Genomic insights into the taxonomy and migration of the Forest Kingfisher Todiramphus macleayii

Heather Johnston†, Jessica Fenker‡, Anna Kearns‡, Alex Drew§, Ian J. Mason§, Craig Moritz§, and Leo Joseph‡

†Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, Australia; ‡Australian National Wildlife Collection, CSIRO National Research Collections Australia, Canberra, Australia

ABSTRACT

The Forest Kingfisher Todiramphus macleayii inhabits eucalypt savannas, rainforests and mangroves across its distribution in Australasia. Two Australian subspecies are consistently recognised but the taxonomic status of resident New Guinean populations is unsettled. Genomic data from populations sampled across the species’ Australian and New Guinean ranges support the recognition of resident New Guinean populations at the subspecies level as T. m. elisabeth. Further work is required to examine island populations that remain unsampled genetically and to place the species in a broader phylogenetic analysis of Todiramphus kingfishers. We also report genetically based detection of a migrant individual in New Guinea either from eastern Australia or the Trans-Fly region of southern New Guinea. Our study provides a first insight into how genetic diversity is structured within this species across its range. It highlights remaining areas for study and illustrates the potential of DNA sequence data in tracking migratory movements of the species.

Introduction

The Forest Kingfisher Todiramphus macleayii occurs primarily in Australia and New Guinea. In Australia it is common in eucalypt woodlands and savannas across monsoonal and subtropical Australia and in New Guinea it also occurs in lowland rainforests. Though unrecorded from seemingly suitable habitat of the Kimberley region of Western Australia, it otherwise ranges east from the Top End of the Northern Territory to subtropical Queensland and northern New South Wales (Figure 1a); Ford 1978; Schodde 1997; Johnstone and Storr 1998; Higgins 1999; eBird https://eBird.org; all eBird records herein accessed 15 December 2021). In New Guinea, it occurs in lowland eastern parts of the island, almost exclusively in Papua New Guinea (PNG) and east to the Bismarck Archipelago (Figure 1a); Mees 1982; Beehler and Pratt 2016; Gregory 2017.

Three subspecies are usually recognised (Clements et al. 2021; Dickinson and Remsen 2013; Del Hoyo and Collar 2014; Gregory 2017; Gill et al. 2022) but the number has long been debated and, as we will show, uncertainty remains (e.g. Mayr 1937; Keast 1957; Mees 1982; Schodde 1997; Higgins 1999). Nominat T. m. macleayii generally refers to populations in Australia’s Northern Territory and some islands to its north and west (e.g. Mees 1982; Schodde 1997; Dickinson and Remsen 2013); they are blue-backed and only some females show a trace of green dorsally. The breeding range of migratory T. m. incinctus is eastern Australia and immediately north of Cape York Peninsula in the Trans-Fly region of New Guinea. It is readily distinguished by extensive greenish dorsal colour of adults of both sexes. Which subspecies occur between core ranges of these two subspecies is mostly unknown (but see Schodde 1997). Lastly, resident blue-backed New Guinean populations are generally assigned to T. m. elisabeth. Two factors have generated debate about whether T. m. elisabeth should be recognised or synonymised with nominate T. m. macleayii: (a) their shared blue dorsal plumage set against (b) the odd ‘leapfrog’ distribution pattern west and east of the populations of T. m. incinctus that results when they are synonymised as T. m. macleayii.

Among recent works, for example, Del Hoyo and Collar (2014) recognised T. m. elisabeth noting that it is barely separable from T. m. macleayii. Gregory (2017), Clements et al. (2021) and Gill et al. (2022) recognised T. m. elisabeth but gave no comments on its differentiation from T. m. macleayii. Conversely, Beehler and Pratt (2016) synonymised T. m. elisabeth with T. m. macleayii.
Figure 1. Genomic variation in Forest Kingfishers (drawn by Julian Teh). (a) Total distribution of the species (modified from Woodall and Kirwan 2020), key localities mentioned in the text, and sampling localities of Forest Kingfisher T. macleayii specimens used in this study (coloured symbols) sampled from five geographic regions — Northern Territory (squares) and Queensland (circles) from Australia, and Trans-Fly (star), Central Province (diamond) and Oro Province (triangle) from Papua New Guinea (PNG). Details of distribution north of Australia as shown broadly reflect available data (e.g. Mees 1982; Dickinson and Remsen 2013) but details especially when and where subspecies may overlap warrant closer study (see Introduction); (b) Results of PCA analysis of SNP data for the first two principal components PC1 and PC 2. Three genetic clusters are identified – Group A (Northern Territory), Group B (Queensland, Trans-Fly and ANWC B57716 from Oro Province) and Group C (Central and Oro Province). Colour coding follows Figure 1(a) — see text for discussion of subspecies assignments. (c) Results of STRUCTURE analysis. Two genetic clusters (K = 2) were determined to be the best fit to the data and highlight the distinctiveness of Group C relative to Groups A and B. Group Labels have been added to Table S1.

No prior population genetics study has been done on this species. Our broad aim was to cast patterns of genomic data in Australian and mainland PNG populations against known patterns of the geographical variation in plumage and debates about subspecific taxonomy. Our specific aim was to describe genetic diversity in resident blue-backed populations of mainland New Guinea and so assess the merit of recognising them as T. m. elisabeth or assigning them to T. m. macleayii.

Materials and methods

Genetic data

We subsampled 34 tissue samples (liver, heart, or muscle) that had been stored at −80°C and held at the Australian National Wildlife Collection (ANWC) into 70% ethanol prior to DNA extraction (Supplementary Material, Table S1). They were from Australia: Northern Territory, including Melville Island (n = 10) and

noting that they followed Mees (1982) who in turn had noted that he synonymised the two reluctantly; his decision was based on examination of specimens at the American Museum of Natural History reported to him by M. LeCroy who could find no differences. Clearly, the question of whether T. m. elisabeth warrants recognition needs reassessment.

In eastern Australia, T. m. incinctus south of ~20°S is at least a partial migrant (Schodde 1997; Higgins 1999). Given its distinctive greenish dorsal plumage (Figure 2), reliable literature and specimen records of its non-breeding season migration span a region from Yamdena (formerly Tanimbar Islands) in the west (Mees 1982) through mainland Papua New Guinea (PNG) to the Bismarck Archipelago in the east (Mayr 1937; Tubb 1945; Schodde and Hitchcock 1968; Coates 1985; Schodde 1997). In eastern lowland PNG, co-occurrence from roughly April-October of green-backed, non-breeding T. m. incinctus and resident blue-backed New Guinean birds e.g. around Port Moresby, is well documented (Tubb 1945; Coates 1985; Figure 2).
Queensland (12 between the tip of Cape York Peninsula and Rockhampton, and PNG: (Trans-Fly (4), Oro Province (4) and Central Province (4). Specimens collected before 1980 lacked frozen tissue samples and so were included in morphological but not genetic analyses.

Our protocols for the extraction of DNA, library preparation and generation of single nucleotide polymorphism (SNP) data essentially followed the Diversity Arrays Technology (DArT) pipeline as described elsewhere (Joseph et al. 2019a, 2019b, 2021). Details are in Supplementary Material but some details follow. Extractions were performed via a salting out method (Miller et al. 1988). Quantification of DNA was carried out initially via gel electrophoresis to confirm the presence of DNA, then using DropSense 96 and DropQuant* (Perkin-Elmer, Melbourne) to determine DNA concentration and Qubit Fluorometric Quantitation for some samples (e.g. failures to read or excessively high). Extractions returning DNA concentrations below thresholds for DArT were reattempted and/or concentrated under vacuum centrifuge. DNA extractions were then sent to DArT for library preparation.

SNPs were called using the standard DArT pipeline and SNP genotyping following Georges et al. (2018) and Wells and Dale (2018) and using a Woodland Kingfisher Halcyon senegalensis reference genome (Genbank Assembly accession number ASM1339759v1). SNPs were filtered by repeatability across replicates (>98%), call rate (<5% missing data), and singletons were removed using the call rate function in dartR (Gruber et al. 2018). A Principal Components Analysis (PCA) was performed to visualise population structure, based on the genetic distance between samples using Euclidean distance to identify population clusters. We ran STRUCTURE (Pritchard et al. 2000) using the same filtering protocol (10,079 SNPs; 34 individuals) 10 times for each value of K between 1 and 5. The model used assumed admixture and correlated allele frequencies. We used clump (Jakobsson and Rosenberg 2007) to summarise the repeated runs for each K and deltaK to select the best value of K (Evanno et al. 2005). The StamPP package in R (Pembleton et al. 2013) was used to generate F_{ST} statistics to compare genetic differentiation between subspecies pairs. Phylogenetic analyses were performed using RAxML, SVD Quartets, and SplitsTree (references in Supplementary Material).

Plumage colouration data

Given that earlier studies have described plumage variation well, at least in the visible spectrum (e.g. Mayr 1937; Keast 1957; Mees 1982; Schodde 1997; Higgins 1999),
we restricted our examination to qualitative visual assessment of colour of the dorsal plumage of all specimens (n = 123) held at the Australian National Wildlife Collection, CSIRO, Canberra. We did not attempt to quantify variation in the size of the white wing-spot because most specimens were available as dried specimens without one accompanying spread wing. For detailed morphometric assessments see Keast (1957) and Higgins (1999).

**Results**

**Genetic variation**

After filtering from 59,992 binary SNPs (40.16% missing data), the number of SNPs reduced to 8,500 (1.85% missing data) (N = 34). The first and second principal components explained 16.7% and 5.4% of the total variation, respectively (Figure 1(b)). Three distinct genetic clusters were evident in PCA and all phylogenetic analyses (Figures S1–S3): Group A comprised all individuals from the Northern Territory, Group B comprised all individuals from Queensland and PNG Trans-Fly as well as one of four samples from Oro Province (sample ANWC B57716), and Group C comprised all other samples from Oro and Central Provinces in PNG. Groups A and B are essentially only separated on the second principal component and show no further substructure. Substructure is evident within Group C between Oro and Central Province specimens along both the first and second principal components (Figure 1(b)). Similarly, STRUCTURE found two genetic clusters (highest deltaK was for K = 2) Again, these corresponded to samples from Australia and Trans-Fly (Groups A and B) on one hand and Oro and Central Province (Group C in PCA) on the other. Again, ANWC B57716, a *T. m. incinctus* specimen from Oro Province, clearly associates with samples from Australia and Trans-Fly (Groups A and B) rather than the other samples from Oro and Central Province in eastern PNG.

The $F_{ST}$ measures of genetic differentiation between Group C and either Group A ($F_{ST} = 0.274$) or Group B ($F_{ST} = 0.240$) were approximately five times higher than that between Group A and B themselves ($F_{ST} = 0.05$). No genetic structure was apparent between geographic regions within Group B ($F_{ST} \leq 0.01$: Queensland, Trans-Fly, and ANWC B 57716 from Oro). Notably, differentiation between Central and Oro samples of Group C ($F_{ST} = 0.113$) was substantially higher than between Groups A and B ($F_{ST} = 0.05$).

Phylogenetic analyses (see Supplementary Material; Figures S1–S3) essentially confirmed the PCA and $F_{ST}$ results for pattern and clustering of the three groups. They also corroborated placement of ANWC B57716 with Group B rather than with other individuals from the Oro Province, which fall in Group C. Relationships among Group B individuals themselves, however, were much less clear and poorly supported (Figures S1 and S3). That is, Group B individuals did not resolve as a single, strongly supported clade despite the apparently high levels of genetic similarity within the group.

**Plumage variation**

The Oro Province specimen ANWC B57716, and all Trans-Fly specimens have green dorsal colouration essentially identical to the Queensland specimens, reinforcing the cohesion of this group (Figure 2(a)). Other specimens from the Central Province savannas (n = 23) were collected between May and September and clearly divided into two groups – greenish-backed Group B birds and blue-backed Group C, some of the latter having been labelled ‘elisabeth’ when collected in the 1960s (Figure 2(b)). We find Group C subtly but readily distinguishable from Group A in having darker, more uniform dorsal blue that is not sexually dimorphic (Figure 2(c)).

**Discussion**

We used genomic data to test the number of taxa that could be recognised within the Forest Kingfisher *T. macleayii* and whether the data could inform migration patterns. We were particularly interested in whether resident blue-backed populations of mainland New Guinea warrant recognition at least as a subspecies *T. m. elisabeth* or should be synonymised with *T. m. macleayii* of Australia’s Top End (see Introduction). Our results robustly and consistently defend support for the resident mainland New Guinean populations as taxonomically distinct from both *T. m. macleayii* (Northern Territory) and *T. m. incinctus* (Queensland and Trans-Fly). They support recognition of these populations at subspecies rank as *T. m. elisabeth*, which would be endemic at least to the lowlands of eastern mainland New Guinea.

Might *T. m. elisabeth* warrant recognition at species rank? It is distinct genetically (Figures 1(b,c) and S1–S3) and phenotypically (Figure 2). The magnitude of its genetic differentiation relative to other subspecies (Figure 1; $F_{ST}$: 0.240, 0.274) is substantial and typical of between-species differences (Roux et al. 2016). Further, habitat differences relative to migrant *T. m. incinctus* are notable. *T. m. elisabeth* inhabits savanna woodland (Bell 1981) whereas non-breeding
migrant *T. m. incinctus* in PNG is ‘essentially a bird of rainforest margins and secondary growth’ (Schodde and Hitchcock 1968). They only overlap in New Guinea when both are not breeding (Tubb 1945; Bell 1981; Coates 1985). We conclude that phylogenetic analysis of all *Todiramphus* and more extensive geographic sampling of *T. macleayii* are needed before changing the taxonomic status of *T. m. elisabeth*. For example, we were unable to sample island populations from Yamdena, Aru, and the Bismarck Archipelago (Figure 1(a)) for which some species-group epithets are available e.g. *insularis* for Aru; *toriu* for Bismarck Archipelago. A broader phylogenetic analysis of *Todiramphus* might also probe reasons for the notable lack of support in our phylogenetic analyses for aligning all Group B individuals together (Figure S3). Possible explanations include gene flow, noisy data or an issue of outgroup selection.

A second key result affirmed the utility of DNA sequences in tracking migrant individuals. We inferred from our data that one specimen (ANWC B57716) collected in Oro Province along the north coast of PNG is likely a migrant *T. m. incinctus* (Figure 1(b,c)). This is consistent with what had already been deduced about this form’s movements from specimens and field observations (Mayr 1937; Tubb 1945; Schodde and Hitchcock 1968; Coates 1985; Schodde 1997). Perhaps notably, ANWC B57716 was a juvenile specimen that grouped with a Trans-Fly specimen (Figure S3).

Other issues concerning movements relate to the eastern and western extremes of the species’ geographical range. At the eastern extreme in the Bismarck Archipelago, the species is usually considered migratory (e.g. Mayr 1937; Mees 1982; Gregory 2017). Two records there (Hoskins, West New Britain Island, May 1984; Galai, West New Britain Island, January 1986) and photographs of an apparently blue-backed bird (Rabaul, East New Britain September 2019), however, are consistent with a resident population or vagrant or migrant *T. m. elisabeth* (https://ebird.org checklists S10394116, S16635584 and S60347107, respectively). To the west, recent Indonesian records and one Australian record from the Timor Sea (Figure 1a) are from between April and October (https://ebird.org, checklists S18998436 and S49132187); these are presumably of non-breeding migrants from Australia but their subspecific identity is unrecorded. There may be resident populations in Indonesia (Coates and Bishop 1997). Although one bird photographed in the Ashmore Reef area in April 1990 appears most likely to be a green-backed *T. m. incinctus*, it could alternatively be a female *T. m. macleayii* (https://ebird.org/checklist/S18998436).

We have provided a first insight into how genetic diversity is structured within the Forest Kingfisher across most of its range. We have highlighted the need for its populations to be included in phylogenetic analyses of all *Todiramphus* kingfishers to better understand how many species should be recognised. Also warranting study are island populations still unsampled for genetic diversity. We have shown how useful DNA sequence data can be in assessing seasonal overlap of migratory and non-migratory populations and the occurrence of the species on islands in the Indo-West Pacific would benefit from this. Similarly, modern analyses of plumage colour in visible and ultraviolet wavelengths and based on all museum specimens worldwide would be of interest.

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No potential conflict of interest was reported by the author(s).

**ORCID**

Jessica Fenker [http://orcid.org/0000-0002-7430-3886](http://orcid.org/0000-0002-7430-3886)

Anna Kearns [http://orcid.org/0000-0002-8502-7442](http://orcid.org/0000-0002-8502-7442)

Craig Moritz [http://orcid.org/0000-0001-5313-7279](http://orcid.org/0000-0001-5313-7279)

Leo Joseph [http://orcid.org/0000-0001-7564-1978](http://orcid.org/0000-0001-7564-1978)

**Data availability of statement**

No proprietary data are associated with this paper. Data are available at figshare (doi:10.6084/m9.figshare.20407503).

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