Molecular Ecology Resources

WILEY

MOLECULAR ECOLOGY RESOURCES

RESOURCE ARTICLE

Chromosome-Scale Genome Assembly Provides Insights Into Condor Evolution and Conservation

Diego De Panis[1,2,3,4] | François Le Dily[5] | Sergio A. Lambertucci[2] | Guillermo Wiemeyer[1,2] | Hernán Dopazo[1] | Marta Gut[6,7] | Tyler S. Alioto[6,7] | Camila J. Mazzoni[3,4] | Ivo Gut[6,7] | Marc Martí-Renom[5,6,8,9] | Julián Padró[2]

[1]Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Universidad de Buenos Aires-CONICET, Ciudad Autónoma de Buenos Aires, Argentina | [2]Grupo de Investigaciones en Biología de la Conservación, INIBIOMA, Universidad Nacional del Comahue-CONICET, Bariloche, Argentina | [3]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany | [4]Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Berlin, Germany | [5]Centre for Genomic Regulation, The Barcelona Institute for Science and Technology, Barcelona, Spain | [6]Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain | [7]Universitat de Barcelona (UB), Barcelona, Spain | [8]ICREA, Barcelona, Spain | [9]Universitat Pompeu Fabra, Barcelona, Spain

Correspondence: Julián Padró (padrojulian@comahue-conicet.gob.ar)

Received: 11 February 2025 | **Revised:** 7 May 2025 | **Accepted:** 21 May 2025

Handling Editor: Joanna Kelley

Funding: This research was supported by the National Council of Scientific and Technical Research of Argentina (CONICET) and funded by the National Agency of Scientific and Technological Promotion (PICT 2020-00132). International mobility funds were provided by the Marie Curie IRSES Project DEANN (PIRSES-GA-2013-612583). We thank IAATE and Alexander von Humboldt-Stiftung for their support to S.A.L. and J.P.

Keywords: comparative genomics | condor | conservation genomics | evolution | genome assembly | vulture

ABSTRACT

Rare species are highly vulnerable to anthropogenic threats due to their unique life-history traits and specialised adaptations. The Andean condor (*Vultur gryphus*), the world's largest soaring bird, exemplifies these challenges with exceptional flight efficiency, delayed maturity, long lifespan, extreme sexual dimorphism and a critical scavenging role. The species faces significant threats, including habitat loss, persecution and poisoning. Meanwhile, conservation efforts have been hindered by knowledge gaps, including limited genetic data. Herein, we present the first chromosome-scale reference genome for the species, a key resource for investigating its evolution and ecology, as well as informing conservation measures. The assembly spans 1.19 Gb with 97.4% completeness, including 29 autosomes and the Z chromosome. High synteny with the California condor (*Gymnogyps californianus*) genome reflects their close evolutionary relationship. Genomic diversity in Andean condors (~0.65He/Kbp; π: 6.73^{e-4}) was lower than in California condors (~0.97 He/Kbp; π: 1.09^{e-3}). Runs of Homozygosity (RoH) analyses revealed a smaller genomic proportion (~15%) with shorter elements in Andean condors (> 5 Mb covering 1.43% of the genome). In contrast, California condors showed a higher genomic proportion (~40%), with longer RoH segments (> 5 Mb covering 7.3% of the genome). Analyses of gene family evolution revealed divergent patterns of expansion and contraction between Andean and California condors, including genes linked to detoxification metabolism, high-altitude adaptation and immune response. Shared genomic trends among avian scavengers highlight convergent evolution in stress response and metabolic pathways. This study provides a key genomic resource for advancing avian research and guiding conservation strategies for threatened vultures.

© 2025 John Wiley & Sons Ltd.

1 | Introduction

Rare species at high trophic levels often exhibit extreme life-history traits or specialised physiological adaptations, making them disproportionately vulnerable to human-induced threats (Orians 1997). These species play essential roles in maintaining ecosystem processes and enhancing functional diversity under changing environmental conditions (Lawler et al. 2003; Mouillot et al. 2013; Wiegand et al. 2020). However, the molecular traits underlying rarity remain poorly understood, threatening the loss of unique genetic diversity and essential ecosystem services.

The Andean condor (*Vultur gryphus*) is a prime example of a rare species due to its specialised ecological roles and remarkable life-history traits. As one of the world's largest flying birds, it boasts an impressive wingspan of up to 3 m and a body mass reaching up to 16 kg (Shepard and Lambertucci 2013; Alarcón et al. 2017). This monotypic species exhibits delayed sexual maturity (reached around eight years of age), has one of the lowest reproductive rates among birds, and can live up to around 75 years (Del Hoyo et al. 1994; Ferguson-Lees and Christie 2001). Contrary to the general tendency of raptors, where females are larger than males (McDonald et al. 2005), Andean condors exhibit extreme sexual dimorphism in the opposite direction, with males up to 50% larger than females (Del Hoyo et al. 1994; Alarcón et al. 2017). Their obligatory scavenger habits demand highly specialised physiology, including highly acidic stomachs and unique immune adaptations to process decayed carcasses (Houston and Cooper 1975; Roggenbuck et al. 2014). Furthermore, Andean condors are extraordinarily adapted for energy-efficient flight, soaring at altitudes above 5000 meters above sea level and covering distances of up to 350 km daily (Lambertucci et al. 2014; Williams et al. 2020; Perrig et al. 2021). Recent bio-logging studies have revealed that these scavengers flap for just 1% of their total flight time, showcasing the lowest movement costs recorded among birds (Williams et al. 2020). These traits enable condors to provide critical ecosystem services across vast regions, such as nutrient recycling and disease regulation (Buechley and Şekercioğlu 2016). While their ability to range over large areas might allow them to avoid localised threats, the gregarious nature of Andean condors leads to concentrated aggregations (often >100 individuals) at key sites, creating population-level vulnerability to anthropogenic threats (Lambertucci et al. 2008). These mass gatherings are particularly susceptible to acute risks such as poisoning events and targeted persecution, where single incidents can impact multiple individuals simultaneously (Plaza and Lambertucci 2020).

The conservation journey of the Andean condor began with the plight of the California condor (*Gymnogyps californianus*), whose drastic population decline in the early 1900s led to its classification as critically endangered (BirdLife International 2021). This crisis spurred extensive research on its Andean counterpart to guide conservation strategies (Toone and Wallace 1994; Wallace and Toone 1992). Initial studies focused on captive breeding and ecology, followed by research into marking, tracking, and release programs (Wallace 1985; Wallace and Temple 1987; Lambertucci 2007). These efforts yielded valuable data on survival, reproduction, feeding behaviour and social structure (e.g., Lieberman et al. 1993; Donázar et al. 1999). While the California condor has benefited from these studies and the consequent intensive conservation efforts, the Andean condor now faces several emerging challenges. The species is currently restricted to the Andean range and Argentina's central mountains, with local extinctions occurring at the periphery of its distribution (Lambertucci 2007; Padró et al. 2020, 2023). According to the International Union for Conservation of Nature (IUCN), the Andean condor is globally classified as 'Vulnerable' but is considered 'Critically Endangered' in its northernmost range (BirdLife International 2021; Padró et al. 2023). Major threats include poaching, poisoning, and collisions with power lines, among others (Plaza and Lambertucci 2020). In response to this conservation need, ex-situ breeding and translocation programs have become the primary conservation strategy to preserve wild populations (e.g., Astore et al. 2017; Lieberman et al. 1993). However, management plans considering the genetic architecture of natural populations are lacking and mostly rely on limited genetic information.

A recent analysis of historic samples has shown that although low mitochondrial DNA diversity largely predates European arrival to the Americas, Andean condors have lost genetic variation, especially with the extirpation of the Atlantic coast population in the early 20th century (Padró et al. 2020). Neutral nuclear genetic markers have shown that despite the dispersal capacity of the species, genetic structure exists and that there is directional dispersal of individuals from southern populations to the north (Padró et al. 2018, 2019, 2023). These findings highlight the relevance of genetic data for management plans. However, they are limited in their ability to resolve fine-scale relationships, detect subtle patterns of local adaptation, assess genetic load, infer demographic events, and understand genome-wide patterns of variation across populations. Moreover, these markers cannot assess genomic offset (the mismatch between current and future genotype-environment associations), vital for predicting population resilience to global change (Chen et al. 2022).

To address these gaps, we developed the first chromosome-scale genome for the Andean condor, a vital resource for advancing modern conservation biology. We analysed population genetic features, including genome-wide heterozygosity, nucleotide diversity and inbreeding, comparing them to those of the Andean condor's closest relative, the California condor. We also conducted exploratory evolutionary analyses of gene family expansion and contraction between the two extant condor species and between condors and Old World vultures, providing insights into the dynamics of gene family evolution across vulture genera and families. This reference genome also facilitates detailed analyses of genetic variation, structural features, and functional elements that drive species survival and adaptation. Furthermore, reference genomes support population health assessments by detecting inbreeding depression, genetic load, and adaptive potential, and they play a crucial role in guiding breeding programs and genetic rescue efforts (Formenti, Theissinger, et al. 2022; Theissinger et al. 2023). By enabling high-resolution analyses of genetic variation, local adaptation, and genomic resilience, this resource marks a significant step toward uncovering the genetic basis of the Andean condor's unique evolutionary pathways and extreme phenotype. It lays the foundation

for advancing conservation genomics and developing informed management strategies to ensure the species' long-term survival.

2 | Methods

2.1 | Sample Information and Sequencing

Blood samples were extracted from a captive male Andean condor (ID: 01C5-218A; ToLID: bVulGry1) originally from Mendoza Province (Argentina), a region containing some of the Americas' highest peaks (up to 6961 masl) and harbouring one of the largest populations in South America (Padró et al. 2018, 2023). Additional details are available under INSDC BioSample accession SAMN19222171.

Total DNA was extracted from blood cell samples following a standard phenol: chloroform extraction protocol for high-molecular-weight (HMW) DNA. Whole genome sequencing was performed using a combination of short and long reads, along with proximity ligation sequencing at the Centre Nacional d'Anàlisi Genòmica (Barcelona, Spain). For long-read sequencing, genomic DNA was used to prepare 1D genomic libraries using the Ligation Sequencing Kit SQK-LSK108 for sequencing on a MinION instrument (Oxford Nanopore Technologies). HMW DNA (1.5 μg) was fragmented by centrifugation in a Covaris G-tube, followed by repair with the NEBNext FFPE Repair Mix (New England Biolabs). End-repair and adenylation were performed using the NEBNext Ultra II End Repair and A-Tailing Module, and MinION AMX adapters were ligated using the NEB Blunt/TA Ligase Master Mix. Sequencing was conducted using R9.4 chemistry on two FLO-MIN106 flow cells over 48 h, with run quality monitored in real time using the MinKNOW platform and base-calling performed concurrently with the Metrichor agent. For short-read sequencing, a PCR-free paired-end library was prepared following the KAPA Library Preparation Kit (Kapa Biosystems) protocol. Sheared DNA (4 μg) was end-repaired, adenylated, and ligated to Illumina-specific indexed adapters. Sequencing was conducted on an Illumina HiSeq 4000 platform in paired-end mode (2×151 bp), with image analysis, base-calling, and quality scoring performed using the Real Time Analysis software (RTA 2.7.6), and FASTQ files generated using CASAVA. To capture chromatin conformation, an in-nuclei Hi-C protocol was performed at the Centre de Regulació Genòmica (Barcelona, Spain). Briefly, cells were cross-linked with 1% formaldehyde and processed as described by Rao et al. (2014). Hi-C libraries were sequenced on an Illumina HiSeq 2000 platform in paired-end mode (2×76 bp) using the TruSeq SBS Kit v4. Image analysis, base-calling, and quality scoring were processed using RTA software (v1.18.66.3), with FASTQ sequence files generated using CASAVA. In total, 65× WGS (Illumina and Oxford Nanopore) and 25× Hi-C data were sequenced to generate the assembly. The sequencing data is available under the Bioproject accession PRJNA1035759.

2.2 | Genome Assembly

The Andean condor genome was assembled using a de novo hybrid pipeline following an iterative approach. The initial assembly served as a reference to guide the scaffolding of the original extended contigs into a second assembly, which in turn acted as an improved reference for a final round of scaffolding to produce the final high-quality assembly (Figure 1a). Briefly, Illumina reads (both WGS and Hi-C) were preprocessed for quality and length using Fastp v0.23.4 (Chen et al. 2018), while ONT reads were corrected with Ratatosk v0.9 (Holley et al. 2021) using the trimmed Illumina WGS reads. The first stage of the assembly pipeline involved a highly accurate contigging step using Platanus v1.2.4 (Kajitani et al. 2014) with trimmed short reads. This was followed by a correction and contig extension step with LongStitch v1.0.5 (Coombe et al. 2021) using corrected long reads. Retained haplotigs were removed using purge_dups v1.2.6 (Guan et al. 2020), and contamination was checked with FCS-GX v0.4 (Astashyn et al. 2024). Finally, scaffolding was performed with YaHS v1.2 (Zhou et al. 2023) using the Hi-C data. The second stage began using the YaHS scaffolds for reference-based scaffolding of the LongStitch extended contigs with RagTag v2.1 (Alonge et al. 2022). The same tool was used for posterior ONT-based correction, followed by haplotigs removal, contamination checking, and additional scaffolding. The process concluded with a quick manual correction using the Hi-C data and Juicebox v2.18 (Durand, Robinson, et al. 2016). The third stage began with the quickly corrected scaffolds, which were used for reference-based scaffolding of the LongStitch-extended contigs with RagTag. This was followed by haplotigs removal, contamination checking, and Hi-C scaffolding. A thorough manual correction was then performed (Durand, Shamim, et al. 2016), culminating in a polishing step using WGS short-read data with DeepVariant v1.6 (Poplin et al. 2018) and BCFtools v1.19 (Li 2011). To confirm the incremental improvement after each step of the pipeline, quality was measured using gfastats v1.3.6 (Formenti, Abueg, et al. 2022) for basic assembly statistics (e.g., size, continuity), BUSCO v4 (Manni et al. 2021) for gene completeness assessment, and Merqury v1.3 (Rhie et al. 2020) for consensus quality and k-mer completeness metrics.

Summary analysis of the assembly was performed using the ERGA-BGE Genome Report ASM Galaxy workflow (De Panis 2024), incorporating tools such as BUSCO, Merqury, and others (see reference for the full list of tools). The genome assembly is available from GenBank under the accession number GCA_039700855.2.

2.3 | Characterisation and Comparative Analysis

The estimated genome size retrieved from Genomes on a Tree for Cathartidae is 1.48 Gb (Challis et al. 2023), while our estimation based on a k-mer profiling of the WGS Illumina reads dataset is 1.22 Gb (Figure S1). The Andean condor has a diploid genome with 80 chromosomes ($2n = 80$), including ZW sex chromosomes in females (Takagi and Sasaki 1974). The karyotype exhibits a pattern typical of many birds, consisting of several pairs of macrochromosomes and numerous microchromosomes, though the distinction between the two is not always clearly defined.

We used the California condor reference genome assembly (RefSeq accession: GCF_018139145.2) for comparative analysis.

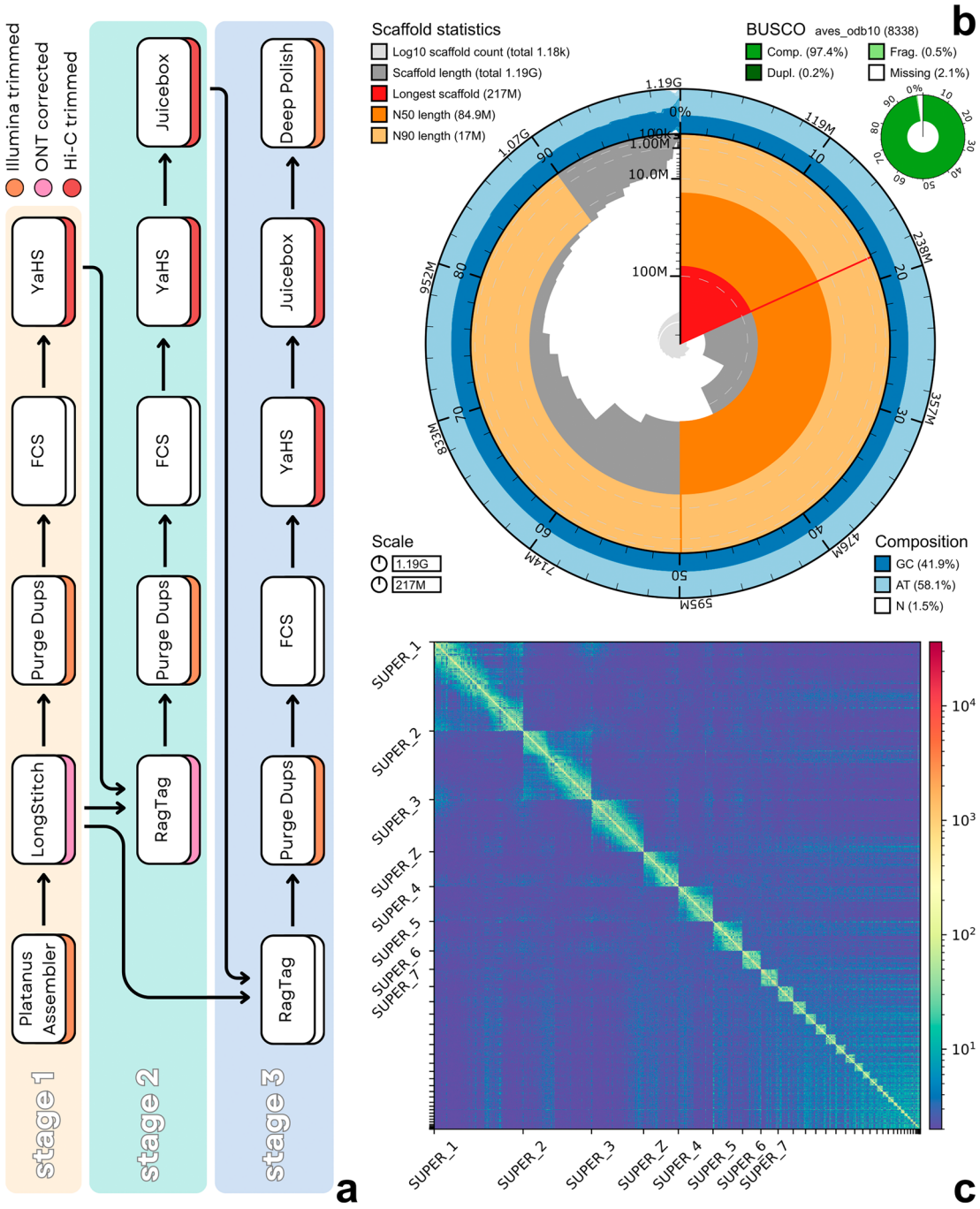

FIGURE 1 | Andean condor genome assembly. Three-stage iterative genome assembly pipeline (a). Snail plot summary of assembly statistics (b). The main plot is divided into 1000 size-ordered bins around the circumference, with each bin representing 0.1% of the 1,190,358,635 bp assembly. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (217,127,986 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 sequence lengths (84,949,164 and 17,024,464 bp), respectively. The pale grey spiral shows the cumulative sequence count on a log scale, with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT, and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the Aves database (odb10) is shown in the top right. Hi-C contact map showing spatial interactions between genome regions (c). The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. The frequency of contacts is shown on a logarithmic heatmap scale. Hi-C matrix bins were merged into a 10 kb bin size for plotting. Names of the eight largest chromosome-scale (SUPER) scaffolds are shown.

Genome-wide synteny analysis was performed using D-GENIES (Cabanettes and Klopp 2018) with default parameters, comparing our Andean condor assembly against the California condor reference.

2.4 | Genetic Diversity and Inbreeding

To explore genome-wide patterns of genetic diversity in Andean (AnCo) and California (CaCo) condors, we analysed all available

WGS Illumina reads data, using the respective genome assemblies as references: AnCo1 (this study) and AnCo2 (SAMN18477436 from Argentina, ancestry/population unknown), CaCo1 and CaCo2 (SAMN18477434 and SAMN18477435, respectively, from Robinson et al. 2021).

First, all reads were quality-trimmed for adapters using Fastp. Subsequent steps were carried out using the jATG pipeline (github.com/diegomics/jATG/tree/devel). Briefly, filtered reads were aligned to the genome assembly with BWA-MEM v2.2.1 (Vasimuddin et al. 2019). PCR duplicates were identified using the MarkDuplicates module, and SNP calling was performed employing the HaplotypeCaller and GenotypeGVCF modules from GATK v4.2.6.1 (der Auwera and O'Connor 2020). Only positions on autosomal scaffolds longer than 5 Mbp were considered for downstream analyses, as these likely represent complete chromosomes, excluding the Z chromosome. SNPs in masked regions, identified with Dfam TE Tools v1.85 via RepeatModeler and RepeatMasker (Flynn et al. 2020), were excluded. Variants were further filtered using BCFtools with GATK's recommended parameter thresholds. SNPs with coverage depth below 8x or greater than twice the average coverage were also removed. Finally, a base-pair resolution gVCF file was generated, with filtered genotypes marked as missing data.

We estimated heterozygosity and runs of homozygosity (RoH) using Darwindow (de Jong et al. 2023). Heterozygosity was calculated using a sliding-window approach with non-overlapping, fixed-length windows of 50 kb. For RoH detection, windows were classified as having low heterozygosity if their values were below one-fifth of the individual's mean heterozygosity. A RoH was defined as two or more consecutive low-heterozygosity windows (minimum length = 100 kb), using default parameters (miss_max = 0.6). This final set of parameters was selected by visually evaluating the fit of RoH and heterozygosity patterns (see Figure S2). This approach has been successfully employed in recent genomic studies (e.g., Bukhman et al. 2024) and offers several advantages for our dataset: it is robust when analysing single individuals without population allele-frequency estimates, avoiding excessive parameterisation that can introduce sensitivity to parameter choices and increase complexity. Moreover, Darwindow allows us to corroborate the fidelity of RoH calls through visual inspection of heterozygosity within the inferred RoH regions. RoHs shorter than 100 kb were interpreted as indicative of background relatedness rather than recent consanguinity. The inbreeding level (FRoH) was calculated as the proportion of the genome within RoHs. Having multiple individuals per species allowed us to assess genome-wide diversity by calculating nucleotide diversity (π) between pairs. Briefly, we used BCFtools to merge the individual base-pair resolution gVCFs, removing all positions with missing genotypes in either individual. Nucleotide diversity was then calculated in 100-kb windows with a 50-kb step size using VCFtools v0.1.16 (Danecek et al. 2011). Genome-wide π estimates were obtained by calculating the weighted mean across all windows, accounting for the differing number of windows per chromosome and excluding scaffolds shorter than 5 Mbp and sex chromosomes. Additionally, we calculated a more conservative estimate using only the large and medium-sized chromosomes. Finally, we converted RoH tract lengths (L, in Mb) into the number of generations to the most recent common ancestor (g) using $g = 100/2rL$ with a recombination rate $r = 2.7$ cM/Mb (Robinson et al. 2021). To estimate meaningful inbreeding events for condors, we consolidated the default RoH bins from Darwindow into long (> 5 Mb), medium (1–5 Mb), and short (< 1 Mb) categories, allowing us to relate RoH length to recent (< 4 generations), historic (4–20 generations), and ancient times (> 20 generations). To express coalescence dates in calendar years, we used IUCN-estimated generation times of approximately 29 years for the Andean condor and 19 years for the California condor, respectively. All plots were generated using R v4.4 (R Core Team 2024).

2.5 | Demographic Analysis

Demographic trajectories for the reference genomes of Andean and California Condors were inferred using the Pairwise Sequentially Markovian Coalescent (PSMC) method (Li and Durbin 2011). Briefly, consensus sequences were derived from the filtered gVCF files generated previously using BCFtools and subsequently converted to the PSMC input format with fq2psmcfa. PSMC was run with default parameters (–N25 –t15 –r5 –p 4 + 25 × 2 + 4 + 6). Following Robinson et al. (2021), we scaled the output using a mutation rate (μ) of 1.4×10^{-8} per site per generation and a generation time of 10 years, reflecting the approximate age at first breeding (demographic trajectories remain consistent across varying mutation rate and generation time parameters). To gain further insight, we also performed the same scaling using the IUCN-estimated generation times. All plots were generated using R v4.4.

2.6 | Evolutionary Analysis of Orthologous Clusters

In addition to the Andean and California condor genomes, we utilised the Chicken reference genome (RefSeq accession: GCF_016699485.2) and Old World vultures genomes, enabling a broad comparative analysis across vulture families (Cathartidae vs. Accipitridae). To mitigate potential biases related to genome assembly quality in our orthologous cluster analysis, we limited our analysis to the highest-quality genomes available: *Gyps himalayensis* (Himalayan vulture, GenBank accession: GCA_021398385.1) and *Gypaetus barbatus* (bearded vulture, GenBank accession: GCA_028022735.1).

First, we generated protein-coding annotations for the four vulture genomes using the Chicken as a high-quality reference. The Chicken genome assembly and its annotation represent one of the most complete and well-curated avian genomic resources available, providing an optimal basis for comparative studies. Additionally, all four vulture genome assemblies are of high quality, ensuring reliable gene predictions. Briefly, we generated lastz-alignment chains between the Chicken genome and each vulture genome using the make_lastz_chains tool (v2.0.8) from TOGA (Kirilenko et al. 2023) and subsequently created the set of homology gene projections using TOGA v1.1.6. To maintain accuracy and minimise the noise in downstream analyses, only TOGA-classified predictions labelled as 'intact', 'partially intact' or 'uncertain loss' were retained (Table S1). Using the longest isoform per transcript, single-copy gene content analysis showed 80.3%–85.4% completeness with BUSCO using the

Aves database, and 81.5%–87.0% completeness with OMArk OMAmer's Neognathae database (Nevers et al. 2025), achieving 91.6%–92.1% consistency (Table S2). By annotating each genome using the same TOGA-based pipeline against a high-quality Chicken reference and retaining only highest-confidence projections, we prioritise conserved genes while minimising artefacts, thus providing a robust and consistent foundation for comparative genomic analyses of vulture species.

Using the annotations generated by TOGA, we conducted exploratory comparative genomic analyses using the OrthoVenn3 platform (Sun et al. 2023). First, orthologous cluster identification was performed with OrthoFinder (Emms and Kelly 2019), a robust and balanced method for grouping orthologous genes into gene families across species. This step enabled the identification of orthogroups and the comparison of evolutionary relationships among the Chicken and the four vulture species. We applied a more stringent DIAMOND e-value cutoff of 1e-5 (default: 1e-2) while maintaining the MCL inflation parameter at 1.5. This helped filter out borderline sequence matches without artificially fragmenting well-supported orthogroups. This setting increased the number of single-copy orthogroups, resulting in cleaner family boundaries for downstream birth-and-death modelling. At the same time, it preserved the overall expansion/contraction patterns observed under default parameters, ensuring that our comparative inferences were driven by biological signals rather than search noise (Table S3). Since the phylogenetic relationships among these species are well established, we performed a phylogenetic analysis to verify the consistency of our comparative genomic approach. OrthoVenn3 constructs phylogenetic trees by identifying highly conserved single-copy genes, which serve as independent evolutionary units for describing evolutionary relationships. Sequence alignment was performed using Muscle (Edgar 2004), and trimAl (Capella-Gutiérrez et al. 2009) was used to extract and trim conserved regions. FastTree2 (Price et al. 2010) was then employed to infer the phylogenetic tree using the maximum likelihood method with the LG + CAT model. The SH test was employed to assess the reliability of each node. The resulting tree (Figure S3) aligned with previously known evolutionary relationships (De Panis et al. 2021), thereby validating the reliability of the dataset and analysis pipeline. Finally, the platform relies on CAFE5 (Mendes et al. 2021) to analyse gene family expansions and contractions relative to the common ancestor, leveraging an ultrametric tree to statistically infer changes in gene family sizes across evolutionary lineages while accounting for phylogenetic history (e.g., Martinů et al. 2024). We used divergence times obtained from TimeTree (http://timetree.org), including: Chicken-Condors, 91 MYA; Chicken-OW vultures, 91 MYA; Andean condor-California condor, 9.5 MYA; and Himalayan vulture-Bearded vulture, 43 MYA. Expanded gene families reflect gene duplications, while contracted families may indicate gene losses, providing insights into lineage-specific adaptations. For each node in the tree (Figure S4), the analysis identifies expanded and contracted gene families and performs a Gene Ontology (GO) enrichment analysis to highlight overrepresented terms. We used significantly enriched GO terms ($p < 0.05$) to describe the functional context of the observed gene family dynamics and shed light on potential evolutionary pressures shaping vulture genomes. It is worth noting that some GO terms may be derived from mammalian annotations, which may not fully capture avian-specific processes but still provide valuable insights into conserved functions. All plots were generated using R v4.4.

3 | Results and Discussion

3.1 | Chromosome-Scale Genome Assembly of the Andean Condor

Our assembly pipeline (Figure 1a) produced a genome of 1,190,358,635 bp, with 90% contained in 18 chromosome-length scaffolds (L90) and N50 of 84.9 Mbp. The single-copy gene content analysis using the Aves database with BUSCO resulted in 97.4% completeness (97.2% single and 0.2% duplicated). 96.94% of Illumina WGS reads k-mers were present in the assembly, and the base accuracy Quality Value (QV) was 59.66 (Figure 1b, Figure S5). Moreover, we obtained 30 chromosome-length scaffolds, referred to as SUPER scaffolds (Figure 1c): six large-sized (> 50 Mbp; SUPER 1 to 5 and Z), nine medium-sized (> 20 and ≤ 50 Mbp; SUPER 6 to 14), six small-sized (> 10 and ≤ 20 Mbp); and nine microchromosome-sized scaffolds (≤ 10 Mbp). In addition, we found a strong collinearity between the Andean and California condor genomes (Figure S6). Overall, our results establish this assembly reference benchmark for comparative and population genomic studies.

3.2 | Contrasting Population Histories of Condor Species

Genome-wide heterozygosity in Andean condors (AnCo1: 0.656 He/Kbp; AnCo2: 0.634 He/Kbp) was circa 1.5-fold lower than in California condors (CaCo1: 1.005 He/Kbp; CaCo2: 0.931 He/Kbp; Figure 2a). Similarly, the Andean condor genome-wide nucleotide diversity (π) was lower compared to the California condor ($6.73e^{-4}$ vs. $1.09e^{-3}$; Figure S7). At the same time, California condors displayed more extensive runs of homozygosity (RoH), indicating higher levels of inbreeding: on average, RoH covered approximately 40% of their genomes, roughly 2.8-fold higher than in Andean condors (Figure 2b). The proportion of long RoH segments (> 5 Mbp) was also notably greater in California condors (CaCo1: 8.95%; CaCo2: 5.64%), particularly in CaCo1, where segments exceeding 10 Mb covered 5.04% of the genome. In contrast, Andean condors exhibited fewer and predominantly shorter RoH regions, with long segments comprising only about 1.43% of their genomes on average. Overall, the different genetic patterns observed in Andean condors compared to California condors, evidenced by reduced nucleotide diversity, decreased heterozygosity, and shorter RoH elements, reflects distinct evolutionary histories, involving both recent/historic and ancient population bottlenecks.

Our estimates of recent-historic inbreeding events, based on the presence and length of RoH and the Time to Most Recent Common Ancestor (Table S4) revealed distinct demographic patterns between species. In Andean condors, the most recent inbreeding signals of long RoH elements (accounting for approximately 12% and 7% of total RoH in AnCo1 and AnCo2, respectively) predominantly date to the early-to-mid-19th century onward, while the majority of short RoH (approximately 67%) reflect historical inbreeding events around the 15th century

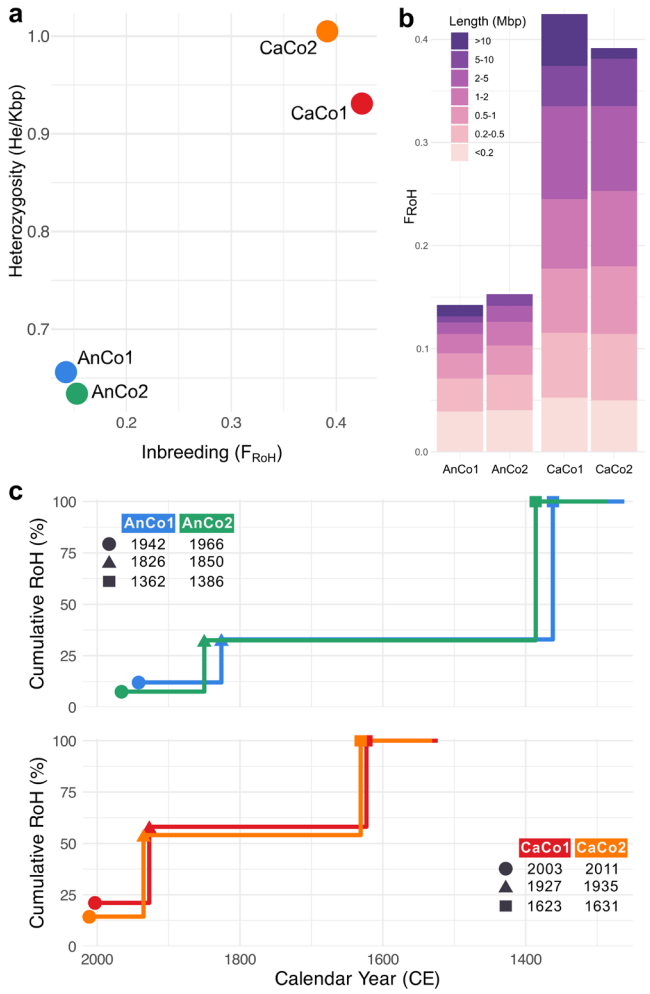

FIGURE 2 | Genome-wide estimations in condors. (a) Genome-wide heterozygosity and inbreeding levels calculated as the fraction of the genome in RoH (F_{RoH}): Andean condor individuals (AnCo1 and AnCo2 in blue and green, respectively) show lower inbreeding and average heterozygosity levels compared to California condor samples (CaCo1 and CaCo2 in orange and red, respectively). (b) Length distribution of the RoH (Mbp) expressed as fractions of the genome in Andean and California condor individuals. (c) Recent inbreeding history inferred from RoH analyses. Step-curves show the cumulative percentage of each individual's genome contained in RoH elements across time. The circles denote each individual's birth year, pointing to the start of the recent inbreeding window (< 4 gens). The triangles depict the start of the historic events (4–20 gens), while the squares indicate the transition to earlier times (> 20 gens). RoH classes were converted to calendar years using IUCN-estimated generation times of 29 and 19 years for Andean and California condors, respectively.

(Figure 2c). California condors exhibited more pronounced recent inbreeding, with long RoH elements (accounting for 14%–21% of total count) accumulating during the early-to-mid-20th century. Intermediate-length segments (37%–40%) can be traced back to the 17th century, while the shortest RoH segments (42%–46%) seem to have originated prior to this time (Figure 2c). These results align with previous studies using historical samples from museum specimens, which identified two potentially important bottleneck periods in both condor species (Padró et al. 2020). The recent decline of Andean condors during the mid-19th to 20th century coincides with the local extinction of the coastal population from eastern Patagonia around that time, while the historic bottleneck of the species is possibly related to European colonisation and the expansion of livestock production in South America during the 17th–18th centuries. Similarly, in California condors, the recent RoH signals match their documented population collapse in the early 20th century, numbering just 22 individuals by 1982 (D'Elia et al. 2016; BirdLife International 2021), while the historic bottlenecks align with the introduction of cattle in California during the 18th century and the commercial over-hunting of marine mammals, a key food source until the late 18th century (Chamberlain et al. 2005; Padró et al. 2020).

Our PSMC demographic analyses provided additional insights into the ancient processes that shaped long-term population trends. Over 1 million years ago, California condor populations were larger (Figure S8) and more genetically diverse than those of Andean condors (D'Elia et al. 2016; Padró et al. 2020). This aligns with fossil evidence showing that California Condors once had a much broader distribution across North America, with Pleistocene records from as far south as Nuevo León (Mexico), extending east to Florida and as far north as New York (Finkelstein et al. 2020), highlighting the species' potential historical adaptability and expansive range. However, the California condor experienced substantial declines during the Middle Pleistocene Transition (approximately 1.25–0.7 Mya), followed by a mid–Pleistocene rebound and sharp reductions from approximately 100,000 to 10,000 years ago (Figure S8), possibly linked to the intensification of Laurentide and Cordilleran glaciations and megafaunal extinctions (Barnosky et al. 2016; Perrig et al. 2019; Tyrberg 2008). These events ultimately contributed to the species' recent population collapse. Meanwhile, Andean condor populations exhibited smaller but relatively stable effective sizes (Figure S8), possibly related to the species' retreat to northern South America during glaciation periods, where carrying capacity and suitable habitat are smaller than in the high Andes in the south of the continent (Naveda-Rodríguez et al. 2016; Padró et al. 2020, 2023).

Overall, the observed patterns align with predictions about how ancestral demography influences extinction risk in wild populations (Kyriazis et al. 2021). Simulation studies show that ancestrally large populations, such as the California condor, often retain higher levels of deleterious variation. When these populations undergo severe contractions, these mutations can drift to homozygosity, unmasking recessive load (van der Valk et al. 2021), such as the recessive chondrodystrophy allele found at high frequency in the remnant population of California condors (Ralls et al. 2000), accelerating extinction risks (Kyriazis et al. 2021). Thus, intensive genetic management remains essential for this species to maximise diversity while minimising mutational load. In contrast, the Andean condor's genomic signatures suggest that its long-term persistence at smaller effective population sizes may have purged many deleterious alleles. However, further population studies are needed to confirm this as hidden recessive mutations can persist in heterozygous form. Importantly, genetic structuring exists across the vast species' range, especially between northern and southern South America (Hendrickson et al. 2003; Padró et al. 2020). The northern region comprises only a few hundred individuals and shows signs of spatial isolation (Naveda-Rodríguez et al. 2016; Padró et al. 2023), while the

southern region sustains larger population sizes totalling thousands of individuals, more interconnected (Padró et al. 2018, 2025; Birdlife International 2021). Thus, developing strategies to enhance population connectivity while characterising genomic variation across populations is critical before implementing translocation programmes. Without proper genomic screening, management efforts risk inadvertently introducing recessive deleterious alleles from larger source populations into small vulnerable groups, a scenario exemplified by Isle Royale wolves (*Canis lupus*), where a migrant from a large population seems to have introduced harmful genetic variation, leading to a catastrophic decline (Robinson et al. 2019).

3.3 | Evolutionary Insights of Genetic Expansions and Contractions

We found a divergent pattern of genetic expansion and contraction in the Andean condor and California condor, relative to their common ancestor, with significant enrichments in functional terms related to high-altitude adaptation, flight efficiency, energy metabolism, development, immune function and detoxification (Figure 3; Table S5).

The Andean condor showed gene expansions related to proteins involved in iron homeostasis (Mitoferrin, GO:0055072), development (MTA3, GO:0010971) and oxidative stress (Glutathione S-transferase; GO:0004364), broadly consistent with the physiological demands of sustained flight and cellular resilience under hypoxic, high-UV conditions (e.g., Singh et al. 2001; Troadec et al. 2011). In contrast, gene family contractions were related to proteins involved in the immune system (Toll-like receptor 2, GO:0002224; Ig heavy chain V, GO:0003823) and detoxification mechanisms (CYP2C8, GO:0006805; glucuronosyltransferase, GO:0052695; E3 ubiquitin-protein ligase, GO:0046685). In addition, homeostatic processes (Endonuclease 8-like 1, GO:000697), muscle development, and vascular systems were also contracted (zinc finger proteins; GO:0001944; ubiquitin-protein ligase PHF7;

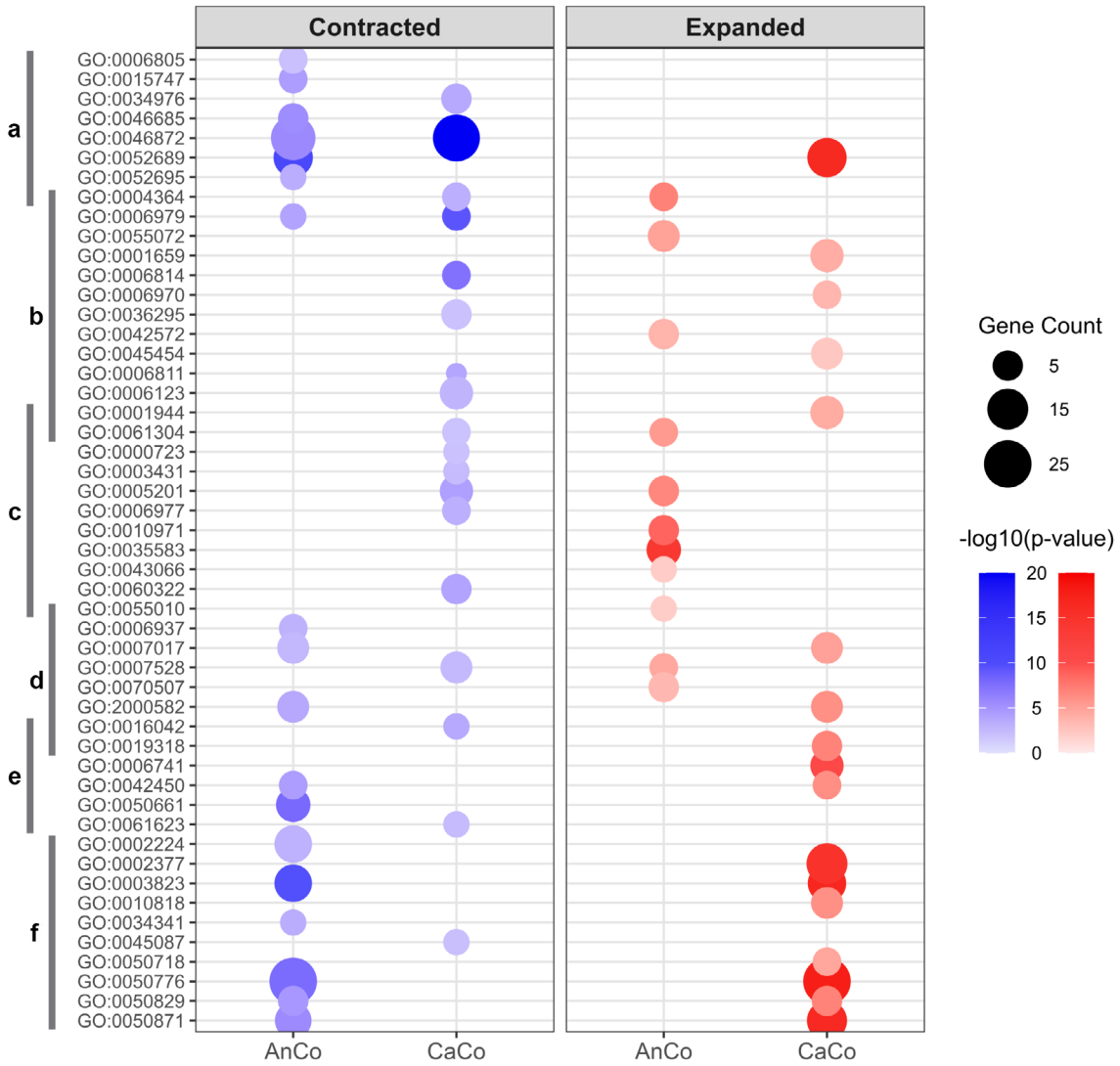

FIGURE 3 | Gene Ontology (GO) IDs enriched in expanded and contracted genes of the Andean (AnCo) and California (CaCo) condors compared to their common ancestor. Out of the 284 unique GO terms retrieved in the analysis, 120 were associated with categories relevant to condor biology and ecology: (a) detoxification/stress, (b) high-altitude adaptation, (c) development, (d) flight, (e) metabolism and (f) immunity. The plot depicts 50 relevant terms.

GO:0046872). On the other hand, the California condor exhibited expansions in genes associated with temperature homeostasis (Arrestin ARRDC3; GO:0001659), possibly reflecting adaptation to its warmer, lower-altitude habitat. In mammals, for example, reduced expression of ARRDC3 leads to increased thermogenesis and energy expenditure (Patwari et al. 2011); thus, its expansion in California condors could suggest a shift toward reduced thermogenic activity and energy conservation under less thermally demanding conditions. Genomic expansion in California condors was especially marked in the immune system (Ig lambda chain V-1, GO:0002377; Ig heavy chain V, GO:0003823; CCL3, GO:0010818; Hexokinase-1, GO:0050718; Gallinacin-7, GO:0050829) which may reflect adaptations to the mammalian diversity in North America, potentially increasing their exposure to a broader spectrum of pathogens (e.g., Marshall 1988; Qiu et al. 2024). Genetic contractions were related to morphological development (Interleukin-11 receptor, GO:0060322), ion transport, and lipid processing (solute carrier family 22; GO:0006811; Arylacetamide deacetylase, GO:0052689) related to the metabolism of xenobiotics and endogenous compounds (e.g., Koepsell 2013). A noticeable gene contraction related to cartilage chondrocyte development (COL27A1: Collagen alpha-1, GO:0003431) was previously associated with chondrodysplasia in mammals (Gonzaga-Jauregui et al. 2015) and thereby possibly important for the genetic management of chondrodystrophy in California condors (Ralls et al. 2000).

Our analyses of general comparative trends between New World (NW) and Old World (OW) vultures suggest potential differences in eco-physiological processes related to detoxification functions and diversification processes (Figure S9; Table S6). For example, OW vultures exhibited expansion of cell-adhesion components (Protocadherin gamma-B5, GO:0007156) and structural proteins like feather keratin (Feather keratin Cos2-3, GO:0005200) recently shown to be involved in the diversification of Banded Penguins (León et al. 2024). Genetic contractions involved detoxification pathways, such as glutathione transferase (Glutathione S-transferase B, GO:0004364) and metalloaminopeptidase enzymes (Xaa-Pro aminopeptidases, GO:0070006), which could potentially align with their documented sensitivity to pharmaceutical toxins such as Non-Steroidal Anti-Inflammatory Drugs (Swan et al. 2006; Ayuso et al. 2021). In contrast, NW vultures showed expansions in sensory signalling pathways, including olfactory receptors (Olfactory receptors 5AS1-14C36, GO:0007608; Olfactory receptors 10A7-C1, GO:0004984) previously suggested to be involved in the adaptive evolution of other avian species, including raptors (e.g., Doyle et al. 2019; Yang et al. 2025). Genomic contractions in NW species were mostly related to the immune system (e.g., MHC class I, GO:0006955) widely associated with avian resistance to pathogens (e.g., Jones et al. 2015; Aguilar et al. 2016). Overall, our analyses highlighted important candidate processes of adaptation, yet it is important to note that neutral processes such as genetic drift may also underlie these gene-family shifts. Thus, future functional assays will be essential to validate the adaptive significance of these patterns.

4 | Conclusion

Our study represents a significant step forward in understanding the genomic architecture of the Andean condor and provides critical insights into the evolutionary divergence between condor species and vulture families. The chromosome-scale reference genome we developed reveals exceptional synteny between the Andean and California condor genomes, underscoring their close evolutionary relationship. The genomic divergence between condor species and vulture families further suggests the influence of habitat and life-history traits, offering new perspectives on how parallel and divergent evolutionary processes may have shaped avian scavenger lineages. Beyond these evolutionary insights, our findings provide essential tools for advancing conservation genomics. This critical resource establishes a benchmark for defining management units, enabling the development of continent-scale conservation strategies. By enabling assessments of fine-scale genetic relationships, local adaptations, RoH hotspots and genomic offsets, it lays the foundation for evidence-based management approaches, including smart-breeding programs, population reinforcement, reintroductions, and genetic rescue. These efforts are critical for ensuring the long-term survival of this iconic scavenger and its vital role in ecosystem health.

Author Contributions

D.D.P., S.A.L., H.D. and J.P. conceived the ideas; G.W. collected the biological sample; D.D.P. performed laboratory procedures; F.L.D. and M.M.-R. performed and supported the Hi-C experiment; M.G., T.S.A. and I.G. prepared libraries, performed sequencing and provided sequencing support; C.J.M. provided bioinformatic resources and data analysis support; D.D.P. analysed the data; J.P. assisted in data analysis. D.D.P. led the writing with J.P. All authors reviewed the final version of the manuscript and gave approval for publication.

Acknowledgements

We thank A. Navarro for helping in this study, and also A. Najenson, C. Corio, J. Ibarra, C. Krenek, and M.B. Ceballos for their assistance during sample and permit processing. We thank T. Carrasco-Valenzuela and L. Arantes for their support on preliminary masking and demographic analysis, respectively. This research was supported by the National Council of Scientific and Technical Research of Argentina (CONICET) and funded by the National Agency of Scientific and Technological Promotion (PICT 2020-00132). International mobility funds were provided by the Marie Curie IRSES Project DEANN (PIRSES-GA-2013-612583). We thank IAATE and Alexander von Humboldt-Stiftung for their support to S.A.L. and J.P. The study was conducted according to the guidelines of the Comité Institucional de Cuidado y Uso de Animales de Laboratorio (CICUAL) of the Facultad de Ciencias Veterinarias-Universidad de Buenos Aires, approved by resolution no. 2013/44. Sample permits were issued by the Argentinean Environmental Ministry under CITES permit no. 1540531. The authors would like to thank the HPC Service of FUB-IT, Freie Universität Berlin, for computing time (https://doi.org/10.17169/refubium-26754).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All raw data and results presented in this manuscript are publicly available, with detailed descriptions and accession numbers provided in the main text and Supporting Information. Additional code and data are available in the GitHub repository: github.com/diegomics/bVulGry.

References

Aguilar, J. R. D., H. Westerdahl, J. M. D. L. Puente, G. Tomás, J. Martínez, and S. Merino. 2016. "MHC-I Provides Both Quantitative Resistance and Susceptibility to Blood Parasites in Blue Tits in the Wild." *Journal of Avian Biology* 47, no. 5: 669–677. https://doi.org/10.1111/jav.00830.

Alarcón, P. A. E., J. M. Morales, J. A. Donázar, J. A. Sánchez-Zapata, F. Hiraldo, and S. A. Lambertucci. 2017. "Sexual-Size Dimorphism Modulates the Trade-Off Between Exploiting Food and Wind Resources in a Large Avian Scavenger." *Scientific Reports* 7: 11461. https://doi.org/10.1038/s41598-017-11855-0.

Alonge, M., L. Lebeigle, M. Kirsche, et al. 2022. "Automated Assembly Scaffolding Using RagTag Elevates a New Tomato System for High-Throughput Genome Editing." *Genome Biology* 23, no. 1: 258. https://doi.org/10.1186/s13059-022-02823-7.

Astashyn, A., E. S. Tvedte, D. Sweeney, et al. 2024. "Rapid and Sensitive Detection of Genome Contamination at Scale With FCS-GX." *Genome Biology* 25, no. 1: 60. https://doi.org/10.1186/s13059-024-03198-7.

Astore, V., R. Estrada, and N. L. Jácome. 2017. "Reintroduction Strategy for the Andean Condor Conservation Program, Argentina." *International Zoo Yearbook* 51, no. 1: 124–136. https://doi.org/10.1111/izy.12140.

Ayuso, P., E. García-Martín, and J. A. Agúndez. 2021. "Variability of the Genes Involved in the Cellular Redox Status and Their Implication in Drug Hypersensitivity Reactions." *Antioxidants* 10, no. 2: 294. https://doi.org/10.3390/antiox10020294.

Barnosky, A. D., E. L. Lindsey, N. A. Villavicencio, et al. 2016. "Variable Impact of Late-Quaternary Megafaunal Extinction in Causing Ecological State Shifts in North and South America." *Proceedings of the National Academy of Sciences of the United States of America* 113, no. 4: 856–861. https://doi.org/10.1073/pnas.1505295112.

BirdLife International. 2021. *Red List Update: The Plight of the Condor*. BirdLife International. https://datazone.birdlife.org/species/factsheet/andean-condor-vultur-gryphus.

Buechley, E. R., and Ç. H. Şekercioğlu. 2016. "The Avian Scavenger Crisis: Looming Extinctions, Trophic Cascades, and Loss of Critical Ecosystem Functions." *Biological Conservation* 198: 220–228. https://doi.org/10.1016/j.biocon.2016.04.001.

Bukhman, Y. V., P. A. Morin, S. Meyer, et al. 2024. "A High-Quality Blue Whale Genome, Segmental Duplications, and Historical Demography." *Molecular Biology and Evolution* 41, no. 3: msae036. https://doi.org/10.1093/molbev/msae036.

Cabanettes, F., and C. Klopp. 2018. "D-GENIES: Dot Plot Large Genomes in an Interactive, Efficient and Simple Way." *PeerJ* 6: e4958. https://doi.org/10.7717/peerj.4958.

Capella-Gutiérrez, S., J. M. Silla-Martínez, and T. Gabaldón. 2009. "trimAl: A Tool for Automated Alignment Trimming in Large-Scale Phylogenetic Analyses." *Bioinformatics* 25, no. 15: 1972–1973. https://doi.org/10.1093/bioinformatics/btp348.

Challis, R., S. Kumar, C. Sotero-Caio, M. Brown, and M. Blaxter. 2023. "Genomes on a Tree (GoaT): A Versatile, Scalable Search Engine for Genomic and Sequencing Project Metadata Across the Eukaryotic Tree of Life." *Wellcome Open Research* 8: 24. https://doi.org/10.12688/wellcomeopenres.18658.1.

Chamberlain, C. P., J. R. Waldbauer, K. Fox-Dobbs, et al. 2005. "Pleistocene to Recent Dietary Shifts in California Condors." *Proceedings of the National Academy of Sciences of the United States of America* 102, no. 46: 16707–16711. https://doi.org/10.1073/pnas.0508529102.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. "Fastp: An Ultra-Fast All-In-One FASTQ Preprocessor." *Bioinformatics* 34, no. 17: i884–i890. https://doi.org/10.1093/bioinformatics/bty560.

Chen, Y., Z. Jiang, P. Fan, et al. 2022. "The Combination of Genomic Offset and Niche Modelling Provides Insights Into Climate Change-Driven Vulnerability." *Nature Communications* 13, no. 1: 4821. https://doi.org/10.1038/s41467-022-32546-z.

Coombe, L., J. X. Li, T. Lo, et al. 2021. "LongStitch: High-Quality Genome Assembly Correction and Scaffolding Using Long Reads." *BMC Bioinformatics* 22, no. 1: 534. https://doi.org/10.1186/s12859-021-04451-7.

Danecek, P., A. Auton, G. Abecasis, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27, no. 15: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

de Jong, M. J., A. Niamir, M. Wolf, et al. 2023. "Range-Wide Whole-Genome Resequencing of the Brown Bear Reveals Drivers of Intraspecies Divergence." *Communications Biology* 6, no. 1: 1–16. https://doi.org/10.1038/s42003-023-04514-w.

De Panis, D. 2024. *ERGA-BGE Genome Report ASM Analyses (One-Asm WGS Illumina PE + HiC)*. WorkflowHub. https://doi.org/10.48546/WORKFLOWHUB.WORKFLOW.1103.2.

De Panis, D., S. A. Lambertucci, G. Wiemeyer, et al. 2021. "Mitogenomic Analysis of Extant Condor Species Provides Insight Into the Molecular Evolution of Vultures." *Scientific Reports* 11, no. 1: 17109. https://doi.org/10.1038/s41598-021-96080-6.

Del Hoyo, J., A. Elliot, and J. Sargatal. 1994. *Handbook of the Birds of the World*. Vol. 1–2. Lynx Edicions.

D'Elia, J., S. M. Haig, T. D. Mullins, and M. P. Miller. 2016. "Ancient DNA Reveals Substantial Genetic Diversity in the California Condor (*Gymnogyps californianus*) Prior to a Population Bottleneck." *Condor* 118, no. 4: 703–714.

der Auwera, G. A. V., and B. D. O'Connor. 2020. *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra*. O'Reilly Media, Inc.

Donázar, J. A., A. Travaini, O. Ceballos, A. Rodríguez, M. Delibes, and F. Hiraldo. 1999. "Effects of Sex-Associated Competitive Asymmetries on Foraging Group Structure and Despotic Distribution in Andean Condors." *Behavioral Ecology and Sociobiology* 45, no. 1: 55–65. https://doi.org/10.1007/s002650050539.

Doyle, J. M., J. R. Willoughby, D. A. Bell, et al. 2019. "Elevated Heterozygosity in Adults Relative to Juveniles Provides Evidence of Viability Selection on Eagles and Falcons." *Journal of Heredity* 110, no. 6: 696–706. https://doi.org/10.1093/jhered/esz048.

Durand, N. C., J. T. Robinson, M. S. Shamim, et al. 2016. "Juicebox Provides a Visualization System for Hi-C Contact Maps With Unlimited Zoom." *Cell Systems* 3, no. 1: 99–101. https://doi.org/10.1016/j.cels.2015.07.012.

Durand, N. C., M. S. Shamim, I. Machol, et al. 2016. "Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments." *Cell Systems* 3, no. 1: 95–98. https://doi.org/10.1016/j.cels.2016.07.002.

Edgar, R. C. 2004. "MUSCLE: Multiple Sequence Alignment With High Accuracy and High Throughput." *Nucleic Acids Research* 32, no. 5: 1792–1797. https://doi.org/10.1093/nar/gkh340.

Emms, D. M., and S. Kelly. 2019. "OrthoFinder: Phylogenetic Orthology Inference for Comparative Genomics." *Genome Biology* 20, no. 1: 238. https://doi.org/10.1186/s13059-019-1832-y.

Ferguson-Lees, J., and D. A. Christie. 2001. *Raptors of the World*. Houghton Mifflin Harcourt.

Finkelstein, M., Z. Kuspa, N. F. Snyder, and N. J. Schmitt. 2020. "California Condor (*Gymnogyps californianus*), Version 1.0." In *Birds of the World*, edited by P. G. Rodewald. Cornell Lab of Ornithology. https://doi.org/10.2173/bow.calcon.01.

Flynn, J. M., R. Hubley, C. Goubert, et al. 2020. "RepeatModeler2 for Automated Genomic Discovery of Transposable Element Families." *Proceedings of the National Academy of Sciences of the United States of America* 117, no. 17: 9451–9457. https://doi.org/10.1073/pnas.1921046117.

Formenti, G., L. Abueg, A. Brajuka, et al. 2022. "Gfastats: Conversion, Evaluation and Manipulation of Genome Sequences Using Assembly Graphs." *Bioinformatics* 38, no. 17: 4214–4216. https://doi.org/10.1093/bioinformatics/btac460.

Formenti, G., K. Theissinger, C. Fernandes, et al. 2022. "The Era of Reference Genomes in Conservation Genomics." *Trends in Ecology & Evolution* 37, no. 3: 197–202. https://doi.org/10.1016/j.tree.2021.11.008.

Gonzaga-Jauregui, C., C. N. Gamble, B. Yuan, et al. 2015. "Mutations in COL27A1 Cause Steel Syndrome and Suggest a Founder Mutation Effect in the Puerto Rican Population." *European Journal of Human Genetics* 23, no. 3: 342–346. https://doi.org/10.1038/ejhg.2014.107.

Guan, D., S. A. McCarthy, J. Wood, K. Howe, Y. Wang, and R. Durbin. 2020. "Identifying and Removing Haplotypic Duplication in Primary Genome Assemblies." *Bioinformatics* 36, no. 9: 2896–2898. https://doi.org/10.1093/bioinformatics/btaa025.

Hendrickson, S. L., R. Bleiweiss, J. C. Matheus, L. S. de Matheus, N. L. Jácome, and E. Pavez. 2003. "Low Genetic Variability in the Geographically Widespread Andean Condor." *Condor* 105, no. 1: 1. https://doi.org/10.1093/condor/105.1.1.

Holley, G., D. Beyter, H. Ingimundardottir, et al. 2021. "Ratatosk: Hybrid Error Correction of Long Reads Enables Accurate Variant Calling and Assembly." *Genome Biology* 22, no. 1: 28. https://doi.org/10.1186/s13059-020-02244-4.

Houston, D. C., and J. E. Cooper. 1975. "The Digestive Tract of the Whiteback Griffon Vulture and Its Role in Disease Transmission Among Wild Ungulates." *Journal of Wildlife Diseases* 11, no. 3: 306–313. https://doi.org/10.7589/0090-3558-11.3.306.

Jones, M. R., Z. A. Cheviron, and M. D. Carling. 2015. "Spatially Variable Coevolution Between a Haemosporidian Parasite and the MHC of a Widely Distributed Passerine." *Ecology and Evolution* 5, no. 5: 1045–1060. https://doi.org/10.1002/ece3.1391.

Kajitani, R., K. Toshimoto, H. Noguchi, et al. 2014. "Efficient De Novo Assembly of Highly Heterozygous Genomes From Whole-Genome Shotgun Short Reads." *Genome Research* 24, no. 8: 1384–1395. https://doi.org/10.1101/gr.170720.113.

Kirilenko, B. M., C. Munegowda, E. Osipova, et al. 2023. "Integrating Gene Annotation With Orthology Inference at Scale." *Science* 380: 3107. https://doi.org/10.1126/science.abn3107.

Koepsell, H. 2013. "The SLC22 Family With Transporters of Organic Cations, Anions and Zwitterions." *Molecular Aspects of Medicine* 34, no. 2–3: 413–435. https://doi.org/10.1016/j.mam.2012.10.010.

Kyriazis, C. C., R. K. Wayne, and K. E. Lohmueller. 2021. "Strongly Deleterious Mutations Are a Primary Determinant of Extinction Risk due to Inbreeding Depression." *Evolution Letters* 5, no. 1: 33–47. https://doi.org/10.1002/evl3.209.

Lambertucci, S. A. 2007. "Biología y Conservación del Cóndor Andino (*Vultur gryphus*) en Argentina." *El Hornero* 22, no. 2: 760. https://doi.org/10.56178/eh.v22i2.760.

Lambertucci, S. A., P. A. E. Alarcón, F. Hiraldo, J. A. Sanchez-Zapata, G. Blanco, and J. A. Donázar. 2014. "Apex Scavenger Movements Call for Transboundary Conservation Policies." *Biological Conservation* 170: 145–150. https://doi.org/10.1016/j.biocon.2013.12.041.

Lambertucci, S. A., N. Luis Jácome, and A. Trejo. 2008. "Use of Communal Roosts by Andean Condors in Northwest Patagonia, Argentina." *Journal of Field Ornithology* 79, no. 2: 138–146. https://doi.org/10.1111/j.1557-9263.2008.00155.x.

Lawler, J. J., D. White, J. C. Sifneos, and L. L. Master. 2003. "Rare Species and the Use of Indicator Groups for Conservation Planning." *Conservation Biology* 17, no. 3: 875–882. https://doi.org/10.1046/j.1523-1739.2003.01638.x.

León, F., E. Pizarro, D. Noll, et al. 2024. "Comparative Genomics Supports Ecologically Induced Selection as a Putative Driver of Banded Penguin Diversification." *Molecular Biology and Evolution* 41, no. 9: msae166. https://doi.org/10.1093/molbev/msae166.

Li, H. 2011. "A Statistical Framework for SNP Calling, Mutation Discovery, Association Mapping and Population Genetical Parameter Estimation From Sequencing Data." *Bioinformatics* 27, no. 21: 2987–2993. https://doi.org/10.1093/bioinformatics/btr509.

Li, H., and R. Durbin. 2011. "Inference of Human Population History From Individual Whole-Genome Sequences." *Nature* 475, no. 7357: 493–496. https://doi.org/10.1038/nature10231.

Lieberman, A., J. V. Rodriguez, J. M. Paez, and J. Wiley. 1993. "The Reintroduction of the Andean Condor Into Colombia, South America: 1989–1991." *Oryx* 27, no. 2: 83–90. https://doi.org/10.1017/S0030605300020603.

Manni, M., M. R. Berkeley, M. Seppey, F. A. Simão, and E. M. Zdobnov. 2021. "BUSCO Update: Novel and Streamlined Workflows Along With Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes." *Molecular Biology and Evolution* 38, no. 10: 4647–4654. https://doi.org/10.1093/molbev/msab199.

Marshall, L. G. 1988. "Land Mammals and the Great American Interchange." *American Scientist* 76: 380–388.

Martinů, J., H. Tarabai, J. Štefka, and V. Hypša. 2024. "Highly Resolved Genomes of Two Closely Related Lineages of the Rodent Louse *Polyplax serrata* With Different Host Specificities." *Genome Biology and Evolution* 16, no. 3: evae045. https://doi.org/10.1093/gbe/evae045.

McDonald, P. G., P. D. Olsen, and A. Cockburn. 2005. "Selection on Body Size in a Raptor With Pronounced Reversed Sexual Size Dimorphism: Are Bigger Females Better?" *Behavioral Ecology* 16, no. 1: 48–56. https://doi.org/10.1093/beheco/arh118.

Mendes, F. K., D. Vanderpool, B. Fulton, and M. W. Hahn. 2021. "CAFE 5 Models Variation in Evolutionary Rates Among Gene Families." *Bioinformatics* 36, no. 22–23: 5516–5518. https://doi.org/10.1093/bioinformatics/btaa1022.

Mouillot, D., D. R. Bellwood, C. Baraloto, et al. 2013. "Rare Species Support Vulnerable Functions in High-Diversity Ecosystems." *PLoS Biology* 11, no. 5: e1001569. https://doi.org/10.1371/journal.pbio.1001569.

Naveda-Rodríguez, A., F. H. Vargas, S. Kohn, and G. Zapata-Ríos. 2016. "Andean Condor (*Vultur gryphus*) in Ecuador: Geographic Distribution, Population Size and Extinction Risk." *PLoS One* 11, no. 3: e0151827. https://doi.org/10.1371/journal.pone.0151827.

Nevers, Y., A. Warwick Vesztrocy, V. Rossier, et al. 2025. "Quality Assessment of Gene Repertoire Annotations With OMArk." *Nature Biotechnology* 43, no. 1: 124–133. https://doi.org/10.1038/s41587-024-02147-w.

Orians, G. H. 1997. "Evolved Consequences of Rarity." In *The Biology of Rarity: Causes and Consequences of Rare—Common Differences*, edited by W. E. Kunin and K. J. Gaston, 190–208. Springer. https://doi.org/10.1007/978-94-011-5874-9_11.

Padró, J., S. A. Lambertucci, P. Alarcon, et al. 2025. "Energy Landscape and Life-History Requirements Shape Habitat Use in an Extreme Soaring Specialist." *Landscape Ecology*. https://doi.org/10.1007/s10980-025-02137-6.

Padró, J., S. A. Lambertucci, P. L. Perrig, and J. N. Pauli. 2018. "Evidence of Genetic Structure in a Wide-Ranging and Highly Mobile Soaring Scavenger, the Andean Condor." *Diversity and Distributions* 24, no. 11: 1534–1544. https://doi.org/10.1111/ddi.12786.

Padró, J., S. A. Lambertucci, P. L. Perrig, and J. N. Pauli. 2020. "Andean and California Condors Possess Dissimilar Genetic Composition but Exhibit Similar Demographic Histories." *Ecology and Evolution* 10, no. 23: 13011–13021. https://doi.org/10.1002/ece3.6887.

Padró, J., J. N. Pauli, P. L. Perrig, and S. A. Lambertucci. 2019. "Genetic Consequences of Social Dynamics in the Andean Condor: The Role of Sex and Age." *Behavioral Ecology and Sociobiology* 73, no. 7: 1–11.

Padró, J., F. H. Vargas, S. A. Lambertucci, et al. 2023. "Demographic Collapse Threatens the Long-Term Persistence of Andean Condors in the Northern Andes." *Biological Conservation* 285: 110217. https://doi.org/10.1016/j.biocon.2023.110217.

Patwari, P., V. Emilsson, E. E. Schadt, et al. 2011. "The Arrestin Domain-Containing 3 Protein Regulates Body Mass and Energy Expenditure." *Cell Metabolism* 14, no. 5: 671–683. https://doi.org/10.1016/j.cmet.2011.08.011.

Perrig, P. L., E. D. Fountain, S. A. Lambertucci, and J. N. Pauli. 2019. "Demography of Avian Scavengers After Pleistocene Megafaunal Extinction." *Scientific Reports* 9, no. 1: 9680. https://doi.org/10.1038/s41598-019-45769-w.

Perrig, P. L., S. A. Lambertucci, P. A. E. Alarcón, et al. 2021. "Limited Sexual Segregation in a Dimorphic Avian Scavenger, the Andean Condor." *Oecologia* 196, no. 1: 77–88. https://doi.org/10.1007/s00442-021-04909-8.

Plaza, P. I., and S. A. Lambertucci. 2020. "Ecology and Conservation of a Rare Species: What Do We Know and What May We Do to Preserve Andean Condors?" *Biological Conservation* 251: 108782. https://doi.org/10.1016/j.biocon.2020.108782.

Poplin, R., P.-C. Chang, D. Alexander, et al. 2018. "A Universal SNP and Small-Indel Variant Caller Using Deep Neural Networks." *Nature Biotechnology* 36, no. 10: 983–987. https://doi.org/10.1038/nbt.4235.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. "FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments." *PLoS One* 5, no. 3: e9490. https://doi.org/10.1371/journal.pone.0009490.

Qiu, Y., C. Lv, J. Chen, et al. 2024. "The Global Distribution and Diversity of Wild-Bird-Associated Pathogens: An Integrated Data Analysis and Modeling Study." *Medicine* 6: 100553. https://doi.org/10.1016/j.medj.2024.11.006.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing (Version 4.4.1) [Computer Software]*. R Foundation for Statistical Computing. https://www.R-project.org.

Ralls, K., J. D. Ballou, B. A. Rideout, and R. Frankham. 2000. "Genetic Management of Chondrodystrophy in California Condors." *Animal Conservation* 3, no. 2: 145–153. https://doi.org/10.1111/j.1469-1795.2000.tb00239.x.

Rao, S. S. P., M. H. Huntley, N. C. Durand, et al. 2014. "A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping." *Cell* 159, no. 7: 1665–1680. https://doi.org/10.1016/j.cell.2014.11.021.

Rhie, A., B. P. Walenz, S. Koren, and A. M. Phillippy. 2020. "Merqury: Reference-Free Quality, Completeness, and Phasing Assessment for Genome Assemblies." *Genome Biology* 21, no. 1: 245. https://doi.org/10.1186/s13059-020-02134-9.

Robinson, J. A., R. C. K. Bowie, O. Dudchenko, et al. 2021. "Genome-Wide Diversity in the California Condor Tracks Its Prehistoric Abundance and Decline." *Current Biology* 31, no. 13: 2939–2946.e5. https://doi.org/10.1016/j.cub.2021.04.035.

Robinson, J. A., J. Räikkönen, L. M. Vucetich, et al. 2019. "Genomic Signatures of Extensive Inbreeding in Isle Royale Wolves, a Population on the Threshold of Extinction." *Science Advances* 5, no. 5: eaau0757. https://doi.org/10.1126/sciadv.aau0757.

Roggenbuck, M., I. Bærholm Schnell, N. Blom, et al. 2014. "The Microbiome of New World Vultures." *Nature Communications* 5, no. 1: 5498. https://doi.org/10.1038/ncomms6498.

Shepard, E. L. C., and S. A. Lambertucci. 2013. "From Daily Movements to Population Distributions: Weather Affects Competitive Ability in a Guild of Soaring Birds." *Journal of the Royal Society Interface* 10, no. 88: 20130612. https://doi.org/10.1098/rsif.2013.0612.

Singh, S. N., P. Vats, M. M. L. Kumria, et al. 2001. "Effect of High Altitude (7,620 m) Exposure on Glutathione and Related Metabolism in Rats." *European Journal of Applied Physiology* 84: 233–237. https://doi.org/10.1007/s004210170010.

Sun, J., F. Lu, Y. Luo, L. Bie, L. Xu, and Y. Wang. 2023. "OrthoVenn3: An Integrated Platform for Exploring and Visualizing Orthologous Data Across Genomes." *Nucleic Acids Research* 51, no. W1: W397–W403. https://doi.org/10.1093/nar/gkad313.

Swan, G. E., R. Cuthbert, M. Quevedo, et al. 2006. "Toxicity of Diclofenac to Gyps Vultures." *Biology Letters* 2: 279–282. https://doi.org/10.1098/rsbl.2005.0425.

Takagi, N., and M. Sasaki. 1974. "A Phylogenetic Study of Bird Karyotypes." *Chromosoma* 46, no. 1: 91–120. https://doi.org/10.1007/BF00332341.

Theissinger, K., C. Fernandes, G. Formenti, et al. 2023. "How Genomics Can Help Biodiversity Conservation." *Trends in Genetics: TIG* 39, no. 7: 545–559. https://doi.org/10.1016/j.tig.2023.01.005.

Toone, W. D., and M. P. Wallace. 1994. *The Extinction in the Wild and Reintroduction of the California Condor (*Gymnogyps californianus*)*, edited by P. J. S. Olney, G. M. Mace, and A. T. C. Feistner, 411–419. Springer. https://doi.org/10.1007/978-94-011-0721-1_23.

Troadec, M. B., D. Warner, J. Wallace, et al. 2011. "Targeted Deletion of the Mouse Mitoferrin1 Gene: From Anemia to Protoporphyria." *Blood* 117, no. 20: 5494–5502. https://doi.org/10.1182/blood-2010-11-319483.

Tyrberg, T. 2008. "The Late Pleistocene Continental Avian Extinction—An Evaluation of the Fossil Evidence." *Oryctos* 7: 249–269.

van der Valk, T., M. de Manuel, T. Marques-Bonet, and K. Guschanski. 2021. "Estimates of Genetic Load Suggest Frequent Purging of Deleterious Alleles in Small Populations." *BioRxiv*: 696831. https://doi.org/10.1101/696831.

Vasimuddin, M., S. Misra, H. Li, and S. Aluru. 2019. *Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems*, 314–324. 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). https://doi.org/10.1109/IPDPS.2019.00041.

Wallace, M. 1985. *Ecological Studies of Andean Condors in Peru*. University of Wisconsin.

Wallace, M., and W. Toone. 1992. *Captive Management for the Long Term Survival of the California Condor*, edited by D. R. McCullough and R. H. Barrett, 766–774. Springer. https://doi.org/10.1007/978-94-011-2868-1_58.

Wallace, M., and S. A. Temple. 1987. "Releasing Captive-Reared Andean Condors to the Wild." *Journal of Wildlife Management* 51, no. 3: 541–550. https://doi.org/10.2307/3801266.

Wiegand, T. P., B. Gentry, Z. McCoy, et al. 2020. "Visualizing Connectivity of Ecological and Evolutionary Concepts—An Exploration of Research on Plant Species Rarity." *Ecology and Evolution* 10, no. 17: 9037–9047. https://doi.org/10.1002/ece3.6633.

Williams, H. J., E. L. C. Shepard, M. D. Holton, P. A. E. Alarcón, R. P. Wilson, and S. A. Lambertucci. 2020. "Physical Limits of Flight Performance in the Heaviest Soaring Bird." *Proceedings of the National Academy of Sciences* 117, no. 30: 17884–17890. https://doi.org/10.1073/pnas.1907360117.

Yang, C., Q. Wang, K. Sun, et al. 2025. "Whole-Genome Resequencing Landscape of Adaptive Evolution in Relict Gull (*Larus relictus*)." *BMC Genomics* 26, no. 1: 67. https://doi.org/10.1186/s12864-025-11257-x.

Zhou, C., S. A. McCarthy, and R. Durbin. 2023. "YaHS: Yet Another Hi-C Scaffolding Tool." *Bioinformatics* 39, no. 1: btac808. https://doi.org/10.1093/bioinformatics/btac808.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.