs in three cohorts (Figure S9A). These included distinct, highly connected family networks, such as one MM group of three full siblings (#14, 15, 21), another MM group of four full siblings (#18, 24, 44, 45), and one GG group of four full siblings (#20, 28, 29, 31). To further explore genetic connections not resolved in this high-confidence pedigree, we utilized the “GetMaybeRel” function, which identified a substantial number of additional dyads (Figure S9B) exhibiting likelihood ratios suggestive of close kinship, many at a level comparable to full siblings.

An analysis of the maternally inherited ND2 gene revealed subtle mitochondrial variations within some of these SNP-defined sibling networks. The following cohorts of birds showed identical ND2 sequences: (1) #2, 7, 12, 41; (2) #3, 4, 6, 8, 9, 13, 18, 45; (3) #16, 17, 24, 44; (4) #14, 15, 21; (5) #5, 22, 25–28, 30–40, 42; (6) #10, 11, 20 (Figure 3).

Estimates using molecular relatedness supported the previous grouping of individuals (Figure S10). The mean TrioML pairwise relatedness values were highest among hybrid (0.35), MM (0.33), and GG individuals (0.27), and lowest within dyads containing MM versus HH (0.24), GG versus HH (0.19), and GG versus MM (0.12) comparisons as expected (Figure S11).

4 | DISCUSSION

Our genomic screening of a large ($n = 45$), private collection of Great Green and Military Macaws demonstrates how modern genomic techniques can dissect complex captive histories to reveal critical, often hidden, conservation

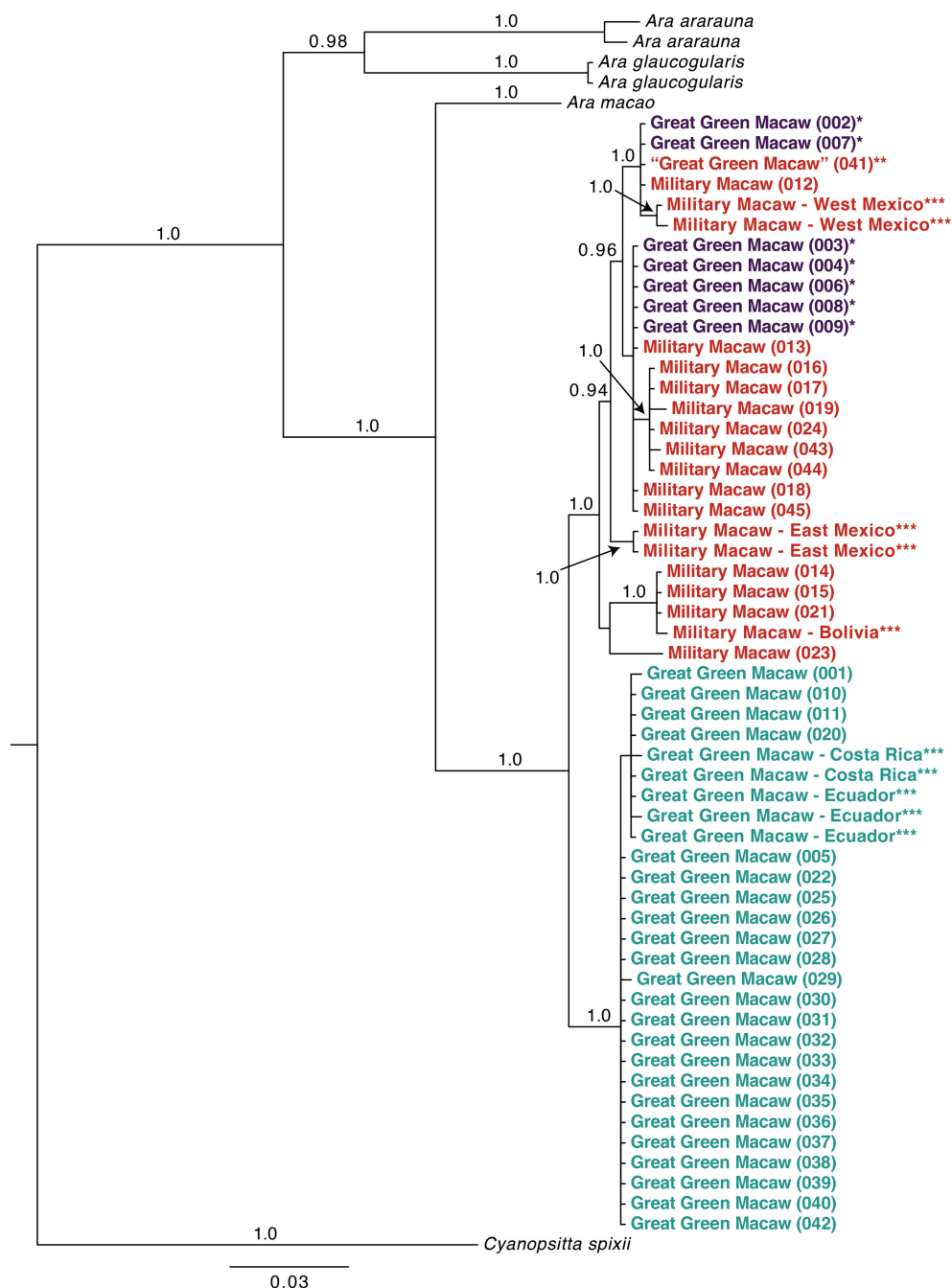


FIGURE 3 50% majority consensus tree resulting from the Bayesian analysis of mtDNA ND2 for sampled Great Green Macaws (*Ara ambiguus*; green), Military Macaws (*Ara militaris*; red), and potential hybrids (dark purple). Sample IDs are provided in parentheses, and nodal support values >0.90 are shown. The tree is rooted using sequences of Scarlet Macaw (*Ara macao*; GenBank accession number: EU327601), Blue-and-Yellow Macaw (*Ara ararauna*; HQ629720 & KF017463), Blue-throated Macaw (*Ara glaucogularis*; HQ270481 & KF017464), and Spix's Macaw (*Cyanopsitta spixii*; EU327614). * potential hybrids; ** misidentified Military Macaw in quotation marks; *** samples with known geographic origin from Eberhard et al. (2015).

challenges (Frankham et al., 2010). We found evidence of hybridization, a cryptic population structure, and pervasive relatedness, all of which compromise the collection's conservation potential if left unmanaged. These findings provide a clear case study with broad applicability, highlighting the urgent need to integrate genomic protocols into the management of ex situ populations, particularly those outside of coordinated international programs.

Nearly half of all parrot species have been reported to hybridize in captivity (McCarthy, 2006) but the challenges identified here are not unique to psittacines.

Cryptic hybridization and undocumented admixture are pervasive issues across diverse threatened taxa represented in the private sector. For example, historical breeding in private aviculture has produced cryptic hybrids among threatened *Lophura* pheasants (Barilani et al., 2007). Similarly, the Asian turtle pet trade frequently produces complex, multi-species hybrids that confound conservation efforts (Stuart & Parham, 2007), while large numbers of captive tigers in private facilities are of unknown or admixed ancestry (Luo et al., 2008). In many of these groups, private collections often house more individuals than accredited zoos, representing a

TABLE 1 Population genetics estimates of two macaw species and their hybrids in captivity.

Cohort	<i>n</i>	Polymorphic loci	H_O	H_E	F
Great Green Macaw (GG)	23	91%	0.450 ± 0.005	0.310 ± 0.003	-0.412 ± 0.032
Military Macaw (MM)	15	88%	0.482 ± 0.005	0.319 ± 0.003	-0.378 ± 0.021
Hybrids (HH)	7	82%	0.511 ± 0.006	0.318 ± 0.003	-0.505 ± 0.017

Note: Shown are the number of samples (*n*), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F) with their standard errors ($\pm$ SE). F were calculated as means of the F_{ind} values from Figure 4.

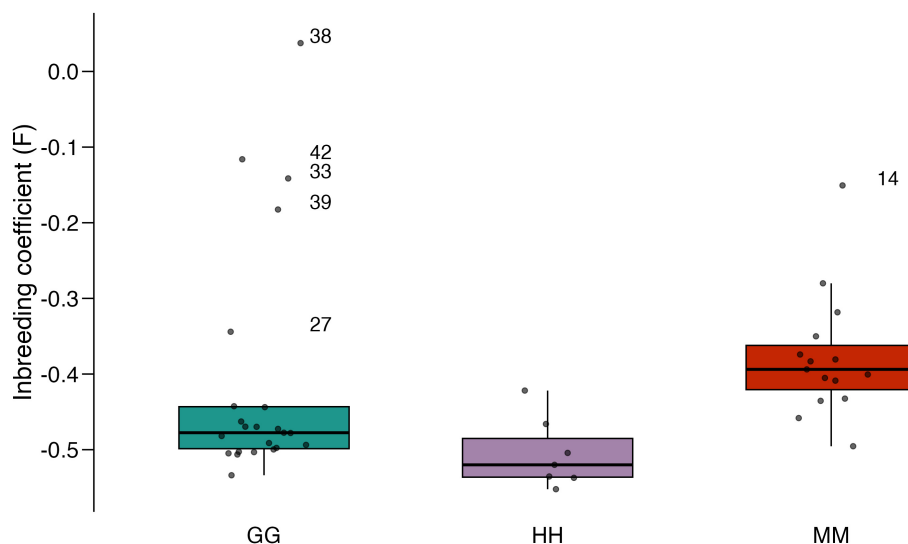


FIGURE 4 Individual inbreeding coefficients (F_{ind}) of Great Green Macaws (*Ara ambiguus*; GG, $n = 23$), Military Macaws (*Ara militaris*; MM, $n = 15$), and identified hybrids (HH, $n = 7$). The F_{ind} coefficient is a genomic estimate of inbreeding based on SNP data, where higher values indicate higher-than-expected homozygosity (inbreeding) and lower values indicate excess heterozygosity (outbreeding) relative to the study population as a whole. Each box represents the interquartile range, containing the middle 50% of F values for each cohort. The horizontal lines mark the median and the whiskers indicate the expected range of variation. Points outside the whiskers are labeled with the individual IDs and considered outliers.

large but genetically ambiguous conservation resource. The genomic auditing framework demonstrated here for macaws provides a blueprint for evaluating and integrating privately held populations across a wide range of endangered taxa.

4.1 | Resolving genetic uncertainties and cryptic histories

A primary challenge in managing captive populations with incomplete documentation is verifying species integrity and ancestry. Historically, many founders in the examined collection were acquired sight-unseen based on seller assurances, a common practice that inadvertently masked the true taxonomic identity of the birds. Because Great Green and Military Macaws share a similar overall appearance, phenotypic identification *ex situ* remains

highly subjective. For example, individual #41 (Figure 2b), originally recorded as a Great Green Macaw, was genomically revealed to be a pure Military Macaw. Similarly, the cryptic hybrids we detected further confounded breeders by exhibiting intermediate phenotypes. Morphological re-evaluation suggests that subtle cues (e.g., specifically intermediate physical size, variations in the overall sheen and shade of the green wing coverts, and differences in central tail feather coloration) may hint at admixture. However, these external characters are subtle, underscoring the necessity of genomic validation to prevent unintentional hybridization in captivity (Allendorf et al., 2001; Müller, 2000).

Interestingly, all seven identified hybrids possessed Military Macaw mitochondrial ND2 haplotypes (Figure 3), indicating that the maternal parent in every crossing event was a Military Macaw. Rather than reflecting an innate biological mating preference, this

unidirectional introgression is an artifact of the collection's history. Our analysis revealed that many of these seven hybrid individuals might be the product of a single, highly prolific mixed pair (a Great Green Macaw male and a Military Macaw female) maintained by a previous breeder. The successful detection of such intricate hybrid origins and kinship patterns demonstrates the critical need for robust genetic analyses to detect and manage potential hybridization risks posed by captive-bred or naturalized parrots to the genetic integrity of wild populations (Kiacz, 2023; Kiacz & Brightsmith, 2021).

Our analysis revealed a distinct genetic substructure within the Military Macaw cohort, with admixture models supporting two groups (Figures 2, S4, S5). By comparing mitochondrial DNA with reference sequences (Figure 3), we traced these lineages to separate geographic origins in Mexico and South America (likely Bolivia), consistent with avicultural knowledge and recent phylogeographic studies (Rivera-Ortiz et al., 2023). Given the highly fragmented natural distribution of this species across Central and South America, it is highly likely that incorporating reference samples from intermediate geographic ranges would reveal additional, geographically distinct clades, providing an even more granular map for tracing founder origins. Nonetheless, the presence of individuals with mixed ancestry between these groups (e.g., #12, 13, and 23 in Figure S5) highlights the long-term complexity of managing captive stocks where subspecies have been inadvertently mixed for generations. For conservation purposes, these distinct lineages may represent separate management units, and failing to account for this structure risks outbreeding depression or the loss of local adaptations. Future efforts to establish comprehensive genomic reference databases will be pivotal for the precise assignment of unknown individuals (Olah et al., 2021).

4.2 | The problem of pervasive relatedness

The challenge of managing breeding colonies in the absence of complete pedigrees or detailed genetic information is most evident in the high levels of relatedness found in this collection. The mean pairwise relatedness within the Military Macaw (0.33) and Great Green Macaw (0.27) cohorts far exceeds that of a wild Great Green Macaw population in Costa Rica (0.14; $n = 13$) and the highly managed European Endangered Species Programme (EEP) population (0.07; $n = 22$) (López-Serna, 2023). These elevated values approach or exceed the theoretical expectation for half-siblings ($r = 0.25$), suggesting a severely limited founder base and a history of breeding among

close relatives (Taylor, 2015). Even when private breeders sourced birds from multiple different states, the probability of acquiring related individuals remained high due to the small total number of founders imported into the country. Furthermore, historical trapping practices often involved harvesting entire clutches of siblings from a single nest or repeatedly raiding the same nests across multiple years (Banchs & Moschione, 2006). These practices, combined with occasional capture biases (such as trapping single-sex flocks) and highly skewed reproductive success in captivity (a few prolific pairs dominate production), rapidly contracted the available gene pool. Because historical records indicate birds were frequently acquired in wholesale regional groups rather than as independent individuals, it is highly likely that the clusters of siblings and closely related individuals identified in our pedigree analyses represent intact familial lineages that were transported and integrated into the facility together.

Our stringent pedigree reconstruction with Sequoia identified 15 full-sibling pairs (and potentially more) distributed across three highly connected family networks (Figure S9). However, an analysis of the maternally inherited ND2 gene revealed subtle mitochondrial variations within these SNP-defined sibling groups. This discordance suggests that these specific individuals are paternal half-siblings—possibly the offspring of a single prolific male and multiple, closely related females. In such pairs, half-siblings inherit such a high proportion of identical-by-descent alleles from their deeply interconnected ancestors that their overall nuclear genetic similarity is artificially inflated, statistically mimicking that of full siblings. This cryptic inbreeding underscores the vital importance of utilizing both bi-parental nuclear and strictly maternal markers to accurately untangle complex relationships in ex situ populations. This situation mirrors the extreme challenges faced in managing species derived from very few founders, such as the Spix's Macaw, where intensive genetic management is the only defense against inbreeding and the loss of diversity (Caparroz et al., 2001; Purchase et al., 2024). The high inbreeding coefficient of some individuals in our study (e.g., MM #14, Figure 4) is a direct consequence of this history and poses a significant threat to fitness and long-term viability.

4.3 | Conservation and management implications

Given the demographic and regulatory difficulties facing this collection, its suitability for direct conservation use within the United States is currently limited. A major compounding factor is the addition of both the Great

Green and Military Macaws to the US ESA as “Endangered” in 2015. This designation effectively bans interstate commerce, rendering the long-term maintenance and coordinated breeding of these species financially unviable for many private aviculturists. Consequently, these macaw populations are slowly aging and disappearing from the US private sector. We acknowledge that for private aviculturists, investing substantial financial resources into advanced genomic testing can be highly discouraging if the results merely confirm that the entire legally accessible population is already heavily inbred. However, while this regulatory reality severely limits the active, nationwide management of these specific flocks, the genomic framework demonstrated here retains broad, critical applicability. It provides a blueprint for evaluating and extracting conservation value from captive populations of other threatened taxa, or for managing ex situ parrot populations in regions where fewer such legal barriers to movement exist. Furthermore, for the birds remaining in the studied collection, the genomic data allowed us to reliably distinguish pure individuals from hybrids and to quantify kinship, providing the necessary information to avoid the fitness consequences often associated with inbreeding (e.g., Hammerly et al., 2016).

Based on these findings, we propose three crucial management actions that respect these legal and logistical constraints:

1. Establish a genomic baseline and coordinate globally: The genetic data should form the basis of a new studbook, correcting all species and subspecies-level misidentifications and documenting kinship and inbreeding coefficients for every individual. Because the strict regulations of the ESA make dynamic, large-scale physical studbook coordination highly difficult within the US, this genomic pedigree should be shared directly with the established International Studbook keepers for the Great Green Macaw (managed in France since 1994) and the Military Macaw (managed in Mexico since 1999). While the physical international transfer of these CITES Appendix I birds from undocumented private collections is often legally and logistically prohibitive, sharing vetted genomic data allows potentially valuable US founder lines to be virtually integrated and co-managed within the global metapopulation, identifying specific individuals whose genetic value justifies the burden of physical transfer.
2. Manage hybrids and subspecies appropriately: The seven hybrid birds have no direct role in conservation breeding and must be managed separately to prevent further gene flow. Similarly, the distinct Mexican and Bolivian lineages within the Military Macaws should

be managed as separate units to preserve their genetic integrity.

3. Implement a genetically-informed breeding plan for remaining flocks: For facilities still legally able to maintain these birds on-site, the relatedness values (Figures S10–11) provide a direct roadmap for forming optimal breeding pairs that minimize kinship and reduce future inbreeding. However, the advanced age of some of the individuals will reduce possibilities for matching pairs. The best approach might be to put the less related individuals into the same aviary and let them form breeding pairs.

While the potential benefits of integrating private collections into formal conservation networks are substantial—namely, accessing untapped genetic reservoirs and leveraging the extensive husbandry expertise of private aviculturists—, this integration can present severe logistical and biological challenges. The private sector's contribution to conservation is frequently undervalued, however, private breeding has generated decades of vital data on incubation requirements, hand-rearing techniques, and veterinary interventions, which field biologists often struggle to collect in the wild (Vigo-Trauco et al., 2021; Voren & Jordan, 1992). Additionally, the presence of these exotic birds in the private sector may directly drive public interest and emotional investment in the conservation of their wild counterparts (Frynta et al., 2010). In range countries for these species, such as Costa Rica and Mexico, conservation planning often relies on integrating formerly private or confiscated birds into highly managed, NGO-led breeding centers under strict governmental oversight (Olah et al., 2026; White Jr et al., 2012).

However, attempting to integrate the less formalized US private sector into conservation programs introduces unique risks that must be carefully mitigated. First, as highlighted by our study flock, the lack of individual background information (e.g., exact age, wild-caught status, historical lineage) necessitates extensive genomic and veterinary auditing before birds can be utilized. Second, transferring birds between private collections and accredited conservation centers carries a high risk of disease transmission if private facilities lack rigorous, standardized veterinary oversight (Fogell et al., 2016). Finally, applying standard genetic management strategies—such as transferring individuals to optimize mean kinship—is biologically complicated, especially in large psittacines. Because many parrots are socially monogamous birds that form long-term pair bonds, forcibly disrupting established pairs to meet genetic targets can induce severe psychological stress and result in prolonged breeding failure (Spoon et al., 2006). Therefore, any integration strategy

must balance genetic optimization with behavioral compatibility, strict biosecurity protocols, and animal welfare.

The main threats to the Critically Endangered Great Green Macaw in the wild are poaching for the illegal wildlife trade, capture for their colorful feathers, and habitat loss and fragmentation (BirdLife International, 2025). Given their rapid decline in the wild, the EEP for the Great Green Macaw was established in 1994, which took measures to improve keeping and breeding requirements for the species in captivity, and a breeding program was started with multiple European institutions (Müller, 2000). Our case study highlights a significant policy gap in the United States, which lacks a coordinated conservation program for this species (and many other threatened parrots) akin to the EEP. Maintaining genetic diversity and maximizing the effective population size are paramount aims of any ex situ conservation plan (Frankham et al., 2010). We strongly urge the adoption of genomic screening as a standard, non-negotiable practice for all captive breeding programs, especially for threatened species with complex histories outside of institutional networks. By providing an unambiguous foundation for evidence-based breeding, these tools are essential for ensuring that captive populations remain viable reservoirs for future conservation action.

AUTHOR CONTRIBUTIONS

J.D.B. conceived idea and collected samples; J.D.B., J.S.J., D.J.B. and J.A.J. designed research; J.A.J. conducted the mtDNA sequencing and analysis; G.O., J.D.B., and J.A.J. performed analyses; J.D.B., J.S.J., J.A.J., G.O. drafted the manuscript, and G.O., J.A.J., J.S.J., D.J.B. revised final manuscript.

ACKNOWLEDGMENTS

This study was conducted and funded by the late Janice Dinegar Boyd, who established and ran Amigos de las Aves USA and the Buffon's Macaw Conservation Center at Hill Country Aviaries in Texas. We acknowledge her life-long dedication to the conservation efforts on parrot species. Kranti Konganti from the Texas A&M Institute for Genome Sciences and Society conducted preliminary raw read analysis. We thank Mark Moore and Rick Jordan for hosting the studied birds at Hill Country Aviaries and for their comments on the results; Sharman Hoppes for taking the samples; Viktor Molnár for providing information on ZIMS records and studbooks; Pere Bover for further insights on the mitochondrial sequence data; and the editors and reviewers for their helpful comments. Open access publishing facilitated by Australian National University, as part of the Wiley - Australian National University agreement via the Council of Australasian University Librarians.

FUNDING INFORMATION

This research was supported by donations from Janice Boyd and Amigos de las Aves, USA. The Buffon's Macaw Conservation Center is funded by the Schubot Center for Avian Health, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University. George Olah was funded by the Australian Research Council DECRA Fellowship (DE230100085) and Loro Parque Fundación (PP-250-2024).

CONFLICT OF INTEREST STATEMENT

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The complete dataset, R-codes, and the ND2 alignment with all the ingroup/outgroup sample sequences are available in FigShare: <https://doi.org/10.6084/m9.figshare.29235725>. All unique ND2 sequences were uploaded to GenBank (accession numbers: PV740797–PV740807).

ORCID

George Olah  <https://orcid.org/0000-0002-7935-8843>
Jeff A. Johnson  <https://orcid.org/0000-0001-7446-5001>
Jeremy S. Johnson  <https://orcid.org/0000-0002-4305-1647>
Donald J. Brightsmith  <https://orcid.org/0000-0002-3306-6490>

REFERENCES

- Abramson, J., Speer, B. L., & Thomsen, J. B. (1995). *The large macaws: Their care, breeding, and conservation*. Raintree Publications.
- Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: Setting conservation guidelines. *Trends in Ecology & Evolution*, 16(11), 613–622. [https://doi.org/10.1016/s0169-5347\(01\)02290-x](https://doi.org/10.1016/s0169-5347(01)02290-x)
- Banchs, R. A., & Moschione, F. N. (2006). Proyecto Elé. In M. L. Bolkovic & D. Ramadori (Eds.), *Manejo de Fauna Silvestre en la Argentina* (pp. 15–25). Dirección de Fauna Silvestre, Secretaría de Ambiente y Desarrollo Sustentable.
- Barilani, M., Sfougaris, A., Giannakopoulos, A., Mucci, N., Tabarroni, C., & Randi, E. (2007). Detecting introgressive hybridisation in rock partridge populations (*Alectoris graeca*) in Greece through Bayesian admixture analyses of multilocus genotypes. *Conservation Genetics*, 8(2), 343–354. <https://doi.org/10.1007/s10592-006-9174-1>
- Berkunsky, I., Quillfeldt, P., Brightsmith, D. J., Abbud, M. C., Aguilar, J. M. R. E., Alemán-Zelaya, U., Aramburú, R. M., Arce Arias, A., Balas McNab, R., Balsby, T. J. S., Barredo Barberena, J. M., Beissinger, S. R., Rosales, M., Berg, K. S., Bianchi, C. A., Blanco, E., Bodrati, A., Bonilla-Ruz, C., Botero-Delgadillo, E., ... Masello, J. F. (2017). Current threats faced by neotropical parrot populations. *Biological Conservation*, 214, 278–287. <https://doi.org/10.1016/j.biocon.2017.08.016>

- BirdLife International. (2019). *Species factsheet: Spix's Macaw Cyanopsitta spixii*. Downloaded from <https://datazone.birdlife.org/species/factsheet/spixs-macaw-cyanopsitta-spixii> on 09/04/2025
- BirdLife International. (2025). *IUCN Red List for birds*. Downloaded from <https://datazone.birdlife.org> 14/05/2025. <https://www.iucnredlist.org>
- BirdLife International and Handbook of the Birds of the World. (2022). Bird species distribution maps of the world. Version 2022.2. Available at <http://datazone.birdlife.org/species/requestdis>
- Boyd, J. D. (2015). The great green (Buffon's macaw): The ambiguous ara (*Ara ambiguus*). *AFA Watchbird*, 41, 31–38.
- Caparroz, R., Miyaki, C. Y., Bampi, M. I., & Wajntal, A. (2001). Analysis of the genetic variability in a sample of the remaining group of Spix's macaw (*Cyanopsitta spixii*, Psittaciformes: Aves) by DNA fingerprinting. *Biological Conservation*, 99(3), 307–311. [https://doi.org/10.1016/S0006-3207\(00\)00196-8](https://doi.org/10.1016/S0006-3207(00)00196-8)
- CITES. (2024). Appendices I, II and III. <https://cites.org/eng/app/index.php>
- Collar, N. J., & Butchart, S. H. M. (2014). Conservation breeding and avian diversity: Chances and challenges. *International Zoo Yearbook*, 48(1), 7–28. <https://doi.org/10.1111/izy.12039>
- Conde, D. A., Staerk, J., Colchero, F., da Silva, R., Schöley, J., Baden, H. M., Juvet, L., Fa, J. E., Syed, H., Jongejans, E., Meiri, S., Gaillard, J.-M., Chamberlain, S., Wilcken, J., Jones, O. R., Dahlgren, J. P., Steiner, U. K., Bland, L. M., Gomez-Mestre, I., ... Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *Proceedings of the National Academy of Sciences of the United States of America*, 116(19), 9658–9664. <https://doi.org/10.1073/pnas.1816367116>
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772. <https://doi.org/10.1038/nmeth.2109>
- Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7(1), 214. <https://doi.org/10.1186/1471-2148-7-214>
- Dunning, J. B. (2008). *CRC handbook of avian body masses*. CRC Press, Taylor & Francis Group.
- Eberhard, J. R., Iñigo-Elias, E. E., Enkerlin-Hoeflich, E., & Cun, E. P. (2015). Phylogeography of the military macaw (*Ara militaris*) and the great green macaw (*A. ambiguus*) based on mtDNA sequence data. *The Wilson Journal of Ornithology*, 127(4), 661–669. <https://doi.org/10.1676/14-185.1>
- Endelman, J. B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *The Plant Genome*, 4(3), 250–255. <https://doi.org/10.3835/plantgenome2011.08.0024>
- Fair, J., Paul, E., Jones, J., & Bies, L. (2023). *Guidelines to the use of wild birds in research*. The Ornithological Council.
- Faust, L. J., Martínez, T. M., Parsons, A. W., White, T. H., Jr., Valentin, R., Vélez-Valentín, J., Ramos-Güivas, B., Nelson, S. S., & Lopez, M. (2025). Assessing population viability and management strategies for species recovery of the critically endangered Puerto Rican parrot. *Animal Conservation*, 28, 104–118. <https://doi.org/10.1111/acv.12987>
- Fjeldsø, J., Krabbe, N., & Ridgely, R. S. (1987). Great green macaw *Ara ambigua* collected in northwest Ecuador, with taxonomic comments on *Ara militaris*. *Bulletin of the British Ornithologists' Club*, 107(1), 28–31.
- Fogell, D. J., Martin, R. O., & Groombridge, J. J. (2016). Beak and feather disease virus in wild and captive parrots: An analysis of geographic and taxonomic distribution and methodological trends. *Archives of Virology*, 161(8), 2059–2074. <https://doi.org/10.1007/s00705-016-2871-2>
- Forshaw, J. M. (2006). *Parrots of the world*. Princeton University Press.
- Frankham, R., Briscoe, D. A., & Ballou, J. D. (2010). *Introduction to conservation genetics* (2nd ed.). Cambridge University Press.
- Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, 6(8), 925–929. <https://doi.org/10.1111/2041-210X.12382>
- Frynta, D., Lišková, S., Bültmann, S., & Burda, H. (2010). Being attractive brings advantages: The case of parrot species in captivity. *PLoS One*, 5(9), e12568. <https://doi.org/10.1371/journal.pone.0012568>
- Galla, S. J., Brown, L., Couch-Lewis, Y. (Ngāi Tahu: Te Hapū o Ngāti Wheke, Ngāti Waewae), Cubrinovska, I., Eason, D., Gooley, R. M., Hamilton, J. A., Heath, J. A., Hauser, S. S., Latch, E. K., Matocq, M. D., Richardson, A., Wold, J. R., Hogg, C. J., Santure, A. W., & Steeves, T. E. (2022). The relevance of pedigrees in the conservation genomics era. *Molecular Ecology*, 31(1), 41–54. <https://doi.org/10.1111/mec.16192>
- Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. *arXiv*. <http://arxiv.org/abs/1207.3907>
- Gonçalves, F., Farooq, H., Harfoot, M., Pires, M. M., Villar, N., Sales, L., Carvalho, C., Bello, C., Emer, C., Bovendorp, R. S., Mendes, C., Beca, G., Lautenschlager, L., Souza, Y., Pedrosa, F., Paz, C., Zipparro, V. B., Akkawi, P., Bercê, W., ... Galetti, M. (2024). A global map of species at risk of extinction due to natural hazards. *Proceedings of the National Academy of Sciences of the United States of America*, 121(26), e2321068121. <https://doi.org/10.1073/pnas.2321068121>
- Hammerly, S. C., de la Cerda, D. A., Bailey, H., & Johnson, J. A. (2016). A pedigree gone bad: Increased offspring survival after using DNA-based relatedness to minimize inbreeding in a captive population. *Animal Conservation*, 19(3), 296–303. <https://doi.org/10.1111/acv.12247>
- Huisman, J. (2017). Pedigree reconstruction from SNP data: Parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources*, 17(5), 1009–1024. <https://doi.org/10.1111/1755-0998.12665>
- IUCN. (2025). IUCN Red List for birds. 2025-1. Downloaded from <https://www.iucnredlist.org> 05/06/2025.
- Jombart, T. (2008). Adegnet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Jombart, T., & Ahmed, I. (2011). Adegnet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27(21), 3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>
- Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, 11(1), 1–15. <https://doi.org/10.1186/1471-2156-11-94>
- Juniper, T., & Parr, M. (1998). *Parrots: A guide to parrots of the world*. Yale University Press. <http://books.google.com.au/books?id=R4TF7Smz7ZQC>
- Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281. <https://doi.org/10.7717/peerj.281>

- Kiacz, S. (2023). Status, distribution, and population modeling of Red-Crowned Parrots and conservation opportunities of naturalized parrots. <https://hdl.handle.net/1969.1/203067>
- Kiacz, S., & Brightsmith, D. J. (2021). Naturalized parrots: Conservation and research opportunities. In *Naturalized parrots of the world: Distribution, ecology, and impacts of the world's most colorful colonizers*. Princeton University Press.
- Knaus, B. J., & Grünwald, N. J. (2017). VCFR: A package to manipulate and visualize variant call format data in R. *Molecular Ecology Resources*, 17(1), 44–53. <https://doi.org/10.1111/1755-0998.12549>
- Lees, C. M., & Wilcken, J. (2009). Sustaining the ark: The challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook*, 43(1), 6–18. <https://doi.org/10.1111/j.1748-1090.2008.00066.x>
- López-Serna, S. (2023). Conservation genetics for the critically endangered Great Green Macaw [Mrs, University of Kent]. <https://doi.org/10.22024/UniKent/01.02.103066>
- Luo, S.-J., Johnson, W. E., Martenson, J., Antunes, A., Martelli, P., Uphyrkina, O., Traylor-Holzer, K., Smith, J. L. D., & O'Brien, S. J. (2008). Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations. *Current Biology*, 18(8), 592–596. <https://doi.org/10.1016/j.cub.2008.03.053>
- McCarthy, E. M. (2006). *Handbook of avian hybrids of the world*. Oxford University Press.
- McClure, C. J. W., Berkunsky, I., Buechley, E. R., Dunn, L., Johnson, J., McCabe, J., Oppel, S., Rolek, B. W., Sutton, L. J., & Gumbs, R. (2023). Conserving the evolutionary history of birds. *Conservation Biology*, 37(6), e14141. <https://doi.org/10.1111/cobi.14141>
- McClure, C. J. W., & Rolek, B. W. (2020). Relative conservation status of bird orders with special attention to raptors. *Frontiers in Ecology and Evolution*, 8, 593941. <https://doi.org/10.3389/fevo.2020.593941>
- Moss, A., & Esson, M. (2010). Visitor interest in zoo animals and the implications for collection planning and zoo education programmes. *Zoo Biology*, 29(6), 715–731. <https://doi.org/10.1002/zoo.20316>
- Müller, M. (2000). Review of the in situ status of the great green or Buffon's macaw *Ara ambigua* and the European endangered species Programme (EEP). *International Zoo Yearbook*, 37(1), 183–190. <https://doi.org/10.1111/j.1748-1090.2000.tb00721.x>
- Olah, G., Butchart, S. H. M., Symes, A., Guzmán, I. M., Cunningham, R., Brightsmith, D. J., & Heinsohn, R. (2016). Ecological and socio-economic factors affecting extinction risk in parrots. *Biodiversity and Conservation*, 25(2), 205–223. <https://doi.org/10.1007/s10531-015-1036-z>
- Olah, G., León-Pérez, R., Berryman, A. J., Asner, G., & Heinsohn, R. (2026). Shifting drivers and predictable corridors of an illegal wildlife trade network. *Proceedings of the National Academy of Sciences of the United States of America*, 123(36), e2609240123. <https://doi.org/10.1073/pnas.2609240123>
- Olah, G., Smith, B. T., Joseph, L., Banks, S. C., & Heinsohn, R. (2021). Advancing genetic methods in the study of parrot biology and conservation. *Diversity*, 13(11), 521. <https://doi.org/10.3390/d13110521>
- Palmer, C. A., Martin, T. E., Durand, S., & Lamont, M. (2018). First observations of the impacts of hurricane Maria on the endemic Imperial Amazon. *Oryx*, 52(3), 410–411. <https://doi.org/10.1017/S0030605318000364>
- Paradis, E., & Schliep, K. (2019). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35(3), 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall, R., & Smouse, P. E. (2012). GenALEX 6.5: Genetic analysis in excel. Population genetic software for teaching and research—An update. *Bioinformatics*, 28(19), 2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, 7(5), e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Pew, J., Muir, P. H., Wang, J., & Frasier, T. R. (2015). Related: An R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, 15(3), 557–561. <https://doi.org/10.1111/1755-0998.12323>
- Prevosti, A., Ocaña, J., & Alonso, G. (1975). Distances between populations of *Drosophila subobscura*, based on chromosome arrangement frequencies. *Theoretical and Applied Genetics*, 45(6), 231–241. <https://doi.org/10.1007/BF00831894>
- Purchase, C., Lugarini, C., Purchase, C., Ferreira, A., Vercillo, U. E., Stafford, M. L., & White, T. H. (2024). Reintroduction of the extinct-in-the-wild Spix's macaw (*Cyanopsitta spixii*) in the caatinga forest domain of Brazil. *Diversity*, 16(2), 2. <https://doi.org/10.3390/d16020080>
- Puritz, J. B., Hollenbeck, C. M., & Gold, J. R. (2014). dDocent: A RAD-seq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ*, 2, e431. <https://doi.org/10.7717/peerj.431>
- Putnam, A. S., Ferrie, G. M., & Ivy, J. A. (2023). Ex situ breeding programs benefit from science-based cooperative management. *Zoo Biology*, 42(1), 5–16. <https://doi.org/10.1002/zoo.21700>
- Rivera-Ortiz, F. A., Sanabria-Urbán, S., Prieto-Torres, D. A., Navarro-Sigüenza, A. G., Arizmendi, M. d. C., & Oyama, K. (2023). Phylogeography of *Ara militaris* (military macaw): Implications for conservation. *Diversity*, 15(10), 10. <https://doi.org/10.3390/d15101035>
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12), 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Smith, B. T., Thom, G., & Joseph, L. (2024). Revised evolutionary and taxonomic synthesis for parrots (order: Psittaciformes) guided by phylogenomic analysis. *Bulletin of the American Museum of Natural History*, 2024(468), 1–89. <https://doi.org/10.1206/0003-0090.468.1.1>
- Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution*, 12(2), 105–114. <https://doi.org/10.1006/mpev.1998.0602>

- Soulé, M., Gilpin, M., Conway, W., & Foose, T. (1986). The millennium ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biology*, 5(2), 101–113. <https://doi.org/10.1002/zoo.1430050205>
- Species360. (2026). Zoological Information Management System (ZIMS). Retrieved on 20/02/2026 from <https://zims.species360.org> [Dataset]
- Spoon, T. R., Millam, J. R., & Owings, D. H. (2006). The importance of mate behavioural compatibility in parenting and reproductive success by cockatiels, *Nymphicus hollandicus*. *Animal Behaviour*, 71(2), 315–326. <https://doi.org/10.1016/j.anbehav.2005.03.034>
- Stojanovic, D., & Heinsohn, R. (2023). Orange-bellied Parrots. *Current Biology*, 33(18), R939–R940. <https://doi.org/10.1016/j.cub.2023.06.075>
- Stuart, B. L., & Parham, J. F. (2007). Recent hybrid origin of three rare Chinese turtles. *Conservation Genetics*, 8(1), 169–175. <https://doi.org/10.1007/s10592-006-9159-0>
- Taylor, H. R. (2015). The use and abuse of genetic marker-based estimates of relatedness and inbreeding. *Ecology and Evolution*, 5(15), 3140–3150. <https://doi.org/10.1002/ece3.1541>
- Vigo-Trauco, G., Garcia-Anleu, R., & Brightsmith, D. J. (2021). Increasing survival of wild macaw chicks using foster parents and supplemental feeding. *Diversity*, 13(3), 121. <https://doi.org/10.3390/d13030121>
- Voren, H., & Jordan, R. (1992). *Parrots: Hand feeding and nursery management*. Silvio Mattacchione.
- Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetics Research*, 89(3), 135–153. <https://doi.org/10.1017/S0016672307008798>
- Ward, P. I., Mosberger, N., Kistler, C., & Fischer, O. (1998). The relationship between popularity and body size in zoo animals. *Conservation Biology*, 12(6), 1408–1411. <https://doi.org/10.1111/j.1523-1739.1998.97402.x>
- Wei, T., & Simko, V. (2021). R package “corrplot”: Visualization of a correlation matrix [Computer software]. <https://github.com/taiyun/corrplot>
- White, T. H., Jr., Collar, N. J., Moorhouse, R. J., Sanz, V., Stolen, E. D., & Brightsmith, D. J. (2012). Psittacine reintroductions: Common denominators of success. *Biological Conservation*, 148(1), 106–115. <https://doi.org/10.1016/j.biocon.2012.01.044>
- Witzenberger, K. A., & Hochkirch, A. (2011). Ex situ conservation genetics: A review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. *Biodiversity and Conservation*, 20(9), 1843–1861. <https://doi.org/10.1007/s10531-011-0074-4>
- Young, A. M., Hobson, E. A., Lackey, L. B., & Wright, T. F. (2012). Survival on the ark: Life-history trends in captive parrots. *Animal Conservation*, 15(1), 28–43. <https://doi.org/10.1111/j.1469-1795.2011.00477.x>
- Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T.-Y. (2017). Ggtree: An r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, 8(1), 28–36. <https://doi.org/10.1111/2041-210X.12628>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Olah, G., Johnson, J. A., Johnson, J. S., Brightsmith, D. J., & Boyd, J. D. (2026). Importance of using genetic data to inform conservation captive breeding: A tale of two species of threatened macaws (*Ara* spp.). *Conservation Science and Practice*, 8(10), e70382. <https://doi.org/10.1111/csp2.70382>