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SHORT COMMUNICATION

PENSOFT.

MUSEUM OF NEW ZEALAND TE PAPA TONGAREWA

DNA sequence from a putative South Island kōkako (*Callaeas cinerea*) feather identifies it as a blackbird (*Turdus merula*)

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The South Island kōkako (*Callaeas cinerea*) is one of five species in the endemic New Zealand wattlebird family (Callaeidae). It is estimated to have diverged from its closest relative, the North Island kōkako (*C. wilsoni*), between 1.5 and 2.7 million years ago (Gibb and Shepherd 2022; Lubbe et al. 2022). South Island kōkako were formerly found in forests throughout the South Island (Salvador et al. 2019) but declined following the introduction of mammalian predators such as ship rats and stoats (Clout and Hay 1981).

South Island kōkako were declared extinct in 2007 (Hitchmough et al. 2007), and at that time there had been no confirmed sightings of this species since 1967. However, its status was later changed from "Extinct" to "Data Deficient" (Robertson et al. 2013) following a sighting in North Westland in 2007 that was accepted by the Ornithological Society of New Zealand's Records Appraisal Committee (Miskelly et al. 2012).

Despite numerous visual and aural reports of South Island kōkako over the last 50+ years (e.g., Milne and Stocker 2014), there has been no definitive evidence, such as a specimen or DNA evidence, to support its ongoing survival. A feather was found by Dave Crouchley in 1986 on Rakiura/Stewart Island during a search for South Island kōkako and subsequently identified as kōkako by John Darby of Otago Museum (Buckingham 1987). This feather has been referenced in scientific literature and popular articles (Buckingham 1987; Nilsson 2002; Evans 2016), in some cases as providing definitive evidence for the survival of this species. Prior to the discovery of this feather, the last accepted record of South Island kōkako from Rakiura/Stewart Island was an observation in 1937 (Harper 2009). Here we report on the DNA identification of this feather, which is now part of the Te Papa collection (NMNZ OR.029399).

The lower 5 mm of the feather calamus was removed for DNA extraction with a sterile razor blade. DNA extraction and PCR setup were performed in a dedicated ancient DNA laboratory located in a separate building from where modern DNA and PCR products are handled. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's instructions but eluting in a final volume of 45 µl buffer AE. A 797 base pair (bp) fragment of the cytochrome oxidase I (COI) locus was amplified and sequenced in five overlapping segments, between 130 bp and 251 bp in length, using the avian-specific primers of Patel et al. (2010). PCR amplification and sequencing were performed in both directions for each of the five amplicons, as described in Shepherd et al. (2020). Potential contamination was monitored through the use of negative extraction and PCR controls, and none was detected.

The resulting COI chromatograms were manually checked and contained no double peaks or other ambiguous nucleotide bases, indicating that the DNA was from a single source. The concatenated sequence (GenBank accession number MZ345595) was compared with DNA sequences in the NCBI GenBank database using a nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This search revealed that the feather DNA sequence was identical to a sequence from a Eurasian blackbird (*Turdus merula*) from Wellington (GenBank accession number MK261909). Three other blackbird sequences differed from our sequence by a single nucleotide substitution: MK262465 from Auckland, GQ482831 from Russia, and KF946897 from the Netherlands.

A PCR test was developed to exclude the possibility that contaminating blackbird DNA was swamping endogenous

South Island kōkako DNA in the feather sample. No COI sequences were publicly available for South Island kōkako at the time this test was designed. Therefore, a COI sequence was obtained using the methods described above from the toepad of a South Island kōkako skin (NMNZ OR.000169) collected from Stewart Island. This South Island kōkako sequence (GenBank accession number MZ345596) differed from the Stewart Island feather DNA sequence at 102 nucleotide sites. Both sequences were used to design two diagnostically discriminating forward PCR primers, each used separately for PCR amplifications with the reverse primer AWCR6 (Patel et al. 2010). Kok1F (5'-TCTGATTCTTCG-GACATCCA-3') was designed to amplify only a 115 bp fragment of South Island kōkako COI, and Turdus1F (5'-GATTCTTTGGCCACCCTGAAGTCTAT-3') to amplify only a 112 bp fragment of blackbird COI. Our PCR amplifications using these two primer sets confirmed their specificity. A PCR product was obtained with the primers Turdus1F and AWCR6 from NMNZ OR.29399; however, no PCR amplicon was obtained from this feather with the South Island kōkako-specific primers Kok1F and AWCR6, indicating that it did not contain any amplifiable South Island kōkako DNA.

The most likely explanation for our result is that the feather is from a blackbird. Blackbirds are very common on Stewart Island (https://www.stewartisland.co.nz/birds/; accessed 22 October 2020), where the feather was found, and the feather color is consistent with it being a blackbird. Furthermore, another putative South Island kōkako feather found at Burton Creek on the West Coast of the South Island was also identified as a blackbird using DNA sequencing (Anon 1996; Ritchie et al. 1997), suggesting morphological similarity between the feathers of the two species.

An alternative explanation is that the blackbird sequence we obtained from specimen NMNZ OR.029399 is the result of contamination, either in the field or in the laboratory. We consider contamination within our laboratory unlikely for several reasons. Contamination has been reported from laboratory reagents, but this tends to derive from domestic animals, especially pigs, cows, and chickens (Leonard et al. 2007). We have never examined or extracted DNA from blackbird specimens in either our ancient DNA or modern DNA facilities. We have used the same COI primers to amplify DNA from over 100 bird specimens with low quantities of DNA, including from subfossil bones (e.g., Tennyson et al. 2015; Shepherd et al. 2022), but have never obtained blackbird DNA sequences from any of them.

Contamination is still a remote possibility, especially if it occurred in the field. Since we could not detect any kōkako DNA with our specific primers, any endogenous kōkako DNA in the feather would have had to degrade prior to contamination with blackbird DNA. Given this small chance of contamination, it is advisable to validate our DNA-based identification using an alternative method, such as protein analysis (Hollemeyer et al. 2002) or a more detailed morphological approach, like the microscopic analysis of feather down (Harwood 2011).

The rediscovery of species and populations thought to be extinct, including birds, is not unheard of. For example, the Bermuda petrel (*Pterodroma cahow*) was assumed extinct for 300 years until its rediscovery in 1951 (Murphy and Mowbray 1951). In New Zealand, the little spotted kiwi (*Apteryx owenii*) was thought to have gone extinct on mainland New Zealand in the late 1970s (Ramstad et al. 2021), but a new population was found on the West Coast of the South Island in 2025.

Regardless of the identity of NMNZ OR.029399, conclusive evidence such as photographs, video, feathers, or droppings for the survival of South Island kōkako should continue to be sought. Emerging methods like environmental DNA (eDNA) assays offer promising new avenues for detecting species' presence but have not detected South Island kōkako DNA to date (https://wilderlab.co/explore; accessed 18 September 2025).

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