

Short communication

Systematic affinities of two enigmatic New Zealand passerines of high conservation priority, the hihi or stitchbird *Notiomystis cincta* and the kokako *Callaeas cinerea*

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Received 22 September 2005; revised 7 January 2006; accepted 23 January 2006

Available online 9 March 2006

1. Introduction

Traditional systematic relations within the Passeriformes, perching birds, have recently been re-shuffled in a major review of the “largest avian radiation” (Barker et al., 2004). This includes now recognising five basal oscine passerine lineages, rather than the traditional view of two major groups, the Passerida and Corvida (Sibley and Ahlquist, 1990). The avian fauna of New Zealand provide key examples in this more complex view. Importantly, this includes the New Zealand wren family (Acanthisittidae), now regarded as a sister taxon to all other passerines (Barker et al., 2002; Ericson et al., 2002). New Zealand’s early split from Gondwana (82–85 Myr ago) may explain the large evolutionary split of the wrens and other speciation events within the endemic avifauna of this country, and these features provide key support for a Gondwanan origin to early passerine diversification (Barker et al., 2002, 2004; Ericson et al., 2002, 2003).

Here, we focus on two enigmatic passerine species of New Zealand, not included in previous systematic studies based on DNA sequence data, but whose relationships are thought to lie within deep splits of the recently re-shuffled phylogeny. First, the hihi is currently classified as the sole representative of an endemic genus, *Notiomystis*, within the honeyeater family (Meliphagidae, Higgins et al., 2001). According to Sibley and Ahlquist’s (1990), DNA–DNA hybridisation distances the honeyeaters rest within the Corvida. Analyses of DNA sequence data, however, have

confidently shown that Corvida is not monophyletic, but includes several groups that branched off from the lineage leading to the large radiation named Passerida (Barker et al., 2002, 2004; Ericson et al., 2002, 2003). The fact that most of these basal lineages of oscine passerines have their main distribution in the Australo-Papuan region has led to the conclusion that the Oscines evolved in this part of the world (Barker et al., 2004; Ericson et al., 2003). This in turn leads to the conclusion that the earliest evolution of passerine birds took place already on the former supercontinent Gondwana in the Late Cretaceous, a view in stark contrast to the long held idea of passerines being one of the youngest groups of birds. The honeyeaters, to which the hihi has been referred (Higgins et al., 2001), constitute one of these basal and ancient groups of oscines (Barker et al., 2002; Ericson et al., 2002). Whether, in fact, hihi are honeyeaters has also been questioned (Driskell, 2001; Higgins et al., 2001). An alternative placement, however, has not been forthcoming.

Second, the kokako is an extant member of an endemic family, the wattle birds (Callaeidae), consisting of three species (one extinct), each of which is placed in its own genus. The systematic relationships of this group to other perching birds have been difficult to determine apart from its obvious ancient split. Sibley and Ahlquist (1990) placed them within the Corvida, yet again this view has been challenged by recent studies. The latest hypothesis of the systematic position of the wattle birds (based on sequences of saddleback, *Philesturnus carunculatus*) is that it groups with the berry-peckers and the genera *Cnemophilus* and *Loboparadisaea* (Barker et al., 2004). The last two taxa were previously regarded as birds-of-paradise but are now known to have no

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close affinities with these (Cracraft and Feinstein, 2000). The grouping of saddleback with the berrypeckers and the cnemophiline birds received low statistical support, however, and the systematic position of the wattlbirds is currently best regarded as unresolved.

Determining the phylogenetic affinities of hihi and kokako to other perching birds is not only of academic merit. Both these species are threatened with extinction and are currently the focus of costly and extensive management (Innes and Flux, 1999; Taylor et al., 2005). The loss of biodiversity worldwide is a continuing crisis which New Zealand has not escaped. New Zealand's native fauna has proven susceptible to disturbances brought with human colonisation (32% of the land and freshwater bird species present when Polynesians settled c. 1000 ybp are now extinct) and many species still extant are threatened to some degree (Department of Conservation, 2000). Resources for conservation are limited and there is a necessity to prioritise use of such limited funds. New Zealand has developed a set of criteria for targeting conservation efforts which include phylogenetic uniqueness as an important component (Barker, 2002; Department of Conservation, 2000). Any change in classification of the hihi and/or kokako will therefore have important ramifications on their management.

Herein, we present an assessment of the degree of phylogenetic uniqueness of both the hihi and kokako based on an analysis of DNA sequences obtained from two protein-coding, nuclear genes. This data can be compared with that published for a wide range of passerine birds by Barker et al. (2002). It is our hope that the results will not only increase our understanding of the evolution of these birds, but also can be utilised in prioritising research and management of them.

2. Materials and methods

2.1. Taxon sampling

The dataset published by Barker et al. (2002) consists of sequences from two nuclear genes (RAG-1 and *c-mos*) obtained from 69 passerines representing almost all traditionally recognised families, including those to which the hihi and kokako are believed to be most closely related. GenBank numbers for the sequences from the hihi (*Notiomystis cincta*) are DQ345904 (RAG-1, 2872 bp) and DQ345902 (*c-mos*, 608 bp). The corresponding numbers from the kokako (*Callaeas cinerea*) are DQ345905 (RAG-1, 2872 bp) and DQ345903 (*c-mos*, 607 bp). We used three non-passerine taxa as outgroups in the analyses: a roller (*Coracias caudatus*), a swift (*Apus apus*), and a fowl (*Gallus gallus*).

2.2. Laboratory procedures

DNA was extracted from tissue or blood specimens using the QIAamp®DNA Mini Kit (QIAGEN®) following

the manufacturer's recommendations. Laboratory procedures for amplifying and sequencing the RAG-1 and *c-mos* genes follow protocols described in Cooper and Penny (1997); Groth and Barrowclough (1999); Barker et al. (2002).

The multiple segments obtained by sequencing with different primers were assembled to complete sequences using SeqMan II™ (DNASTAR®). When nucleotides could not be unambiguously determined, the IUPAC coding system was used. The sequences were aligned by eye with MegAlign™ (DNASTAR®). The aligned datasets became 2902 bp long for RAG-1 and 586 bp for *c-mos* (after removing a 36 bp long region where the alignment is ambiguous). The DNA segments from the two genes were analysed both separately and in combination.

2.3. Phylogenetic inference and model selection

Bayesian inference and Markov chain Monte Carlo (MCMC) were used for estimating phylogenetic hypothesis from our DNA data (Huelsenbeck et al., 2001). The models for nucleotide substitutions used in the analyses were selected for each gene individually by using Akaike Information Criterion (AIC, Akaike, 1973) and the program MrModeltest (Nylander, 2002) in conjunction with PAUP* (Swofford, 1998).

The posterior probabilities of trees and parameters in the substitution models were approximated with MCMC and Metropolis coupling using the program MrBayes (Ronquist and Huelsenbeck, 2003). Analyses were performed for both the individual gene partitions and the combined dataset. In the analysis of the combined dataset the models selected for the individual gene partition were used, but the topology was constrained to be the same. One cold and three incrementally heated chains were run for 2.5 million generations, with a random starting tree. Trees were sampled every 100th generation, and the trees sampled during the burn-in phase (i.e., before the chain had reached its apparent target distribution) were discarded. Four runs, starting from different, randomly chosen trees, were made to ensure that the individual runs had converged on the same target distribution (Huelsenbeck et al., 2002). After checking for convergence, final inference was made from the concatenated output from the four runs.

3. Results and discussion

The Bayesian analysis of the concatenated dataset resulted in a phylogenetic tree that agrees almost completely with the likelihood tree in Barker et al. (2002, Fig. 1). The only differences concern taxa that are directly involved in the position of hihi and kokako in the tree, and we will restrict our comments on the tree to these results. Hihi and kokako grouped together with high support in the tree based on the combined dataset (Fig. 1) and in the RAG-1 tree (Supplementary material). In the *c-mos* tree, however, they group neither with each other nor with any other

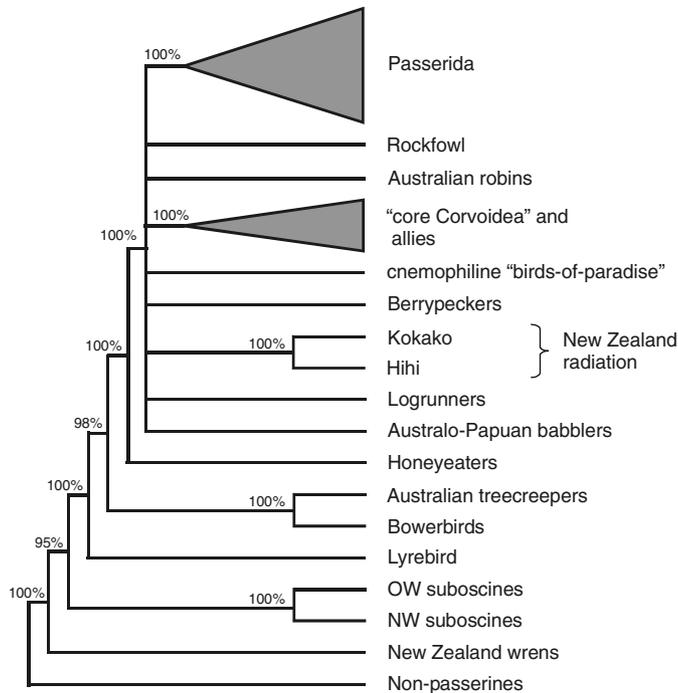


Fig. 1. Phylogenetic tree showing the systematic position of two enigmatic, New Zealand bird species, the hihi (*Notiomystis cincta*) and the kokako (*Callaeas cinerea*). The tree was derived from 3486 bp obtained from 71 species representing all major groups of passerines. The DNA sequences derive from two nuclear, protein-coding genes (RAG-1 and *c-mos*). To enhance visibility, the tree has been simplified to only show clades that received posterior probabilities of 95% or higher in the Bayesian analysis. The tree indicates hihi and kokako group strongly together as a single clade within the newly described paraphyly of the oscine radiation.

taxon. Instead both are part of a largely unresolved polytomy that also includes essentially all groups of oscine passerines except the most basal lineages. In relation to the remaining taxa it is only clear that hihi and kokako are not part of the “core Corvoidea” (sensu Barker et al., 2004) or Passerida (sensu Ericson et al., 2003). A further indication that they are not part of the Passerida radiation is that neither hihi nor kokako possess the insertion in the *c-myc* gene that is proposed to be a synapomorphy for this clade (Ericson and Johansson, 2003; Ericson et al., 2000) (P. Ericson pers. obs., data not shown).

Neither of the individual gene trees contradicts the systematic position of hihi and kokako suggested by the analysis of the combined dataset. The RAG-1 tree essentially gives the same result and groups these two New Zealand birds together with a 100% posterior probability (Supplementary material). The higher-level relationships among oscine passerines are largely unresolved in the *c-mos* tree above the level of lyrebirds, Australian treecreepers, and bowerbirds (Supplementary material). This dataset does not group hihi or kokako together either. However, the *c-mos* tree is not incompatible with the RAG-1 and the combined trees after nodes with posterior probabilities lower than 95% having been collapsed. Posterior probability values reflect the amount of data supporting a certain

node and the 95% cut off value has been empirically shown to be a good estimate for clades that can be considered “reliable” (Whittingham et al., 2002). Parts of a tree topology that involves nodes with lower support than 95% are often unstable.

The analysis of Barker et al. (2004) suggested an affinity of the wattlebirds with a group of birds occurring in New Guinea: three genera of berrypeckers (*Melanocharitidae*: *Melanocharis*, *Oedistoma* and *Toxoramphus*) and two cnemophiline “birds-of-paradise” (*Cnemophilus* and *Loboparasidaea*). In their analysis, however, this hypothesis received low statistical support (Bayesian posterior probability <0.95) and in our analysis hihi and kokako do not group with the berrypeckers. We have not been able to fully test Barker et al.’s (2004) hypothesis, as no cnemophiline “birds-of-paradise” are included in our dataset. Barker et al. (2004) also used another species of wattlebird (saddleback) than us, but the small (1%) sequence distance in RAG-1 unambiguously shows that kokako and saddleback are closely related (the corresponding difference between kokako and hihi is 4%). We conclude that hihi and kokako are members of an ancient radiation of birds in New Zealand to which the saddleback also belongs. It is uncertain when the ancestor of this group reached New Zealand, although this seemingly happened in the early Tertiary. Although the ancestor most probably left from Australia, this must remain unknown until the sister group to this New Zealand radiation is identified.

Evidence for a separate and ancient radiation of avian taxa in New Zealand, that includes both hihi and kokako, has important repercussions on management. Most importantly, the increased taxonomic uniqueness these species contribute to biodiversity requires consideration in priority setting by the New Zealand government (Barker, 2002; Department of Conservation, 2000). Given their vulnerability, hihi have recently been elevated in conservation status to ‘nationally endangered’ which places them in a category of species considered most at risk (Hitchmough, 2002; Taylor et al., 2005). Despite this, the hihi’s profile remains lower than that of charismatic species like kiwi and kakapo, and it is recognised as essential to raise public awareness of this species and the current recovery effort (objective 2 of hihi recovery plan, Taylor et al., 2005). Removing hihi from a relatively species rich and commonly seen Australasian family, which includes the New Zealand tui (*Prosthemadera novaseeland*) (honeyeaters, see Driskell and Christidis, 2003) certainly aids in this respect. The taxonomic uniqueness of the Callaeidae has afforded kokako (and saddleback) high national conservation profile and our results certainly substantiate their claims as some of New Zealand’s more iconic bird taxa.

Acknowledgments

We thank the New Zealand Department of Conservation and hihi recovery team leader, Richard Griffiths, for supporting this research. This work was supported by an

IoZ Research Fellowship and Royal Society Research Grant to JGE, and a Grant (No. 621-2004-2913) from the Swedish Research Council to PE. We are also grateful to Jan Ohlson and Dario Zuccon for sequencing the samples and Rose Thorogood for comments on drafts of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2006.01.026](https://doi.org/10.1016/j.ympev.2006.01.026).

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