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Ancient mitochondrial genomes unveil the origins and evolutionary history of New Zealand's enigmatic takahē and moho

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Abstract

Many avian species endemic to Aotearoa New Zealand were driven to extinction or reduced to relict populations following successive waves of human arrival, due to hunting, habitat destruction and the introduction of mammalian predators. Among the affected species were the large flightless South Island takahē (*Porphyrio hochstetteri*) and the moho (North Island takahē; *P. mantelli*), with the latter rendered extinct and the former reduced to a single relictual population. Little is known about the evolutionary history of these species prior to their decline and/or extinction. Here we sequenced mitochondrial genomes from takahē and moho subfossils (12 takahē and 4 moho) and retrieved comparable sequence data from takahē museum skins ($n = 5$) and contemporary individuals ($n = 17$) to examine the phylogeny and recent evolutionary history of these species. Our analyses suggest that prehistoric takahē populations lacked deep phylogeographic structure, in contrast to moho, which exhibited significant spatial genetic structure, albeit based on limited sample sizes ($n = 4$). Temporal genetic comparisons show that takahē have lost much of their mitochondrial genetic diversity, likely due to a sudden demographic decline soon after human arrival (~750 years ago). Time-calibrated phylogenetic analyses strongly support a sister species relationship between takahē and moho, suggesting these flightless taxa diverged around 1.5 million years ago, following a single colonisation of New Zealand by a flighted *Porphyrio* ancestor approximately 4 million years ago. This study highlights the utility of palaeogenetic approaches for informing the conservation and systematic understanding of endangered species whose ranges have been severely restricted by anthropogenic impacts.

KEYWORDS
genetic bottleneck, human impact, phylogeny, phylogeography, quaternary, Rallidae

1 | INTRODUCTION

Human arrival in Aotearoa New Zealand approximately 750 years ago (Bunbury et al., 2022; Wilmshurst et al., 2008) significantly impacted the archipelago's biodiversity (Bromham et al., 2012; Rawlence et al., 2022). Hunting practices, habitat destruction and the introduction of mammalian predators contributed to the declines and/or extinctions of numerous native and endemic species (Allentoft et al., 2014; Perry, Wilmshurst, & McGlone, 2014; Worthy & Holdaway, 2002). Affected avifaunal taxa included the moho 1848 (*Porphyrio mantelli*) and takahē (*P. hochstetteri*)—two large flightless rails endemic to New Zealand's North and South islands, respectively. Although originally described as separate species within their own genus *Notornis* (Owen, 1848; Meyer, 1883), takahē and moho were long considered subspecies within *Porphyrio* (Kinsky, 1970), until morphometric differences between the two taxa led to their reclassification as separate species, as moho were found to be significantly larger and more slender than takahē (Trewick, 1996). This taxonomic separation was subsequently corroborated by genetic evidence, which suggested that takahē and moho were paraphyletic within *Porphyrio*, based upon the analysis of short 12S mitochondrial DNA fragments (Trewick, 1997). This morphometric and genetic evidence suggested that takahē and moho evolved from distinct volant ancestors that independently colonised New Zealand (Trewick, 1997).

There exist several avian sister species pairs in New Zealand with taxa endemic to the North and South Island, including adzebill (*Aptornis* spp.), giant moa (*Dinornis* spp.), New Zealand robins (*Petroica* spp.) and New Zealand wattlebirds (Callaeidae) (Boast et al., 2019; Bunce et al., 2009; Kearns et al., 2019; Lubbe et al., 2022; Parker et al., 2014). The suggested non-sister relationship of takahē and moho (Trewick, 1997) would thus represent a surprising exception to this common phylogenetic pattern. Molecular estimates of the divergence times between many of these North–South Island species pairs have been dated to the Plio- Pleistocene (5.3–0.012 Mya) and may have been promoted by the intermittent formation and closure of marine straits between the two landmasses, including the Manawatu Strait (3–1 Mya; Trewick & Bland, 2012) and the Cook Strait (0.5 Mya; Lewis et al., 1994). Molecular divergence dating suggests that takahē and moho diverged from their respective common ancestors ~2–2.5 Mya (Garcia-Ramirez & Trewick, 2015). Colonisation of New Zealand may have been aided by the onset of the Pleistocene Ice Ages from ~2.58 Mya, which were marked by the periodic expansion of open grassland–shrublands facilitating the establishment of open-habitat adapted species that dispersed from Australia (Rawlence et al., 2019).

Quaternary palaeontological and archaeological records suggest that both takahē and moho were widespread across their respective islands at the time of human arrival (Trewick & Worthy, 2001). However, the rarity of reported encounters of these taxa by Europeans suggests that both had undergone dramatic declines prior to European arrival in the 19th century (Grueber & Jamieson, 2011). Indeed, while moho may have persisted until the late 19th century (Phillipps, 1959), this species is definitively known only from subfossil and archaeological material. Takahē were similarly considered extinct between the late 1800s and early 1900s, until the 1948 discovery of a relict population in the southern South Island (Murchison Mountains, Fiordland) (Lee & Jamieson, 2001). This species is now heavily managed on predator-free islands, fenced mainland ecosanctuaries and two predator-controlled mainland localities (Hunter-Ayad et al., 2021).

Species range retractions are often accompanied by genetic bottlenecks and demographic declines, which can lead to reductions in adaptive potential and heightened extinction risks (Frankham, 2005). For endemic New Zealand birds, such genetic bottlenecks have often been linked to anthropogenic impacts (Ramstad et al., 2013; Rawlence et al., 2015; Taylor et al., 2007), and/or pre-human climatic and environmental changes during the Late Quaternary (Bunce et al., 2009; Dussex et al., 2014, 2018; Verry et al., 2022). Palaeogenetic analyses have often enhanced our understanding of such bottlenecks (Dussex et al., 2015, 2018, 2021; Ramstad et al., 2021; Verry et al., 2022) through the sampling of populations across different regions and time periods. The contemporary takahē population exhibits low levels of genetic diversity, consistent with a severe population bottleneck (Jamieson et al., 2003; Lettink et al., 2002). Palaeogenetic analyses of takahē skins collected during the mid–late 1800s and mid-1900s detected similarly low levels of genetic diversity (Grueber & Jamieson, 2011). These initial findings suggest that the inferred genetic bottleneck may pre-date European arrival. A comparable pre-European decline scenario has been suggested for the similar-sized kākāpō, which underwent two separate declines associated with both Polynesian and European arrival (Dussex et al., 2018; Seersholm et al., 2018). Kākāpō genomes have also suggested a decline associated with the onset of the Last Glacial Maximum (~30 Kya; Dussex et al., 2021). However, the precise timings and durations of bottlenecks inferred for *Porphyrio* lineages remain poorly understood.

Here, we present mitochondrial genome sequences from takahē and moho, including living, historic, archaeological and subfossil takahē, and the first moho mitochondrial genome. We analyse this temporal dataset to test for phylogeographic and demographic shifts related to anthropogenic and abiotic disturbance, and to clarify phylogenetic relationships among *Porphyrio* taxa. Specifically, we test the hypotheses that (1) takahē and moho constitute sister species, reflecting a single loss of flight from a volant common ancestor (similar to much of New Zealand's avifauna) and (2) that the low genetic diversity of takahē stems from a pre-European anthropogenic bottleneck.

2 | MATERIALS AND METHODS

2.1 | Modern takahē mitochondrial genome sequencing

Genomic DNA was extracted from 17 Seutin buffered (Seutin et al., 1991) takahē blood samples using the Qiagen MagAttract HMW DNA extraction kit, following the manufacturer's instructions

for blood. These samples originate from individuals born at the Burwood Takahē Centre during the 2014–2015 breeding season. Long-range PCR was used to amplify the entire takahē mitochondrial genome in two overlapping fragments using the primer pair Tak16SF-LR and TaktLeuR-LR (~9.3 Kbp including primers), alongside TaktSerF-LR and Tak16SR-LR (~7.8 Kbp including primers) (Table S1) and the Kapa LongRange HotStart PCR kit. The reaction mix contained 1× KAPA LongRange Buffer, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM dNTPs, 0.675 U KAPA LongRange HotStart DNA Polymerase and 1 μL of genomic DNA, made up to a total volume of 25 μL with distilled water. The thermocycling conditions used were 95°C for 3 min, followed by 10 cycles of 95°C 25 s, 55 or 57°C 15 s (55°C for Tak16SF-LR + tLeuTakR-LR, 57°C for tSerTakF-LR + Tak16SR-LR), 68°C 10 min, and 27 cycles of 95°C 25 s, 55 or 57°C 15 s (primer/temperature combinations as above), 68°C 10 min + 20 s per cycle, with a final extension step of 68°C for 10 min. Successful PCRs were identified via gel electrophoresis using a 2% agarose gel stained with SYBRSafe, visualised under blue light.

Long-range PCR products were sheared to an average fragment size of 200 bp using a Bioruptor-Pico (Diagenode) with 13 cycles of 30 s ON and 30 s OFF. Sheared PCR products were purified using homemade SPRI-beads (MagNA; Rohland & Reich, 2012), quantified using a Nanodrop spectrophotometer and converted into DNA libraries following the protocol of Meyer and Kircher (2010), with 100 ng of each sheared fragment as template. qPCR was used to determine the optimal number of PCR cycles for indexing as per Gansauge and Meyer (2013). Each library was divided into four indexing PCRs to minimise PCR bias, with each reaction containing 5 μL of DNA library, 1× PCR Buffer II (Applied Biosystems), 2.5 mM $MgCl_2$, 0.8 mg/μL BSA, 0.2 μM of each indexing primer (P5 and P7, with each featuring a seven-nucleotide barcode; see Gansauge & Meyer, 2013), 0.25 mM dNTPs and 2 U of Amplitaq Gold DNA Polymerase, made up to 25 μL with distilled water. Indexing PCRs were pooled by sample, purified with MagNA beads (Rohland & Reich, 2012) and quantified via Qubit and Qiaxcel (Qiagen). Indexed libraries were pooled in equimolar concentrations and sent to the Otago Genomics facility (University of Otago) for sequencing on the Illumina MiSeq sequencing platform using 2 × 75 bp sequencing chemistry (Table S2).

2.2 | Ancient takahē and moho mitochondrial genome sequencing

Pleistocene and Holocene subfossil, and archaeological, takahē and moho bones covering the geographic and temporal distribution of these species were sampled from New Zealand museum collections (Figure 1a; Table S3). Only common elements with the same left or right orientation were sampled from each individual deposit to ensure sample independence. Bones were sub-sampled using a low-speed drill to directly obtain bone powder, or a small fragment was removed using a radial cutting disk. Solid fragments were ground to powder using a sterile mortar and pestle in a dedicated ancient DNA facility (described below). Where possible, previously published radiocarbon dates (Table S3) were used to estimate the age of the sampled bones, with calibrated dates calculated using the SHCal20 calibration curve (Hogg et al., 2020) in OxCal (v4.4; Ramsey, 2009). When multiple dates were available for the same site, the average calibrated age was calculated. Furthermore, we also included toepad samples from four of the five known takahē skins dating to the 19th century (it should be noted that the holotype of takahē is not destroyed or lost [Ballance, 2023; Grueber & Jamieson, 2011] and is in the collections of the Museum of Zoology, Senckenberg Natural History Collection, Dresden; Martin Päckert, personal communication), and one toepad from a specimen collected post-rediscovery. All five of these specimens were previously examined by Grueber and Jamieson (2011) who analysed microsatellites and partial mitochondrial control region fragments (Table S4).

Ancient DNA extractions, PCR set-up and DNA library preparations were performed within a purpose-built ancient DNA laboratory at the University of Otago (Otago Palaeogenetics Laboratory). These facilities are physically isolated from other molecular laboratories and subject to regular decontamination with bleach (sodium hypochlorite) and UV irradiation (Knapp et al., 2012). Strict ancient DNA protocols were followed to minimise contamination of samples with exogenous DNA. Downstream processes (e.g. library indexing and hybridisation capture enrichment) were undertaken within a separate modern genetics laboratory. Ancient DNA extractions from bone followed Rohland et al. (2010), while ancient DNA extractions from toepad tissue followed Verry et al. (2019). DNA extracts were screened for endogenous mitochondrial DNA via PCR of an 85 bp fragment of 12S using the primers 12Shf and 12SKr (Cooper et al., 2001) and/or a 199 bp fragment of the control region using Takdloop1 and Takdloop4 (Grueber & Jamieson, 2011; see Supporting Information for details). Successful samples were utilised for downstream processes (i.e. DNA library preparation and hybridisation capture enrichment). The exception to this protocol was the specimen WCM 18003 (F1c Cave) which was analysed separately within the ancient DNA facility at Massey University, Palmerston North, following the extraction protocol of Delsuc et al. (2016) with library preparation as described in Gibb et al. (2015). See the Supporting Information for full methodological details relevant to this specimen.

Double-stranded DNA libraries were prepared from 16 μL of DNA extract following the BEST protocol of Carøe et al. (2018), with modifications, as stipulated in Mak et al. (2017). A negative library build was included with every batch of seven samples. qPCR was used to determine the optimal number of PCR cycles for indexing PCR using the primer pair IS7/IS8 (Meyer & Kircher, 2010) and the qPCR protocol of Gansauge and Meyer (2013). For indexing amplification, each library was divided into 4 × 25 μL reactions each containing 5 μL of DNA library, 1× PCR Buffer II, 2.5 mM $MgCl_2$, 0.8 mg/μL BSA, 0.2 μM of each forward and reverse indexing primer (P5 and P7, with each featuring a seven-nucleotide barcode; see Gansauge & Meyer, 2013), 0.25 mM dNTPs, and 2 U of Amplitaq Gold DNA Polymerase, made up to 25 μL with distilled water. The

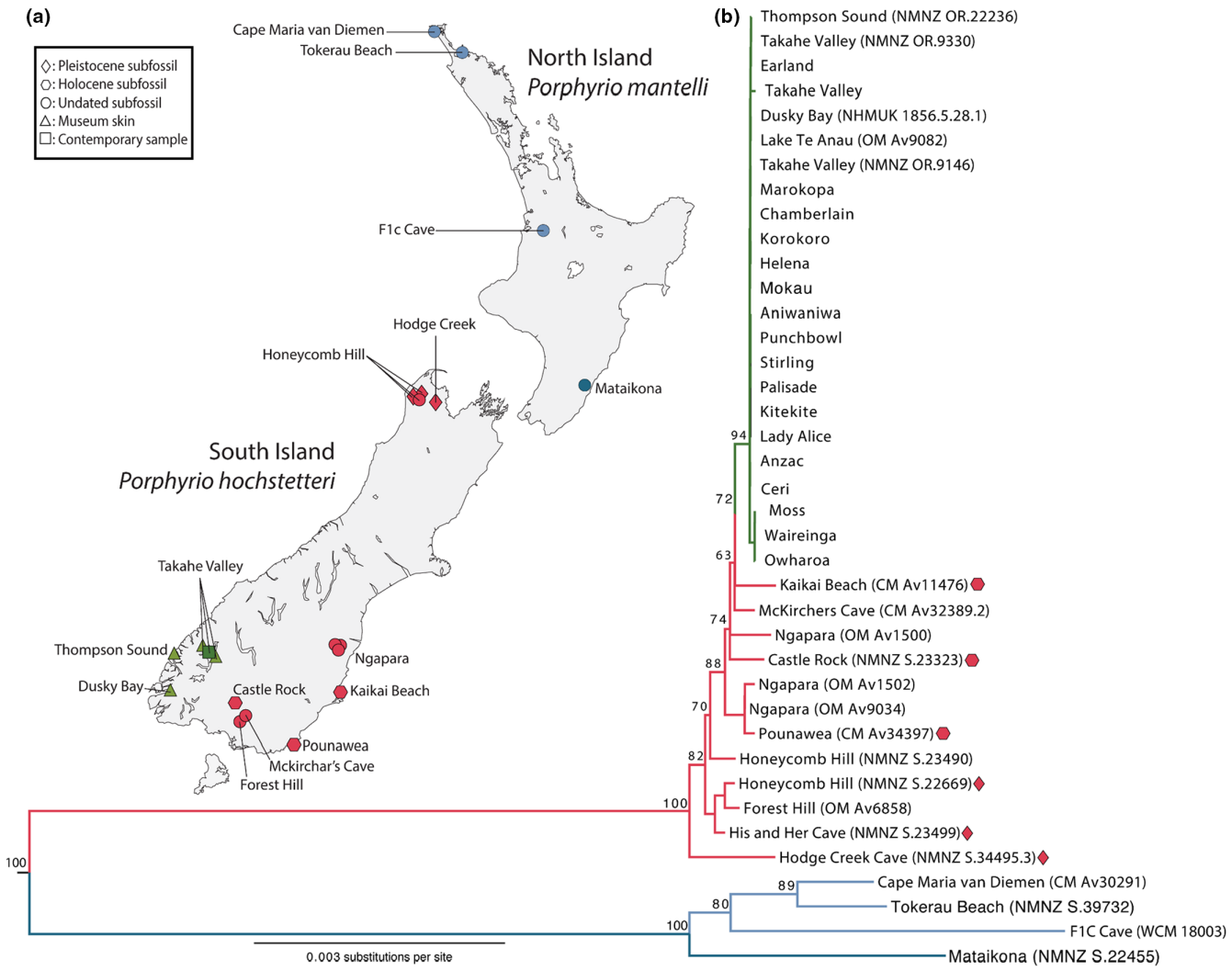

FIGURE 1 (a) Map of Aotearoa New Zealand illustrating the geographic and temporal distribution of the takahē/moho mitochondrial genomes analysed in this study. Colours are indicative of either sample type (takahē) or phylogeographic structure (moho; see panel b). (b) Maximum likelihood phylogeny reconstructed from takahē and moho mitochondrial genomes. Node values denote bootstrap support values. Tip labels represent the recorded collection locality of the specimen, followed by the museum registration number in brackets. Specimens with associated radiocarbon dates are denoted by symbols as per panel a.

thermocycling profile for indexing PCR consisted of 95°C 5 min; 12–23 cycles of 95°C 30 s, 60°C 30 s, 72°C 1 min, followed by 10 min at 72°C. The four separate reactions for each library were pooled and purified using the Qiagen MinElute PCR purification kit. For a select few samples where initial sequencing of double-stranded DNA libraries indicated the presence of poorly preserved endogenous DNA (NMNZ S.39732 and CM Av30291), single-stranded DNA libraries were also constructed, following the protocol of Gansauge et al. (2017). As above, qPCR was used to determine the optimal number of indexing PCR cycles to maximise library complexity, followed by indexing PCR and purification.

Indexed DNA libraries were enriched for takahē and moho mitochondrial DNA following the protocol of González Fortes and Paijmans (2019), using sheared long-range takahē mitochondrial PCR products (detailed above) as bait. Briefly, ~500 ng of DNA library was hybridised with 125 ng of bait at 65°C for 48 h; hybridised bait-DNA molecules were subsequently bound to Dynabeads™ M-270 Streptavidin beads, washed with BWT buffer (1 M NaCl, 5 mM Tris–HCl, 0.5 mM EDTA, 0.05% Tween-20), Hot-Wash buffer (1 × AmpliTaq Gold Buffer; 2.5 mM $MgCl_2$) for 2 min at 50°C, and TE Buffer. The DNA was then eluted from the beads in 21 μL of TET buffer via incubation at 95°C for 5 min (for a detailed methodology, please see the Supporting Information). As the concentration of each library was significantly reduced following hybridisation capture, libraries were reamplified using the reamplification primers of Meyer and Kircher (2010; IS5/IS6). The qPCR protocol described above was used to determine the optimal number of reamplification cycles to avoid overamplifying the libraries. Captured libraries were then split into four separate PCR reactions containing 5 μL of captured library, 1 × PCR Buffer II (Applied Biosystems), 2.5 mM $MgCl_2$, 0.8 mg/μL BSA, 0.5 μM of each primer (IS5/IS6), 0.25 mM dNTPs and 1.25 U AmpliTaq

Gold DNA polymerase, made up to 25 μL with distilled water. Thermocycling conditions were identical to those of the indexing PCR. PCR reactions were then pooled by library, purified using AMPure XP beads, and quantified via Qubit and Qiaxcel (Qiagen). Reamplified dsDNA libraries were pooled in equimolar ratios and sent to the Otago Genomics facility (University of Otago) for sequencing on one lane of the Illumina HiSeq2500 sequencing platform using 2 × 75 bp sequencing chemistry (Tables S3 and S4). The two ssDNA libraries were sequenced in a separate pool across four lanes of a single Nextseq550 flowcell also using 2 × 75 bp sequencing chemistry at the Kinghorn Centre for Clinical Genomics (Garvan Institute of Medical Research).

2.3 | Sequence processing

Sequencing reads were provided demultiplexed. Demultiplexed reads were subsequently processed using the Paleomix pipeline (Schubert et al., 2014), with sequencing data from ancient DNA libraries processed as follows: briefly, sequencing adapters and low-quality bases were trimmed, overlapping paired-end reads collapsed, and reads <25 bp discarded, using AdapterRemoval2 (v2.3.1; Schubert et al., 2016). Collapsed reads were mapped to the takahē mitochondrial genome (GenBank accession number: EF532934; Morgan-Richards et al., 2008) using BWA (v0.7.15; Li & Durbin, 2010) with the backtrack algorithm, seed disabled, a relaxed edit distance (option -*n* = 0.01), and increased number of allowed gap opens (option -o = 2), settings previously suggested to aid the mapping of ancient DNA (Schubert et al., 2012). PCR duplicates were removed from the resulting alignment using the markduplicates command in Picard (v2.1.0: https://github.com/broadinstitute/picard). mapDamage2.0 (v2.0.9: Jónsson et al., 2013) was used to visualise post-mortem DNA damage patterns consistent with authentic ancient DNA (an increase in C-T transitions towards the 5′ end of one DNA strand and an increase in G-A transitions towards the 5′ end of the opposite strand for double-stranded DNA libraries). The final alignment was imported into Geneious Prime (2020.0.4; https://www.geneious.com) to visually inspect the bam file and generate a consensus sequence, calling nucleotides covered by at least three unique reads. IUPAC ambiguities were called for bases with insufficient coverage, or where no 75% consensus base was observed.

Sequencing data originating from contemporary samples were processed in a similar manner using the same pipelines and programmes, except that both paired-end and collapsed reads were mapped to the takahē mitochondrial genome reference using BWA with default parameters. Additionally, mapDamage was not used as no DNA damage is expected in well-preserved, contemporary samples.

The WCM 18003 (F1c Cave) sample, which was not enriched prior to sequencing, therefore, had a much lower proportion of endogenous DNA. The resulting reads were trimmed of any 3′ adapter sequence using cutadapt v1.1 (Martin, 2011) and mapped to the Cape Maria van Diemen (CM Av30291) mitochondrial genome sequence in Geneious. Mapped reads were checked by eye, and all conflicting or highly divergent reads were manually removed.

Consensus sequences were aligned using MAFFT (Katoh & Standley, 2013), as implemented within Geneious Prime, yielding a 16,989 bp mitochondrial genome dataset containing sequences from 39 individuals.

2.4 | Phylogeographic structure within takahē and moho

We partitioned our mitochondrial genome dataset into six different partitions (first, second and third codon positions of protein-coding genes, tRNAs, rRNAs and the mitochondrial control region) to account for different modes of molecular evolution. A Bayesian phylogeny was constructed in BEAST2 (v2.6.2; Bouckaert et al., 2019) using a birth–death tree prior, a strict molecular clock, and 10 million MCMC generations, sampling every 1000 generations with the first 10% discarded as burn-in. Modeltest-NG (v0.1.7; Darriba et al., 2020) was used to determine the most appropriate model of nucleotide substitution for each data partition (Table S5), while nested sampling analysis was used to determine the most appropriate tree prior and molecular clock model (Russel et al., 2019; Table S6). MCMC convergence and effective sample sizes (all >200) were checked in Tracer (v1.7; Rambaut et al., 2018). Bayesian phylogenetic trees were summarised using TreeAnnotator (v2.6.2) to create a maximum clade credibility tree, which was visualised in FigTree (v1.4.3; https://github.com/rambaut/figtree/releases). Using the same partitioned dataset, a maximum likelihood tree was built using IQ-TREE (v1.6.12; Minh et al., 2020). ModelFinder (Kalyaanamoorthy et al., 2017) was used to implement the most appropriate nucleotide substitution model for each partition, as implemented in IQ-TREE (see Table S5), alongside 1000 ultrafast bootstrap replicates (Hoang et al., 2018). The final bootstrap consensus tree was visualised using FigTree (v.1.4.3). A median-joining haplotype network was constructed in PopART (Leigh & Bryant, 2015), using the complete mitochondrial genome dataset, masking sites with missing data.

We constructed a temporal haplotype network for takahē using the TempNet script (Prost & Anderson, 2011) in R to illustrate temporal changes in genetic diversity. We first removed ambiguous sites from the alignment and excluded WCM 18003 from this analysis as it contained significantly more ambiguous sites than our other sequences. The temporal network utilised three broad time bins: subfossil and archaeological bones (16,000–500 years BP), historical museum specimens (1849–1958) and contemporary samples (2015–present). To further examine temporal changes in genetic diversity, we constructed an additional TempNetwork, using only dated specimens with four time bins: Pleistocene subfossils (16,000–11,650 ya), Holocene subfossils and archaeological bones (11,650 ya – 1450 CE), historical museum skins (1800–1970 CE) and contemporary samples (present day).

2.5 | Takahē demographic history

To calculate summary statistics and estimates of genetic diversity in takahē, we excluded moho sequences from the analysis due to limited sample sizes and then removed ambiguous nucleotides from the mitochondrial genome alignment (resulting in an alignment of 14,054 bp). Summary statistics and estimates of genetic diversity (e.g. haplotype frequency, number of segregating sites, average number of nucleotide substitutions, haplotype diversity and nucleotide diversity) were subsequently calculated in DnaSP (v6.12; Rozas et al., 2017), grouping samples by specimen type (subfossil/archaeological, historical museum skin, contemporary).

We employed multiple methods to examine the demographic history of takahē only, as we did not obtain enough moho sequence data to reliably investigate their demographic history. First, we calculated Tajima's D-statistic and Fu's F-statistic in Arlequin (v3.5.2.2; Excoffier et al., 2005), utilising 5000 coalescent simulations to assess the statistical significance of each and the same dataset/sample grouping as the summary statistics/estimates of genetic diversity. Second, a Bayesian skyline plot was constructed using BEAST2, to investigate temporal changes in the effective population size of the takahē. We partitioned the data using the same six partitions as in the previous Bayesian phylogenetic analyses and used tip dating to incorporate the age of the specimens into the analysis (when known, see Tables S3 and S4). We performed a date randomisation test (DRT) using the TipDatingBeast package in R (Rieux & Khatchikian, 2017) to determine whether these tip dates contained enough information to calibrate the molecular clock and reliably estimate evolutionary rates. Modeltest-NG (Darriba et al., 2020) was used to determine the most appropriate nucleotide substitution model for each partition (Table S5). We utilised a strict molecular clock, coalescent Bayesian skyline tree prior and 10 million MCMC generations, sampling every 1000 generations with the first 10% discarded as burn-in. MCMC convergence and effective sample sizes (all >200) were checked in Tracer (v1.7; Rambaut et al., 2018), which was also used to generate the skyline plot.

2.6 | Porphyrio phylogenetics and molecular dating

To re-examine the phylogenetic relationships of both the takahē and moho, we retrieved *Porphyrio* DNA sequences for four mitochondrial genes (12S, 16S, cytochrome b, Control Region) from GenBank (Table S7), representing species/subspecies from throughout the *Porphyrio* phylogeny (Garcia-Ramirez & Trewick, 2015), including the Americas, Africa, Eurasia, and the Pacific. We also incorporated relevant portions of the newly generated moho mitochondrial genome from CM Av30291, as this specimen had the highest sequencing depth/coverage of the moho specimens successfully sequenced (Table S3), resulting in a complete mitochondrial genome with minimal expected errors. *Amaurornis flavirostra* was used as an outgroup to root the tree, following Garcia-Ramirez and Trewick (2015). DNA sequences for each gene were aligned and concatenated in Geneious Prime, resulting in a 3252 bp dataset. We estimated divergence dates by constructing a time-calibrated phylogeny in BEAST2, implementing a lognormal relaxed molecular clock and birth–death tree prior. The most appropriate nucleotide substitution model for the dataset was determined using Modeltest-NG (Darriba et al., 2020). We constrained the height of the tree (the divergence between *A. flavirostra* and *Porphyrio*) to between 28 and 39 Mya based on the divergence estimate calculated by Garcia-Ramirez et al. (2014) using a TMRCA prior with a normal distribution (mean = 34 Mya, 95% CI = 28–39 Mya). A prior was also placed upon the origin of *Porphyrio* based on the analysis of Garcia-Ramirez et al. (2014), using a TMRCA prior with a normal distribution between 11 and 20 Mya (mean = 15.5, 95% CI = 11–20 Mya), following Garcia-Ramirez and Trewick (2015). The MCMC was run for 20 million generations, sampling every 1000 generations with the first 10% discarded as burn-in. MCMC convergence and effective sample sizes (all >200) were checked in Tracer (v1.7; Rambaut et al., 2018). Bayesian phylogenetic trees were summarised using TreeAnnotator (v2.6.2) to create a maximum clade credibility tree, which was visualised in FigTree (v1.4.3).

3 | RESULTS

3.1 | Sequencing results

We successfully retrieved mitochondrial genomes from 12 takahē and 4 moho bones, 5 takahē museum skins and 17 contemporary takahē blood samples. The 20 dated takahē and moho samples ranged in age from 15,900 to 63 years BP (Tables S2–S4). The sequencing coverage for 16 subfossil and archaeological specimens ranged between 59.3% and 100% (13 samples with over 95% coverage), with a mean sequencing depth of 26.2× (5.3–95.5×; Table S3), while sequencing coverage for historical museum skins ranged from 98.4% to 100%, with a mean sequencing depth of 1221.8× (9.2–2921.1×; Table S4). All contemporary samples yielded complete mitochondrial genomes with high sequencing depth (134.9–183.5×) (Table S2). All ancient DNA libraries exhibited post-mortem DNA damage patterns consistent with ancient DNA (Figure S1).

3.2 | Phylogeographic structure within takahē and moho

Near-identical tree topologies were inferred by the maximum likelihood and Bayesian inference phylogenies (Figure 1b and Figure S2). The only major difference between phylogenies was the position of the Kaikai Beach (CM Av11476) and McKirchers Cave (CM Av32389.2) specimens, with moderate support (BS = 63–72, PP = 0.6–0.65). We found significant genetic divergence between takahē and moho, and relatively deep genetic structure among moho, with the central North Island moho more closely related to

the northern individuals than the southern one. Little phylogeographic structure was detected within takahē, with closely related lineages found at both ends of the South Island (i.e. NMNZ S.23669 Honeycomb Hill [northern South Island] and OM Av6858 Forest Hill [southern South Island]; Figure 1).

However, we report temporal phylogenetic structure within takahē. Takahē specimens from the Pleistocene and Pleistocene–Holocene transition (e.g. Honeycomb Hill and Hodges Creek in northern South Island) cluster closer to the most recent common ancestor of takahē, while late Holocene and archaeological specimens form a moderately supported clade nested within the phylogeny, sister to closely related post 19th-century individuals (Figure 1b). The phylogenetic position of undated specimens from Forest Hill and Honeycomb Hill (NMNZ S.23490 and OM Av6858), in combination with taphonomic data (Worthy, 1998; Worthy & Cresswell, 1993) suggests they are Pleistocene in age. Haplotype networks (Figure 2) exhibit the same genetic divergence between takahē and moho and illustrate the close relationships between the various takahē haplotypes of all ages.

The temporal haplotype network (TempNet; Figure 2b) illustrates the loss of takahē mitochondrial genetic diversity through time, a finding corroborated by our estimates of temporal genetic diversity (see Table 1). Our analyses suggest that a diverse range of haplotypes found within the Holocene takahē population are lost after human arrival and the restriction of takahē to southern New Zealand (Figure 2). Genetic turnover is also observed between takahē populations over the Pleistocene–Holocene transition (Figure S3), but this result may be explained by sampling biases (discussed below).

3.3 | Takahē demographic history

Summary statistics and estimates of mitochondrial genetic diversity from subfossil/archaeological takahē confirm that takahē populations once contained higher levels of genetic diversity, prior to a population bottleneck post-Polynesian arrival (Table 1; see analyses of demographic history below). We identified 11 different haplotypes in our subfossil/archaeological takahē dataset, while only 3 haplotypes are present in individuals originating from the 1800s onwards (Table 1). Haplotypes from subfossil samples are more diverse than those found in more recent individuals, separated by multiple substitutions, while haplotypes detected post-1800s are all closely related, separated from other such haplotypes by only one or two mutations across the entire mitochondrial genome (Figure 2). This is also reflected in the increased levels of haplotype and nucleotide diversity found in the subfossil/archaeological individuals (Table 1).

All estimates of Tajima's *D*-statistic and Fu's *F*-statistic were negative, yet only a single estimate was considered statistically significant (Table 1). A negative Tajima's *D* value suggests that the ancient takahē population contained an excess of rare alleles, consistent with a selective sweep or population expansion following a recent bottleneck. In contrast, the Bayesian skyline plot suggests that the effective population size of takahē has remained stable throughout the last 20 Kya, before a sudden decline beginning approximately 0.6 Kya approximately coincident with the arrival of Polynesians (Figure 3). Although the date randomisation test (Figure S4) suggests that our dataset contains sufficient temporal information to calibrate the molecular clock, limited sample sizes may not have the

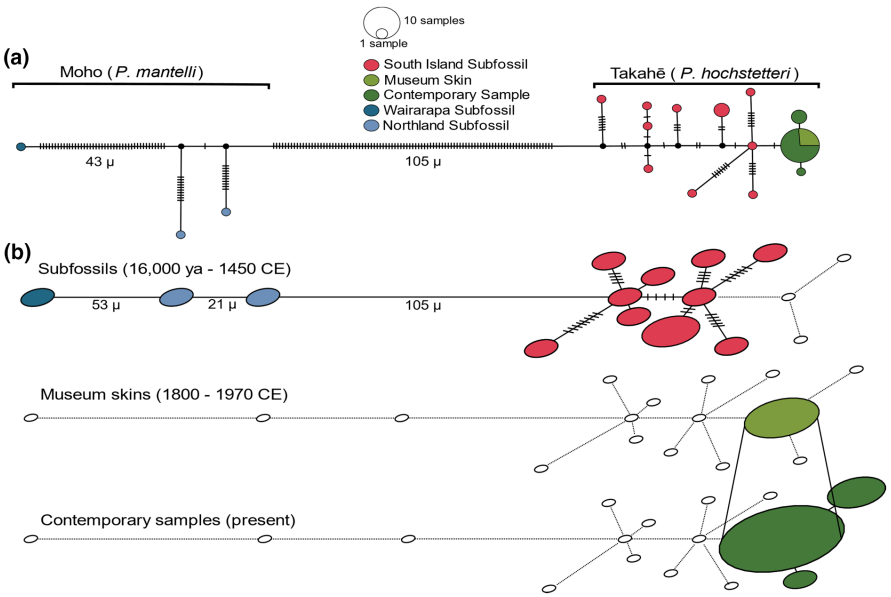

FIGURE 2 (a) Median-joining haplotype network constructed from takahē mitochondrial genomes, masking sites with missing data. Circle size is proportional to haplotype frequency, haplotype colours correspond to geography and time period as in Figure 1. Mutations are represented by hatch marks. (b) Temporal haplotype network (TempNet) of 11,584 bp of takahē mitochondrial DNA utilising three discrete time periods based upon the sample type used (subfossil bones, museum specimens/skins and contemporary samples). Circle size is proportional to haplotype frequency, white circles represent haplotypes absent from a given time period, while haplotypes shared between time periods are linked.

TABLE 1 Summary statistics and genetic diversity estimates calculated from 14,054 bp of South Island takahē mitochondrial DNA.

	n	H	HD	π (SD)	S	K	TD (*p*-value)	FF (*p*-value)
Takahē subfossils	12	11	0.985	0.00081 (0.00012)	53	11.439	**−1.60 (.041)**	−2.42 (.096)
Museum specimens	5	1	–	–	–	–	–	–
Contemporary samples	17	3	0.386	0.00003 (0.00001)	2	0.405	−0.74 (.242)	−0.674 (.207)
Museum specimens + Contemporary samples	23	3	0.312	0.00003 (0.00001)	2	0.324	−0.90 (.203)	−0.916 (.159)

Note: Sample size (*n*), haplotype frequency (H), haplotype diversity (HD), nucleotide diversity (π), frequency of segregating sites (S), average number of nucleotide substitutions (*k*), Tajima's *D*-statistic (TD) and Fu's *F*-statistic (*FF*). Bold values indicate statistical significance.

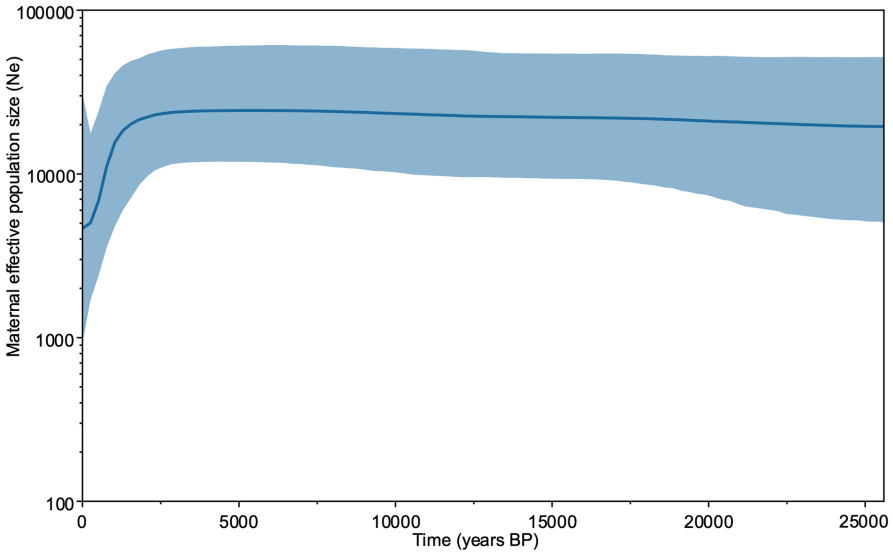

FIGURE 3 Bayesian skyline plot depicting changes in the effective population size of South Island takahē through time. The solid line depicts the median value, while shaded areas represent 95% HPD intervals.

power to resolve less recent demographic changes (e.g. those associated with the Last Glacial Maximum 19–29 Kya).

3.4 | Porphyrio phylogenetics and molecular dating

Contrary to previous studies that recovered a paraphyletic relationship between takahē and moho (Garcia-Ramirez & Trewick, 2015; Trewick, 1997), our phylogenetic analyses of significantly longer sequences and hence greater phylogenetic signal recovered a strongly supported monophyletic sister species relationship between the two species (Figure 4). Other relationships among *Porphyrio* taxa are consistent with Garcia-Ramirez and Trewick (2015). The molecular dating analysis estimated that takahē/moho diverged from other *Porphyrio* taxa ~4 Mya (95% HPD = 2.5–5.5 Mya) consistent with previous studies (Garcia-Ramirez et al., 2014; Garcia-Ramirez & Trewick, 2015), while takahē and moho diverged from each other ~1.5 Mya (0.8–2.5 Mya).

4 | DISCUSSION

4.1 | Temporal structure and population demography of takahē

Phylogenetic analyses did not detect phylogeographic structure within takahē (Figures 1b and 2). Repeated Pleistocene glaciations do not appear to have structured takahē populations in the same manner as other South Island endemic avian species (Bunce et al., 2009; Dussex et al., 2018; Rawlence et al., 2012; Weir et al., 2016; Weston & Robertson, 2015) by isolating populations within distinct refugia. The takahē subfossil record suggests they favoured edge habitats (Trewick & Worthy, 2001), which would have been widespread throughout the Pleistocene, with much of the eastern and southern South Island consisting of a mosaic of open grasslands and shrubland with small patches of forest (Newnham et al., 2013). The palaeontological site of Forest Hill in southern New Zealand has been interpreted as a time-averaged pitfall deposit containing the remains of vertebrates dating to the past 12 Kya based on moa faunal assemblage and taphonomy (Worthy, 1998). If the takahē bones from this site date to the last glacial period, then takahē occupied both ends of the South Island during this time. Geographically intermediary subfossils dating to the last glacial period prior to the LGM (29–19 Kya), where ancient DNA sequencing was unsuccessful, have been found in central South Island fossil deposits (e.g. Tuarangi station; Worthy, 1997b). However, the late Pleistocene (12–15 Kya) takahē fossil record is scarce and mostly restricted to the northwestern South Island (e.g. the Honeycomb Hill and Hodge Creek cave systems; Trewick & Worthy, 2001; Worthy, 1997a; Worthy & Cresswell, 1993). Takahē appear highly adaptable to different environments, with translocated individuals able to take advantage of novel food resources (Hunter-Ayad et al., 2021). This adaptability, coupled with abundant favourable habitats, may have prompted the

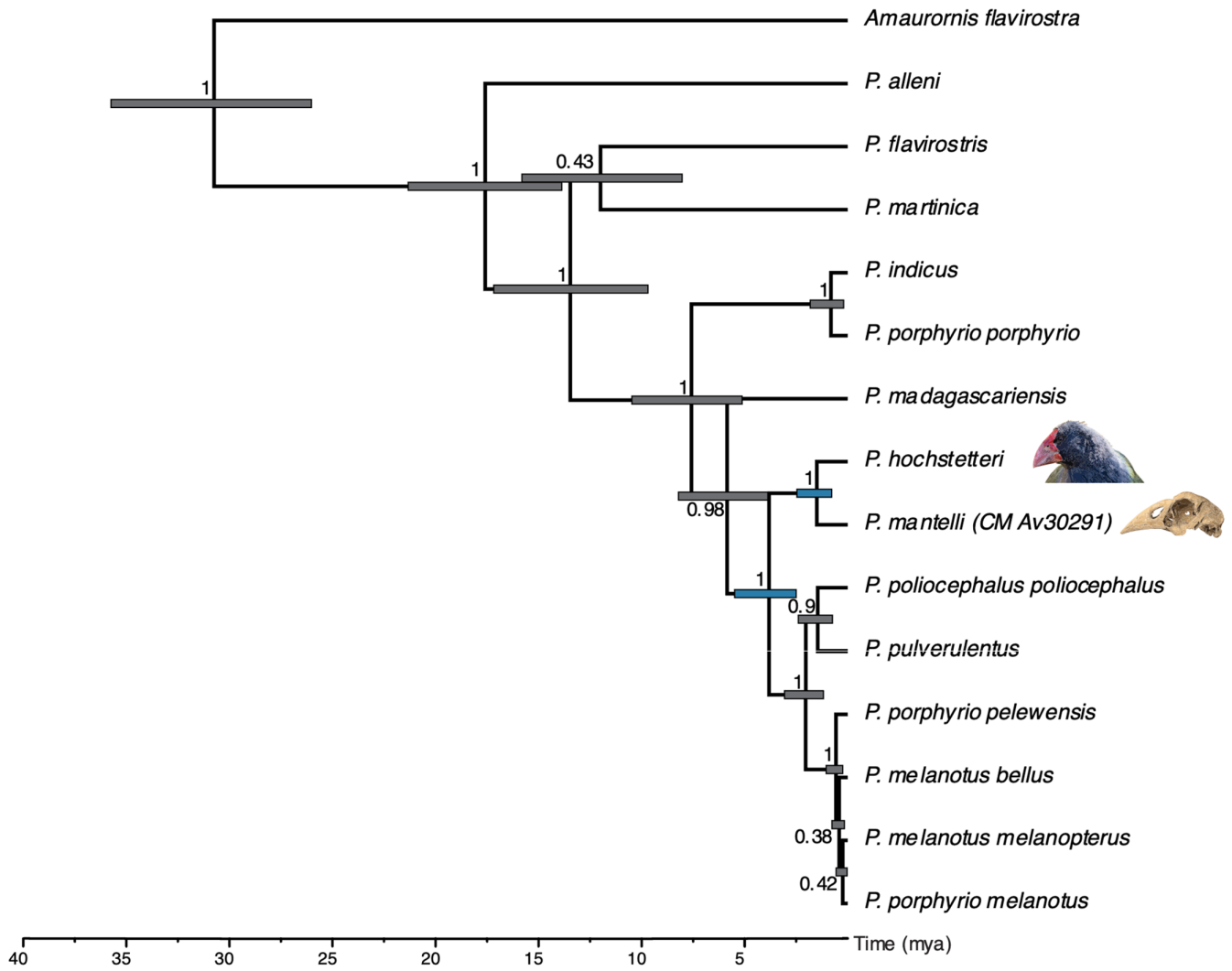

FIGURE 4 Bayesian time-calibrated phylogeny of *Porphyrio* using four mitochondrial genes. Node labels denote posterior probability values, while node bars represent the 95% HPD interval for node ages. HPD intervals relating to takahē/moho are coloured. The positions of the takahē (*P. hochstetteri*) and moho (*P. mantelli*) are illustrated.

formation of a well-connected takahē metapopulation throughout the South Island leading to the occurrence of closely related genetic lineages at either end of the island during the last glacial period.

Geographic and temporal disjunctions within the takahē subfossil and archaeological record (see below) make it difficult to confidently infer any biological response of takahē to Quaternary climatic and environmental change. In contrast to subfossils dating to the Pleistocene and Pleistocene–Holocene transition (see also Boast, 2019 for a high altitude takahē subfossil from Euphrates Cave potentially of early Holocene age), takahē from the late Holocene are found throughout the eastern and southern South Island but are near-absent from north-western regions. This suggests that by the early Holocene, the range of takahē may have become largely restricted to eastern and southern regions. In addition, sampled Holocene-aged takahē represent different genetic lineages to those found in older subfossils (Figure S3). This genetic and geographic disjunction between Holocene and older takahē subfossils is potentially related to local extinction and population bottleneck/genetic turnover across the Pleistocene–Holocene transition ~11.7 Kya (Figure 1 and Figure S2). Takahē may have been pushed out of the north-western South Island (or forced to higher altitudes) as the climate warmed during the Pleistocene–Holocene transition and their preferred open shrubland–grassland habitat was replaced by forest at lower altitudes. This appears to be the case for crested moa (*Pachyornis australis*; a species which also prefers open vegetation and occurred in similar altitudinal fossil sites), which tracked its preferred sub-alpine habitat through time, as younger subfossils are found at higher elevations than older remains (Rawlence et al., 2012). If takahē were unable to track changes in the distribution of their preferred habitat, the population may have gone locally extinct, potentially explaining the changes in temporal structure and geographic distribution across the Pleistocene–Holocene transition. However, we do not see evidence of a population bottleneck in our Bayesian Skyline analysis. Low sample sizes and a limited number of dated individuals mean there may not be enough power within our dataset to reject the null assumption of constant population size

during this time period as these analyses typically perform poorly with low information content datasets (Ho & Shapiro, 2011).

Preliminary mitochondrial control region sequence data from many of the specimens analysed herein (including several that were not successful for mitochondrial genome recovery) broadly support our mitochondrial genomic analyses, with a common haplotype found across the eastern and southern South Island during the late Holocene (Mas-Carrió, 2016). Mitochondrial genome data provide increased resolution, revealing a number of closely related haplotypes throughout the same areas at the same time. Additional mitochondrial genomes from Holocene specimens from the eastern South Island are likely to be closely related to other Holocene-aged mitochondrial genomes presented here, which would suggest that takahē populations were well connected during the Holocene.

The widespread eastern and southern South Island distribution of takahē during the Holocene, including at the time of Polynesian arrival in the late 13th century, stands in stark contrast to their relict historical and contemporary distribution. This is accompanied by a significant population genetic bottleneck, as seen in our population genetic summary statistics and Bayesian Skyline Plot, where we reconstructed a rapid decline beginning at approximately 0.6 Kya. This also supports previous preliminary analyses of a small fragment of the mitochondrial control region (Grueber & Jamieson, 2011; Mas-Carrió, 2016) which demonstrated that historical and contemporary samples shared the same haplotype, indicative of a pre-European bottleneck.

The decline in distribution and genetic diversity in takahē had almost certainly occurred by the time of European settlement in the late 18th century and was no doubt caused by human impact. Many of New Zealand's endemic birds became extinct during the early Māori period (1280–1450 CE) as east Polynesian arrivals burned forests, hunted naïve K-selected slow breeding species, and introduced the Pacific rat (kiore) and Polynesian dog (kuri; Greig & Rawlence, 2021; Perry, Wheeler, et al., 2014; Perry, Wilmshurst, & McGlone, 2014; Tennyson & Martinson, 2006). Takahē bones have been found in archaeological middens dated to this period but not thereafter (Trewick & Worthy, 2001), strongly suggesting the drastic decline of takahē occurred shortly after Polynesian settlement. Multiple life history traits such as flightlessness and large body size increase the extinction risk of takahē/moho (Garcia-Ramirez & Di Marco, 2020). Indeed, modelling analyses have suggested that even low levels of human pressure may have been sufficient to cause the extinction of several large vertebrate taxa in New Zealand (Holdaway et al., 2014; Rawlence et al., 2016). In contrast to the sustained human population pressure in the North Island that likely led to the extinction of moho, the reduced human pressure in the southern South Island (Waters et al., 2017) apparently allowed takahē to persist as a small isolated population in the remote, mountainous Fiordland region. Populations of kākāpō persisted in this same area until the 1980s, following their extirpation from other areas of New Zealand (Powlesland et al., 2006). Kākāpō were also significantly impacted by anthropogenic influences, with genetic bottlenecks associated with both Polynesian and European arrival (Dussex et al., 2021; Seersholm et al., 2018). The small effective population size of takahē over the last 600 years has likely meant that genetic drift has further reduced the amount of genetic diversity present within this relict population.

Intriguingly, we have not detected the closely related historical/contemporary genetic lineages present in the living population in our widespread subfossil and archaeological specimens. This could be because (1) modern haplotypes were restricted to Fiordland; (2) modern haplotypes were rare during the Pleistocene and Holocene, and became fixed due to genetic drift in a small bottlenecked population after Polynesian arrival; or (3) de novo mutations occurred in the bottlenecked population and drifted to near fixation. The rarity of subfossil and archaeological takahē remains from their relict distribution in Fiordland (e.g. no takahē remains described by Coutts, 1977; remains noted by Duff, 1977 but potentially not collected) makes it difficult to test these hypotheses, though it appears unlikely that the modern haplotypes were restricted to Fiordland, given the lack of phylogeographic structure in takahē.

4.2 | Phylogeographic structure within moho

Phylogeographic structure between extinct moho populations was detected, with specimens from the central and northern North Island distinct from more southerly populations. Central North Island volcanism (i.e. eruptions in the Taupō volcanic zone) is often implicated as the cause of phylogeographic structure in other species inhabiting this region (e.g. Shepherd et al., 2007) including analogous flightless birds (Bemmels et al., 2022; Bunce et al., 2009). However, the large geographic distance between the sequenced moho specimens means we cannot rule out a simple isolation-by-distance model or discern phylogeographic barriers. Additional palaeogenetic data from other North Island sites will likely illuminate phylogeographic patterns in moho.

4.3 | Takahē and moho originate from a single colonisation of New Zealand by a *Porphyrio* ancestor

While previous morphological and molecular studies suggested that takahē and moho resulted from two separate colonisations of New Zealand by volant *Porphyrio* ancestors (Garcia-Ramirez & Trewick, 2015; Trewick, 1996, 1997), our phylogenetic analysis strongly supports a sister species relationship (Figure 4). The mitochondrial 12S moho sequence published by Trewick (1997) exhibits multiple cytosine to thymine mutations that are not observed within any of our sequences. These are indicative of post-mortem DNA damage (Hofreiter et al., 2001) and (along with the little phylogenetic signal provided by the short sequence length analysed) likely led to the distinct phylogenetic position of the moho in the analyses conducted by Trewick (1997). The additional mitochondrial Cytochrome B data sequenced by Garcia-Ramirez and Trewick (2015) do not exhibit post-mortem DNA damage patterns, further suggesting that the erroneous 12S sequence was the cause of the previously observed paraphyly between takahē and moho. Our sequences

(derived from high-throughput DNA sequencing) have enabled us to account for DNA damage by only calling a consensus sequence for regions with sufficient sequencing depth (>3) and generate enough DNA sequence data to confidently estimate phylogeny. Our results highlight the strengths of high-throughput DNA sequencing in the study of ancient DNA and the importance of best-practice standards within the field (Orlando et al., 2021).

A sister species relationship between takahē and moho implies that New Zealand was colonised only once by a single *Porphyrio* ancestor which subsequently evolved into the two species. A single dispersal event and subsequent North–South Island divergence is consistent with other avian sister species pairs endemic to New Zealand (e.g. Boast et al., 2019). Our molecular dating suggests that the common ancestor of takahē and moho colonised New Zealand between ~4 and 1.5 Mya. This interval includes the Miocene–Pliocene transition, a period where additional open habitats were beginning to form throughout New Zealand due to rain shadow effects from the uplift of the Southern Alps, and global climatic cooling (Heenan & McGlone, 2013; Rawlence et al., 2019). The formation of open habitat may have facilitated the colonisation of New Zealand by the common ancestor of takahē and moho by providing it with suitable habitat to which it could adapt and establish viable populations. This scenario has been posited as the driver of speciation within multiple genera of birds exhibiting Australian and New Zealand sister species pairs (see Rawlence et al., 2019).

The glacial/interglacial cycles of the Pleistocene are implicated in the divergence between takahē and moho ~1.5 Mya. This divergence date is largely consistent with the formation of an overland connection between the North and South Islands as Pliocene sea straits in central New Zealand (e.g. the Manawatū Strait) closed, in combination with lowered glacial sea levels (Bunce et al., 2009; Trewick & Bland, 2012). Such a connection would have enabled takahē or moho ancestors to disperse from one landmass to the other, before the subsequent formation of the Cook Strait (~500 Kya; Lewis et al., 1994) and rising sea levels during warm interglacials separated the two islands. This hypothesis has been proposed to explain the divergence between other North–South Island sister species pairs of flightless birds such as the giant moa (*Dinornis* spp.; Bunce et al., 2009), adzebill (*Aptornis* spp.; Boast et al., 2019) and kiwi (*Apteryx* spp.; Weir et al., 2016) with mean divergence dates of approximately 1.5–2 Mya. These comparable divergence time estimates between North and South Island sister species pairs of flightless birds suggest that the common ancestor of takahē/moho may have become flightless by this time.

5 | CONCLUSIONS AND CONSERVATION IMPLICATIONS

The application of palaeogenetic techniques to the remains of the takahē and moho has provided new insights into their evolutionary history. Our results suggest that takahē and moho are sister species, resulting from a single colonisation of New Zealand by a volant common ancestor approximately four Mya, with subsequent divergence of the North and South Island forms roughly 1.5 Mya. Colonisation of New Zealand may have been aided by the formation of new habitats, while island speciation is potentially related to the formation of marine straits and separation of the two landmasses. We find no evidence of strong phylogeographic structure among takahē populations, in contrast to that found in moho, and report evidence of temporal genetic structuring within takahē. This temporal structure is suggestive of local extirpation or genetic turnover of takahē in the north-western part of their range during the Pleistocene–Holocene transition, but biases within the fossil record and biomolecular preservation make it difficult to determine population dynamics during this period. Finally, we present the first direct evidence for lost mitochondrial genetic diversity in takahē across the Pleistocene–Holocene transition, and as a direct consequence of the arrival of humans to New Zealand.

The presence of low genetic diversity within takahē due to past genetic/population bottlenecks means that the conservation and maintenance of their remaining genetic diversity becomes particularly important, in order to maximise their adaptive potential to novel threats and changing environments (e.g. disease, climate change). Maintenance of genetic variation within takahē remains a key goal of the takahē recovery programme (Greaves et al., 2020), with takahē at various secure sites (e.g. offshore islands, fenced ecosanctuaries) managed as a metapopulation, such that individuals are translocated between sites in order to minimise inbreeding and the loss of genetic diversity due to drift. Although some genetic diversity was likely lost during the founding of some of these populations (Grueber & Jamieson, 2008), modelling suggests that current management strategies for takahē will conserve genetic variation in the future (Greaves et al., 2020). While it may one day be possible to restore some of the lost genetic diversity identified herein to takahē populations through genome-editing techniques (e.g. CRISPR-CAS9), this would require significant support from both the New Zealand public and conservation practitioners (Taylor et al., 2017).

Mitochondrial DNA is non-recombining and therefore constitutes a singular locus, with a singular evolutionary history. Singular loci may not be representative of the true evolutionary history of a given lineage due to factors such as introgression, signal saturation and incomplete lineage sorting (Ballard & Whitlock, 2004; Funk & Omland, 2003; Phillips & Zakaria, 2021). On the other hand, ancient mitochondrial genomes are cost-efficient for sequencing and can provide a high degree of phylogenetic resolution (Phillips & Zakaria, 2021). We speculate that analysis of nuclear genetic variation would be congruent to our well-supported mitochondrial DNA analyses, illustrating the loss of genetic diversity within takahē following their decline and population bottleneck and the evolutionary relationships within this group. Analysis of genome-wide nuclear DNA from takahē would provide additional insights into levels of genetic variation and inbreeding, population structure and demography by sampling multiple independent points across the genome, providing increased resolution over the single, non-recombining genetic marker used here. A high-quality takahē reference genome has recently been assembled (Cheng et al., 2022), facilitating the use of genomic data to inform the management and conservation of takahē. Results from

the analysis of genomic data will be particularly relevant to takahē conservation as they can facilitate the conservation of genetic variation by providing an in-depth picture of genetic diversity and relatedness within the population (but see also Grueber et al., 2010, 2011; Grueber & Jamieson, 2008 for earlier takahē studies utilising pedigrees and microsatellites). In addition, demographic reconstructions using genomic approaches (e.g. PSMC; Li & Durbin, 2011) may improve our understanding of takahē population dynamics throughout the last glacial period and transition into the present interglacial.

Genomic approaches can also be applied to ancient DNA. Although the retrieval of high-coverage genome sequences from subfossils is often challenging/uneconomical due to low levels of endogenous DNA within such remains, hybridisation capture techniques can be used to economically retrieve genome-wide loci from subfossils (Suchan et al., 2022). Additionally, avian museum skins frequently contain high levels of endogenous DNA (Irestedt et al., 2022), facilitating the recovery of genomic sequences via shotgun sequencing (Dussex et al., 2021). Genome sequencing of the museum skins analysed herein may illuminate inbreeding/heterozygosity levels shortly after the time of European colonisation, while hybridisation capture techniques may be necessary to recover genomic data from takahē/moho subfossils and determine the amount of genetic diversity present within their populations prior to their population/genetic bottleneck and extinction, respectively. Because conventional ancient DNA sampling techniques are destructive, we did not sample every available subfossil takahē/moho individual. We instead attempted to maximise temporal and geographical coverage while minimising sampling effort. Additional subfossils remain to be tested in the future, where improved DNA extraction, library preparation and hybridisation capture techniques may improve the amount of endogenous DNA retrieved from such subfossils.

Finally, this study also highlights the potential benefits of palaeontology for informing evidence-based conservation management decisions, such as the suitability of translocation sites. Takahē have recently been translocated to the Gouland Downs region of Kahurangi National Park in the north-western South Island. This area was chosen as it contains large areas of open tussock habitat suitable for takahē, low densities of invasive predators, and because takahē subfossils are found in the north-western South Island (Hunter, 2022). Our results suggest that takahē populations (and their unique genetic lineages) inhabiting this region may have been extirpated following the Pleistocene–Holocene transition and become locally extinct, indicating that this area may not be the most suitable habitat for takahē. Ecological niche and species distribution modelling has been used to try and identify areas of suitable habitat for takahē translocations/reintroductions (Hunter-Ayad et al., 2021), but these models are currently based upon their relict distribution in the open tussock habitat of the Murchison Mountains, not their natural pre-human distribution and edge habitat preferences (Hunter-Ayad et al., 2021). This relict distribution likely provides a poor ecological baseline and predictor of takahē occurrence, as it simply reflects where takahē were able to survive following successive waves of human arrival, the destruction of much of their natural habitat and the introduction of mammalian predators. A proxy for their natural distribution is available via the fossil record, which could be used to better inform the management of takahē populations. Takahē subfossils have been found in a variety of different habitats and altitudes across the South Island, including formerly forested areas and coastal sand dunes (Trewick & Worthy, 2001). They are rarely found above the treeline (Boast, 2019), despite the existence of many potential subfossil deposits containing other avian species (Trewick & Worthy, 2001), suggesting that they do not favour the sub-alpine tussock grassland habitats of their relict range. Ecological niche and species distribution models incorporating the takahē fossil record may be able to identify additional areas of suitable habitat for takahē in the South Island and also in the North Island, where takahē could potentially be introduced as an ecological surrogate of the extinct moho. However, the most important criterion for selection of a translocation site is (and will remain) predator control (Hunter-Ayad et al., 2021).

AUTHOR CONTRIBUTIONS

NJR and JMW conceived the project and provided support, supervision, materials and funding. BCR provided samples. AJFV, EM-C and GCG conducted the laboratory work. AJFV, GCG and LD analysed the data. AJFV wrote the initial manuscript draft. All authors edited the manuscript and approved its content for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Consensus mitochondrial genome sequences have been deposited on GenBank, accession numbers: OR753214, OR750879–OR750915. Raw sequencing data have been deposited on DRYAD (DOI: 10.5061/dryad.j0zpc86mz).

BENEFIT SHARING STATEMENT

Benefits Generated: The results of this research will help improve evidence-based conservation management of this taonga (treasured) species by tangata whenua and the New Zealand Department of Conservation. Benefits from this research also accrue from the sharing of our data and results on public databases as described above.

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SUPPORTING INFORMATION

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

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