




Article

No Signs of Genetic Erosion in a 19th Century Genome of the Extinct Paradise Parrot (*Psephotellus pulcherrimus*)

Martin Irestedt ^{1,*}, Per G. P. Ericson ¹, Ulf S. Johansson ², Paul Oliver ^{3,4}, Leo Joseph ⁵
and Mozes P. K. Blom ^{1,6,*}

¹ Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden; per.ericson@nrm.se

² Department of Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden; ulf.johansson@nrm.se

³ Environmental Futures Research Institute, Griffith University, Nathan 4111, Australia; p.oliver@griffith.edu.au

⁴ Biodiversity and Geosciences Program, Queensland Museum, South Brisbane 4101, Australia

⁵ Australian National Wildlife Collection, CSIRO National Research Collections Australia, Canberra ACT 2601, Australia; Leo.Joseph@csiro.au

⁶ Museum für Naturkunde, Leibniz Institut für Evolutions- und Biodiversitätsforschung, 10115 Berlin, Germany

* Correspondence: martin.irestedt@nrm.se (M.I.); mozes.blom@gmail.com (M.P.K.B.)

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Abstract: The Paradise Parrot, *Psephotellus pulcherrimus*, was a charismatic Australian bird that became extinct around 1928. While many extrinsic factors have been proposed to explain its disappearance, it remains unclear as to what extent genetic erosion might have contributed to the species' demise. In this study, we use whole-genome resequencing to reconstruct a 15x coverage genome based on a historical museum specimen and shed further light on the evolutionary history that preceded the extinction of the Paradise Parrot. By comparing the genetic diversity of this genome with genomes from extant endangered birds, we show that during the species' dramatic decline in the second half of the 19th century, the Paradise Parrot was genetically more diverse than individuals from species that are currently classified as endangered. Furthermore, demographic analyses suggest that the population size of the Paradise Parrot changed with temperature fluctuations during the last glacial cycle. We also confirm that the Golden-shouldered Parrot, *Psephotellus chrysopterygius*, is the closest living relative of this extinct parrot. Overall, our study highlights the importance of museum collections as repositories of biodiversity across time and demonstrates how historical specimens can provide a broader context on the circumstances that lead to species extinctions.

Keywords: *Psephotellus pulcherrimus*; museomics; genome-wide heterozygosity; genetic erosion

1. Introduction

The Paradise Parrot, *Psephotellus pulcherrimus* of central eastern Australia, was first described in 1845 by Gould [1]. Less than 100 years later, this iconic parrot was extinct. Even among parrots, the Paradise Parrot was strikingly colorful and Gould [2] wrote: “The graceful form of this Parrakeet [sic], combined with the extreme brilliancy of its plumage, renders it one of the most lovely of the Psittacidae yet discovered; and in whatever light we regard it, whether as a beautiful ornament to our cabinets or a desirable additional to our aviaries, it is still an object of no ordinary interest”. Extensive published requests for information about the species in Australian newspapers, particularly by the

naturalist A. H. Chisholm (1890–1977), multiple advertised expeditions to search for its existence and numerous unconfirmed sightings during the 20th century have made the reputation of the Paradise Parrot an ornithological equivalent of the illustrious Tasmanian tiger *Thylacinus cynocephalus* (see [3]).

The Paradise Parrot was a medium-sized parrot that inhabited grassy woodlands of eastern Australia and was mostly found in southeast Queensland. With the Golden-shouldered Parrot (*Psephotellus chrysopterygius*) and the Hooded Parrot (*Psephotellus dissimilis*), it was one of three tropical or subtropical woodland species that nest in termite mounds and feed on seeds from native grasses [3–6]. The fourth species assigned to *Psephotellus*, the Mulga Parrot *P. varius* of the Australian arid zone, nests in tree hollows. Male Paradise Parrots were turquoise and greenish ventrally, they had red on the undertail-coverts, forehead and on a prominent wing patch, a brownish back and black cap. Females were generally duller copies of the males, the scarlet patches reduced to smaller, red and less bright shoulder and belly patches (e.g., [3–6]; Figure 1). The species' historical distribution was reviewed by Olsen [3], confirmed records only being from southeastern Queensland but its range possibly extended into the northernmost parts of New South Wales. Historical sightings from Cape York Peninsula are almost certainly of misidentified Golden-shouldered Parrot or are otherwise dubious [3].



Figure 1. Photos of the male Paradise Parrot specimen (NRM 561897) held at the Swedish Museum of Natural history showing its ventral (above) and dorsal (below) plumage. This is the sample that was used to sequence the Paradise Parrot's genome.

The Paradise Parrot was probably at least locally abundant within its fairly restricted range before it declined dramatically during the second half of the 19th century [3]. From the 20th century onwards, there are few reliable records [3]. However, in December 1920, Cyril Jerrard recorded a small population at the Burnett River in southeast Queensland and he recorded the presence of an isolated group there until 1927 (a neighbor reported one bird in December 1928). These are the last confirmed records of the species. Extracts of Jerrard's unpublished notes, including photos of living Paradise Parrots, can be found at <http://www.nla.gov.au/pub/paradiseparrot/> [7]. A thorough review

of unconfirmed sightings after the 1920s, including a putative clutch of eggs, suggests that records past 1928 are all are questionable [3].

The extinction of the Paradise Parrot was probably precipitated by a variety of factors: trapping for the aviary trade, predation by introduced mammals; the severe drought of 1902; land clearing; pastoralism; changed fire regimes, and the destruction of termite mounds (nesting sites) [3]. During the same period, many other bird species with diets similar to the Paradise Parrot have also declined or disappeared from the region. While several species have now largely recovered (e.g., Turquoise Parrot *Neophema pulchella* [6]) others still decline or are feared to be locally extinct in this region (e.g., Black-throated Finch *Poephila cincta* and Star Finch *Neochmia ruficauda* [8,9]). The ramifications of long-term vegetation change, particularly due to overstocking, are likely the most important reasons causing the Paradise Parrot's extinction [3].

While many hypotheses for the Paradise Parrot's decline have thus been proposed, one aspect that currently remains uncertain is to what extent genetic erosion might have contributed to the species' demise. Genetic erosion, such as through inbreeding depression or genetic drift, may affect a species' viability [10–13]. Genome-wide patterns of sequence variation between historical and extant populations are now widely used to detect such signs of genetic erosion in conservation genetics (e.g., [14]). Unfortunately, in the case of the Paradise Parrot, no fresh tissue samples are available for genetic analyses but with the advent of High-Throughput sequencing it has now also become tenable to assay genetic variation from historical collections [15]. Museum collections are therefore unique sources for genetic data from the past and they make it possible to compare extant and historical populations to study how species have responded to environmental change (e.g., [15]) or how genetic diversity has changed through time (e.g., [16]). Here we use museomics to sequence the genome of a single Paradise Parrot collected during the second half of the 19th century, the time when the species' population likely crashed. By contrasting the genome-wide genetic diversity of this genome with corresponding levels in other avian species, especially extant ones that are currently endangered, we provide an estimate of whether the Paradise Parrot suffered from genetic erosion at the time of its dramatic decline in order to place the factors discussed by Olsen [3] in a broader context. We also use this genome to assess the species' population history through approximately the last 100,000 years and evaluate existing hypotheses about its phylogenetic affinity.

2. Materials and Methods

2.1. Extraction, Library Preparation and Sample Information

Genomic DNA was extracted from a toepad sample from a Paradise Parrot study skin kept at the Swedish Museum of Natural History (NRM 561897, Figure 1) following the protocol described in Irestedt et al. [17]. Like many other study skins of Paradise Parrots in natural history collections, the specimen carries little metadata. Label data gives the date it arrived at the museum (17 November 1902) and the locality as Brisbane, Queensland. The specimen was one of 105 specimens that arrived at the Swedish Museum of Natural History at the same time, all labeled with the same locality and listing J. H. Nicholson as the collector/donor. Most likely this person was John Henry Nicholson (1838–1923) and Brisbane not where these birds were collected, but the port from where the shipment was sent. Thus, the specimen is likely to have been collected many years, or even decades before 1902, and is most likely not from Brisbane at all. However, as the first specimen of the Paradise Parrot was collected by John Gillbert 1848 we know for sure that it almost certainly was collected during the second half of the 19th century. We could thus conclude that the specimen was collected during the period when the species started to decline, but also before it had become extremely rare during the first decades of the 20th century.

Following DNA extraction, the protocol published by Meyer and Kircher [18] was used to create sequencing libraries suitable for Illumina sequencing. In brief, library preparation consisted of blunt-end repair, adapter ligation, adapter fill-in and was followed by four independent index PCRs to

reduce PCR bias. All libraries were pooled at equal ratio with another museum sample and sequenced on a single Illumina HiSeq X lane. The raw reads have been deposited at the NCBI Sequence Read Archive (SRA), accession number PRJNA530123.

2.2. Filtering of Raw Reads and Mapping

Illumina sequencing reads were processed using a custom-designed workflow available at <https://github.com/mozesblom> [19] to remove adapter contamination, low-quality bases and low-complexity reads. Overlapping read pairs were merged using PEAR [20] and SuperDeduper [21] was used to remove PCR duplicates. Trimming and adapter removal was done with TRIMMOMATIC (v.0.32 [22]; default settings) and overall quality and length distribution of sequence reads was inspected with FASTQC (v.0.11.5 [23]) before and after the cleaning. DNA degradation patterns in historical specimens could lead to a consistent signal of erroneous substitutions. As degradation almost exclusively occurs at the ends of DNA-fragments we first investigated how the trimming of reads would affect the number of recovered variants. We first deleted 5 bp from both ends and repeated the analyses by removing 10 bp from each end. We found that the number of variants called drop considerably after the trimming of the ends, but we found almost identical results after the removal of 5 bp and 10 bp, respectively. We thus based all analyses herein on reads from which 5 bp had been removed from both ends.

We used BWA mem v. 0.7.12 [24] to map the polished reads against the whole genome of *Agapornis roseicollis* (GCA002631895; [25]) as well as a mitochondrial genome obtained from another individual of *Psephotellus pulcherrimus* (NC031358; [26]). High-quality SNP's were called from the whole-genome BAM-file using mpileup in samtools v. 0.1.9 [27]. We applied a minimum variant quality of 30 and a minimum genotype depth of 12x using bcftools v. 1.1 (<https://samtools.github.io/bcftools>) [28] and vcftools v.0.1.12 [29]. We also filtered variants with a minor allele frequency (MAF) of 10% or less (i.e., at least two out of twelve reads support either of the two alleles). We used HMMER v.3.2.1. (<http://hmmmer.org>) [30] to search for sequence homologs of nuclear genes across the whole genome. In the first step we built probabilistic query profiles using hidden Markov models and alignments of three nuclear genes (c-mos, RAG 1 and ZENK) (Table 1). These alignments consist of sequences obtained in a phylogenetic analysis of Australasian platycercine parrots [31], the clade to which *Psephotellus* parrots belong [32]. We searched where in the mapped genome these queries had their best fit and from the list of suggested genomic coordinates we selected the ones with the lowest “sequence E-value”, i.e., the expected number of false positives (non-homologous sequences) that scored this well or better. The queries resulted in recovery of the whole query-sequence for all three genes and these were then parsed out from the mapped genome based on the identified genomic coordinates.

Table 1. Sequences used by [31] that have been downloaded from GenBank and used in HMMER and in the phylogenetic analyses.

Species	Rag1	ZENK	c-mos	cytb	ND2
<i>Psephotellus chrysopterygius</i>	JF807991	JF807977	JF807963	JX442436	JX442400
<i>Psephotellus dissimilis</i>	GQ505210	GQ505155	GQ505101	JX442415	JX442384
<i>Psephotus haematonotus</i>	JX442403	JX442379	JX442404	JX442435	JX442399
<i>Psephotellus varius</i>	GQ505209	GQ505154	GQ505100	JX442414	JX442383
<i>Purpureicephalus spurius</i>	GQ505204	GQ505150	GQ505096	JX442410	JX442381
<i>Platycercus venustus</i>	GQ505207	GQ505153	GQ505098	JX442413	JX442382
<i>Lathamus discolor</i>	GQ505211	GQ505156	GQ505102	JX442416	JX442385

2.3. Phylogenetic Analyses and Divergence Time Estimation

For the phylogenetic analysis, we added the three nuclear and two mitochondrial genes obtained for the species by Schweizer et al. [31], pruned to only include those species that are the putative closest relatives to the Paradise Parrot. Best-fit maximum-likelihood trees were estimated individually for each of the three nuclear genes and the two mitochondrial genes using RAxML v. 7.4.7 [33]

applying the General Time Reversible model of nucleotide substitution. We also estimated best-fit maximum-likelihood trees for the concatenated data sets consisting of all five genes (totaling 4870 bp). Substitution models used in these reconstructions were selected using jModelTest v.2.1.10 [34,35]. Based on the individual gene trees estimated for the five genes we calculated a species tree using MP-EST [36]. Bootstrap values for the species tree were estimated in MP-EST after submitting 100 bootstrap trees per gene obtained in the RAxML analysis of the individual genes. A rough estimate of the divergence time between the Paradise Parrot and its closest relatives was obtained by applying the 2.1% per million year divergence in avian mitochondrial genes (e.g., [37]). Genetic p-distances were calculated for the cytochrome *b* gene in MEGA X [38].

2.4. Population Estimates

We studied the demographic history of the Paradise Parrot using the pairwise sequentially Markovian coalescent (PSMC) model to estimate changes in effective population size [39]. Each diploid genome is a collection of hundreds of thousands independent loci, each with its own time to the most recent common ancestor (TMRCA) between the two alleles an individual carry. By estimating the TMRCA of the two alleles at each locus a distribution of TMRCA across the genome is created. As the rate of coalescent events is inversely proportional to the effective population size, PSMC identifies periods of change in the effective population size. For example, when many loci coalesce at the same time, it is a sign of small effective population size at that particular time. TMRCA distribution was scaled into years by generation time and average mutation rate. Unfortunately, the general biology and life-history parameters for the Paradise Parrot are largely unknown. Here we have used 4,1 years that is the estimated average generation length of the closely related Golden-shouldered Parrot [9] and a genomic mutation rate of 2.1×10^{-9} per site per year [36], Figure 2. The parameters for the PSMC analysis were set to “-N30 -t5 -r5 -p 4 + 30 × 2 + 4 + 6 + 19” following recommendations by [40]. We performed bootstrapping of PSMC by splitting the Paradise Parrot genome into shorter segments from which new sequences were constructed through random sampling with replacement. The information on the demographic history provided by the PSMC analysis should be interpreted with some caution, as the amount of sequence data available does not entirely fulfil recommendations for what is needed to make reliable inference of population size dynamics over time. Based on empirical observations [40], a sequencing depth of 18x or above is suggested to obtain stable results. The mapping of Paradise Parrot resulted in a slightly lower sequencing depth, 15.3x.

The genome-wide rate of heterozygosity was estimated with bcftools stats. The rate was calculated as the observed number of heterozygous sites divided by the total number of variants in the sample after filtering out all variants with a read-depth below 15 and above 30 (see Supplementary Material Figure S1). Larger stretches with homozygous sites were searched by using the -homozyg option of PLINK v. 1.9b4.9 [41] and the following settings to call a segment as homozygote: a sliding window of 500 kbp, a minimum of 10 SNPs per window, a threshold of 0.05 for overlapping homozygote windows, and a maximum of 15 missing sites and one heterozygote position.

3. Results

3.1. Mapped Genomes and Population Genetics

The PSMC-analysis of effective population size through the last 100,000 years (Figure 2) suggest that the Paradise Parrot had a relatively high effective population size around 100 kya, from which it decreased to a minimum around ~30 kya. After that the effective population size appears to have been stable with a slight increase. The genome-wide level of heterozygosity in the Paradise Parrot was estimated to 1.75 SNPs per thousand nucleotides (Table 2), and no long runs of homozygous sites were found in a genome-wide scan.

Table 2. Estimated genome-wide levels of heterozygosity in three parrot species. The table shows that the genome-wide heterozygosity in the Paradise Parrot is substantially lower than in the abundant Australian budgerigar but almost twice as high as in the endangered New Zealand Kea.

Species	SNP rate (10^{-3})	Ref
Kea, <i>Nestor notabilis</i>	0.91	[42]
Budgerigar, <i>Melopsittacus undulatus</i>	4.31	[43]
Paradise parrot, <i>Psephotellus pulcherrimus</i>	1.70	This study

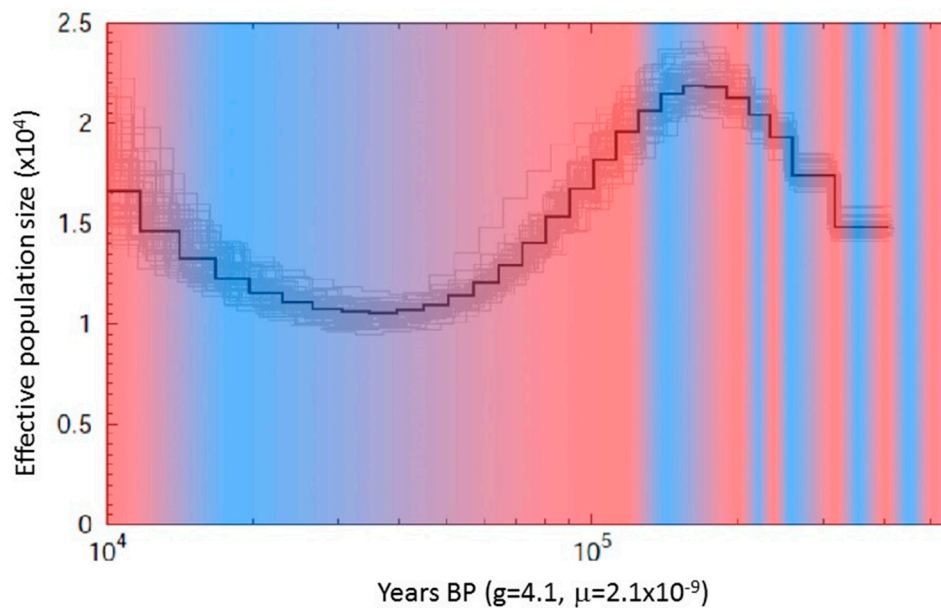


Figure 2. The change in effective population size over time for the Paradise Parrot was derived by the pairwise sequential Markovian coalescent model (PSMC). The x axis gives a log scale of the time in years, applying a genome mutation rate of 2.1×10^{-9} per site and generation time of 4.1 years. The bold black line shows the effective population size through time. The thin grey lines represent 100 rounds of bootstrapped sequences. The background color indicates past temperature fluctuations in Australia [44] where pink indicate warmer periods and blue colder periods.

3.2. Phylogenetic Relationships and Divergence Estimates

The phylogenetic trees obtained from the analyses of the concatenated dataset as well as of the individual loci recovered are shown in the Supplementary Material (Figures S2–S8). All analyses recovered the three termite mound-nesting *Psephotellus* parrots (the Paradise Parrot *Psephotellus pulcherrimus*, the Golden-shouldered Parrot *Psephotellus chrysopterygius* and the Hooded Parrot *Psephotellus dissimilis*) as monophyletic, and the Paradise Parrot and golden-shouldered parrot as sister species within this clade (see Supplementary Material). Overall, the tree obtained from the concatenated data set has an identical topology to that obtained by [31], when taking into account that we have pruned several taxa from their data set.

The genetic distances in the mitochondrial cytochrome b for all species included in the phylogenetic analyses are summarized in Table 3. The genetic distance between the Paradise Parrot and Golden-shouldered Parrot was 1.77%. Based on the mitochondrial DNA clock (which for birds is normally based solely on divergence rates in the cytochrome b gene) of 2.1% for birds e.g., [33] a very rough estimate suggest that these two species diverged from each other slightly less than one million years ago.

Table 3. The uncorrected genetic p-distances for the mitochondrial cytochrome *b* gene between species included in the phylogenetic analyses.

	JX442416	JX442435	JX442413	JX442410	JX442414	JX442415	JX442436
<i>Lathamus discolor</i> JX442416							
<i>Psephotus haematonotus</i> JX442435	12.67%						
<i>Platycercus venustus</i> JX442413	11.99%	10.16%					
<i>Purpureicephalus spurius</i> JX442410	12.10%	10.96%	10.05%				
<i>Psephotellus varius</i> JX442414	10.62%	9.93%	8.11%	8.45%			
<i>Psephotellus dissimilis</i> JX442415	12.10%	10.39%	9.36%	9.59%	7.99%		
<i>Psephotellus chrysopterygius</i> JX442436	11.53%	11.19%	9.93%	9.02%	7.99%	6.85%	
<i>Psephotellus pulcherrimus</i> this study	11.31%	11.31%	11.09%	9.76%	7.76%	6.21%	1.77%

4. Discussion

Species that become extinct may go through a long-term, gradual decline in population size that ends in a period when they have very small populations. Analyses on 38 avian genomes revealed that several threatened species (listed in the IUCN Red List of Threatened Species) showed long-term reduction in population size before their recent declines [45]. Small population size may result in reduction of genetic diversity that in turn could affect fertility, resistance to diseases and/or the capacity to adapt to changing environments [11–13]. As natural selection is less efficient in small populations, the risk of fixation of deleterious alleles might also increase in small populations [10]. Consequently, the reduction of genetic diversity and the concomitant reduction in genetic fitness may potentially be a factor that undermines the viability of such a population and pushes it to extinction. However, species may also go extinct following rapid declines due to extrinsic factors such as habitat loss or anthropogenically induced disease. Losses of genetic diversity and fitness likely then have no significant impact on the extinction process.

The Paradise Parrot's fluctuation in population size through time (Figure 2) correlates to some degree with the annual temperature fluctuation during the last glacial cycle [44]. Its lowest effective population size is estimated to have been close to the Last Glacial Maximum ca 20 kya. Although the vegetation history in this part of Australia is poorly known, pollen deposits from the Redhead Lagoon (slightly south of the known distribution of the Paradise Parrot) suggest an open woodland vegetation during the period when the Paradise Parrot had its highest effective population size [46]. Around the Last Glacial Maximum the pollen deposits at Redhead Lagoon suggest a grassland-dominated landscape, but they also indicate a harsher and dryer environment with a less complex vegetation community than today [46]. It is likely that the drier climate around Last Glacial Maximum was suboptimal for the Paradise Parrot. This could explain its lower population size during that period. As indicated by [3] the Paradise Parrot probably had very specific food and breeding requirements, this may have made the Paradise Parrot particularly vulnerable for environmental changes. Even though our analysis of population size changes through time indicates that the Paradise Parrot was affected by past temperature fluctuation, the population is estimated to have been rather stable in size during the last 25 ky. The results suggest a weakly increasing trend in population size towards the present time. At least, the results suggest that the Paradise Parrot was not declining during this period. However, this method provides no estimate for the last 10 ky, i.e., during the period of recent human impact on the environment.

Li et al. [47] found that threatened species, of which some had gone through severe population bottlenecks, on average had considerably lower genome-wide levels of heterozygosity than common and abundant species. Although the genome-wide level of heterozygosity in the Paradise Parrot genome is not as high as in the abundant Australian Budgerigar, it is almost twice as high as in the now endangered New Zealand Kea (Table 2). It is also higher than the genome-wide level of heterozygosity found in almost all endangered and vulnerable species listed by [47]. It is within the range of what is found for species falling in the IUCN category "least concerned". This is an additional sign that

the Paradise Parrot was likely in a relatively good “genetic shape” at the time of its dramatic decline. This is further supported by the absence of large regions of homozygosity in the Paradise Parrot genome, which would have indicated inbreeding. As the decline of the Paradise Parrot coincides with the major expansion of pastoral settlement in the region [3], it is reasonable to infer that it was mainly changing land use, and particularly the effect of grazing on the seeding of native grasses [3] that changed the Paradise Parrot’s natural environments and drove it to extinction within a few decades. As Olsen [3] writes, “The parrot’s days were numbered by the time it was discovered. Its country, the rich grassland in Australia, was seized and overused within a few decades. There is no absolute proof for any of these claims, but they can be deduced from the trail of evidence left by explorers, natural history collections, ornithologists, bird-lovers and aviculturists”. That anthropogenic land use has had large effect on the granivorous avifauna in this region is also indicated by the decline and local extinction of other seed-eating open woodland species [8,9]. Franklin et al. [48] have also shown that there is a correlation between grazing and decline of granivorous birds in northern Australia.

Within the trio of Australian termite mound-nesting parrots the Golden-shouldered Parrot and the Hooded Parrot are morphologically most similar. However, based on mtDNA sequence data Christidis and Norman [49] suggested that the Paradise Parrot and the Golden-shouldered Parrot were the two closest relatives within this trio. This notion is also strongly supported by our analyses. The recognition of the Paradise Parrot as a unique species has rarely been contested (though see [50,51]) and the observed genetic distance between the paradise and hooded parrots e.g., [52] coupled with its distinctive phenotype and isolated geographic range, confirm that the world has lost a truly unique parrot species.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/11/4/58/s1>, Figures S1–S5: individual gene trees, Figure S6: RAxML tree from concatenated data set, Figure S7: MP-EST tree based on 5 genes, Figure S8: Estimated rate of heterozygosity versus read depth.

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