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Inferring population connectivity across the range of the purple-crowned fairy-wren (*Malurus coronatus*) from mitochondrial DNA and morphology: implications for conservation management

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Abstract. Knowledge of population structure and patterns of connectivity is required to implement effective conservation measures for the purple-crowned fairy-wren (*Malurus coronatus*), a threatened endemic of northern Australia. This study aimed to identify barriers to dispersal across the distribution of *M. coronatus*, investigate the impact that the recent declines may have on population connectivity, and propose conservation actions to maintain natural patterns of gene flow. Analysis of mitochondrial DNA sequences from 87 *M. coronatus* identified two phylogenetic clusters that corresponded with the phenotypically defined western (*M. c. coronatus*) and eastern (*M. c. macgillivrayi*) subspecies. The genetic divergence between these subspecies was consistent with isolation by a natural barrier to gene flow, and supports their separate conservation management. Within the declining *M. c. coronatus*, the lack of genetic divergence and only slight morphological difference between remnant populations indicates that populations were recently linked by gene flow. It is likely that widespread habitat degradation and the recent extirpation of *M. c. coronatus* from the Ord River will disrupt connectivity between, and dynamics within, remnant populations. To prevent further declines, conservation of *M. coronatus* must preserve areas of quality habitat and restore connectivity between isolated populations.

Additional keywords: conservation biology, northern Australia, phylogeography, wildlife management.

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Introduction

Conservation managers require knowledge of population structure to implement effective conservation measures for threatened species (Thrall *et al*. 2000; Segelbacher *et al*. 2010). This information can be used to identify populations that warrant separate management (Paetkau 1999; Palsboll *et al*. 2007), prioritise conservation actions (McDonald-Madden *et al*. 2008), and enhance restoration, translocation, and captive-breeding programs (Montgomery *et al*. 1997; Moritz 1999). Furthermore, maintaining population structure within threatened species may allow preservation of their evolutionary potential (Crandall *et al*. 2000; Moritz 2002).

Knowledge of population genetic structure is required to aid conservation management of the purple-crowned fairy-wren (*Malurus coronatus*). The purple-crowned fairy-wren is a riparian habitat specialist that occurs in patches of dense river-fringing vegetation in northern Australia (Fig. 1). The species has declined across parts of its range and is threatened by the degradation and fragmentation of riparian vegetation caused by the grazing of introduced herbivores, weed incursion and repeated intense fires (Smith and Johnstone 1977; Rowley and Russell 1993; Garnett *et al*. 2011; Skroblin and Legge 2011). Conservation initiatives have been suggested to halt population declines and restore connectivity (Rowley 1993; van Doorn 2007; Skroblin and Legge 2010, 2011). However, an understanding of predegradation population structure is required to ensure that management actions, if possible, enhance natural patterns of gene flow and maintain any adaptive divergence between populations (Moritz 1994; Crandall *et al*. 2000).

Two subspecies of the purple-crowned fairy-wren are recognised (Schodde 1982; Higgins *et al*. 2001) and receive separate conservation management listings (Garnett *et al*. 2011). The eastern form, *M. c. macgillivrayi*, occurring around the Gulf of Carpentaria, is considered to be Near Threatened (Garnett *et al*.

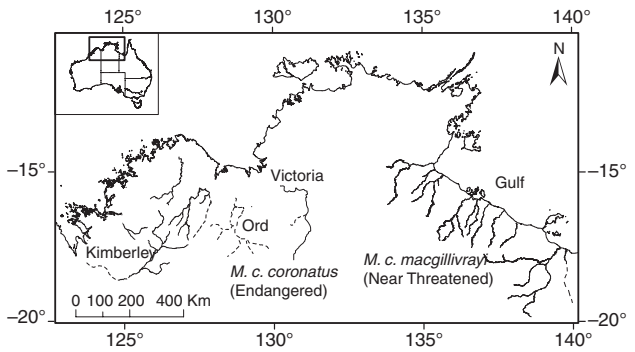

Fig. 1. The occurrence and status of *Malurus coronatus* on rivers in northern Australia. The western subspecies, *M. c. coronatus*, occurs in the Kimberley, Ord and Victoria River districts, while the eastern subspecies, *M. c. macgillivrayi*, occurs on rivers that drain into the Gulf of Carpentaria. Rivers that are drawn with dashed lines are those where the species once occurred but is now presumed to be extinct following Rowley and Russell (1993) and Skroblin and Legge (2010).

2011), although population dynamics are unknown. By contrast, the western form, *M. c. coronatus*, occurring in the Kimberley and Victoria River area, is listed as Vulnerable nationally (*Environment Protection and Biodiversity Conservation Act 1999* (*Aust.*)) (DEWHA 2009), but has recently been described as Endangered in the 'Action Plan for Australian Birds 2010' (Garnett *et al*. 2011) on the basis of a series of studies revealing declines in its distribution (Rowley 1993; van Doorn 2007; Skroblin and Legge 2010). The two subspecies are separated by a natural break of ~300 km of unsuitable habitat (Rowley 1993), and have been split on the basis of differences in plumage colouration and body size of museum skins (Schodde 1982; Higgins *et al*. 2001). Although subspecies that are defined by phenotypic differences are often used as units for conservation management, their use could misdirect conservation effort if they represent geographic divisions of character clines that are weakly associated with underlying genetic diversity (Avise *et al*. 1987; Zink 2004; Remsen 2005; Rising *et al*. 2009). The conservation of the purple-crowned fairy-wren may therefore benefit from genetic analysis to assess the appropriateness of the phenotypically delineated subspecies, and therefore their separate conservation rankings, as well as identify any further population divisions that were not evident in previous analyses of morphology (Zink 2004; Phillimore and Owens 2006).

Without an understanding of historical connectivity across the range of *M. coronatus*, it is difficult to gauge the effect that anthropogenic degradation of riparian habitat (occurring since the early 20th century) has had on population dynamics, and hence determine appropriate management strategies. Observations of limited flight capabilities and a strong adherence to dense river-fringing vegetation (Rowley and Russell 1993) suggest that the species has a limited potential for dispersal between widely spaced waterways or patches of isolated habitat (Rowley 1993). Of particular interest is the impact that the recent disappearance of purple-crowned fairy-wrens from the Ord River system, previously the centre of the range of *M. c. coronatus* (Skroblin and Legge 2010), will have on extant populations in the western Kimberley and Victoria River (Fig. 1). The impact of this decline may be limited if movement between the western Kimberley and Victoria River was historically restricted by biogeographical barriers that have been found to separate populations of other terrestrial and aquatic species within the vicinity of the Ord River region (Unmack 2001; Bowman *et al*. 2010; Fujita *et al*. 2010; Melville *et al*. 2011; Potter *et al*. 2012). Conservation strategies for extant populations of *M. c. coronatus*, such as translocations, should be informed by the natural connectivity between remnant populations.

Here we investigate the broad-scale population structure of *M. coronatus* to aid in determining management priorities and strategies for effective conservation. We first employ phylogenetic analyses (Bayesian, maximum likelihood and statistical parsimony) to describe genetic structure and identify any phylogeographic clusters of individuals across the species' range. We thereby aim to identify natural barriers to dispersal across the distribution of *M. coronatus*, and gain an understanding of how populations were connected before the habitat degradation that has occurred within the past 150 years (Rowley 1993; National Land and Resources Audit 2002). We specifically test the hypothesis that the phenotypic subspecies of *M. coronatus* represent distinct evolutionary lineages, thereby verifying the appropriateness of delineation between populations in the Kimberley and Victoria River districts (*M. c. coronatus*) versus those in the Gulf (*M. c. macgillivrayi*) for conservation purposes. Finally, we use both genetic and morphological data to investigate the impact that the recent extirpation of purple-crowned fairy-wrens from the Ord River system, in the centre of the distribution of *M. c. coronatus*, may have on population dynamics within the subspecies. The similarity of fairy-wrens (both morphologically and genetically) in the western Kimberley with those on the Victoria River will increase understanding of historical connectivity and may inform whether potential actions to restore connectivity or translocations between these areas would be appropriate. An understanding of population structure within *M. coronatus* will allow managers to implement actions that conserve natural patterns of connectivity between populations and their evolutionary potential.

Materials and methods

Genetic sampling

Blood samples were attained from 87 live purple-crowned fairy-wrens from throughout the three districts where the species occurs: Victoria River ($n = 29$), Kimberley ($n = 19$), and Gulf ($n = 39$) (Table 1; Fig. 2). Birds were captured in mist-nets, and small blood samples (~10–20 μL) were obtained by pricking the brachial vein or the sinus close to the inside crook of the wing with a needle. The resulting spot of blood was drawn off using a hematocrit tube and stored in 70% ethanol. Sampling effort was designed to maximise genetic variation. Samples were collected from purple-crowned fairy-wrens on seven rivers in the Kimberley, from three sites on the Victoria River (each separated by ~100 km of river distance) and on six rivers in the Gulf. Only one individual per territorial group was sampled.

DNA extraction, genotyping and sequencing

DNA was extracted from blood samples using a conventional proteinase K, ammonium acetate and ethanol protocol. The

Table 1. Geographical locations at which genetic samples of *Malurus coronatus* were collected
The first letter in the sample codes represent the district from which the birds were sampled (G, Gulf; K, Kimberley; V, Victoria River). The site ID represents the river from which the birds were sampled and corresponds with Fig. 2

Site name	*N*	Sample code	Site ID	Latitude (°S)	Longitude (°E)
M. coronatus coronatus	*48*				
Kimberley					
Wood River	1	K1	WOD	16.96	126.85
Chapman River	3	K2, K3, K4	CHAP	16.51	126.67
Blackfellow Creek	2	K5, K6	BFC	16.68	126.85
Fitzroy River	3	K7, K8, K19	FIT	17.43	126.39
Throssell River	3	K9, K10, K11	THR	17.45	126.05
Adcock River	1	K12	ADC	17.47	126.02
Durack River	6	K13–K18	DUR	16.25	127.18
Victoria River					
Big Horse Creek	9	V1–V9	BHC	15.62	130.42
Victoria River Crossing	8	V22–V29	VBR	15.62	131.13
Dashwood Crossing	12	V10–V21	DWC	16.33	131.11
M. coronatus macgillivrayi	*39*				
Gulf					
Limmen	2	G1, G2	LIM	15.48	135.39
Towns River	9	G3–G11	TWN	15.04	135.18
Cox River	10	G12–G21	COX	15.32	135.34
Calvert River	12	G22–G33	CAL	16.73	137.41
Robinson River	2	G34, G35	ROB	16.46	137.05
McArthur River	4	G36–G39	MCA	16.78	135.75

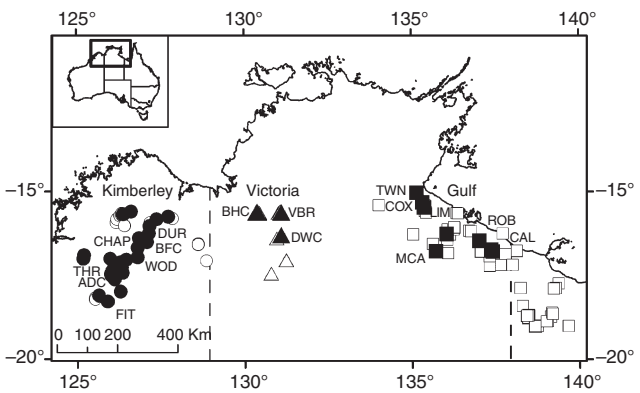

Fig. 2. Sightings of *M. coronatus* with the locations where morphological and genetic samples were collected. Three districts are indicated: Kimberley (○), Victoria River (△), and Gulf (□). Open symbols represent locations where the species has been sighted since 1996 (Skroblin and Legge 2010). The species is considered to occur at these localities except for the Ord River in the Kimberley where it has recently disappeared. Black symbols represent locations where morphology was measured. Genetic samples were collected at sites that are labelled with three-letter codes (Table 1).

approximate concentration of DNA was determined by electrophoresis in a 2% agarose gel stained with 160 ng L^{-1} ethidium bromide and visualised under UV light. The *NADH dehydrogenase subunit 2* (*ND2*) mitochondrial gene was sequenced for use in all genetic analyses. *ND2* was utilised as it is highly appropriate for detecting patterns of recent evolution or isolation of the sort anticipated to occur in the purple-crowned fairy-wren (Rubinoff and Holland 2005; Zink and Barrowclough 2008; Zink 2010). The suitability of mtDNA for investigating recent phylogeographical divergence is due to its smaller effective population size and faster rate of molecular evolution than nuclear DNA (Avise *et al*. 1987; Moritz *et al*. 1987), which leads to both shorter coalescence times and higher diversity (Zink and Barrowclough 2008).

The ND2 region was amplified with the primers L5215 (5′-TATCGGGCCCATACCCCGAAAAT-3′: Hackett 1996) and H6313 (5′-ACTCTTRTTTAAGGCTTTGAAGGC-3′: Sorenson *et al*. 1999). The expected size of the amplified product was ~1000 bp. The PCR amplification was performed in a 20-μL reaction containing 2.0 μL of 10× PCR buffer, 1.2 μL of 50 mM $MgCl_2$, 1.0 μL dNTPs (10 mM), 1.0 μL of each of the forward and reverse primer (10 pmol), 0.5 units of Taq (Qiagen), 12.75 μL of double-distilled H_2O and 1.0 μL of template DNA (50–100 ngs mL^{-1}). Amplification was carried out using 94°C (5 min), 15 cycles of 94°C (30 s), 58°C (30 s), 72°C (1 min), followed by 25 cycles of 94°C (30 s), 62°C (30 s) and 72°C (1 min), and final extension at 72°C (10 min). A negative control was run for every amplification. PCR products were purified by ammonium acetate (4 M) and ethanol (100%) precipitation. Cycle-sequencing reactions contained 0.75 μL of BigDye (Applied Biosystems), 3 μL of 5× buffer 0.32 μL of primer 14.05 μL of double-distilled water and 2 μL of purified PCR product. Cycle-sequencing consisted of 25 cycles at 94°C (5 s), 50°C (10 s) and 60°C (4 min). The product was precipitated using sodium acetate and ethanol. The pelleted DNA was washed three times in 70% ethanol. DNA pellets were dried before addition of 20 μL of HiDi formamide and sequencing on an ABI 3100 autosequencer (Applied Biosystems).

Sequence editing

Sequence data were edited and aligned using Geneious Pro 4.6.4 (Drummond *et al*. 2010). The vertebrate mitochondrial genetic

code was used to translate aligned ND2 sequences into amino acids. To certify that sequences were of mitochondrial origin, we checked for internal stop codons and calculated nucleotide diversity statistics. Following Zink *et al*. (2006), the McDonald–Kreitman test was used in DnaSP ver. 5.10 (Librado and Rozas 2009) to test for neutrality of ND2, and hence check that our phylogenetic inferences were not compromised by natural selection.

Phylogenetic analysis

Phylogenetic analyses were conducted to describe genetic structure and identify any phylogeographic clusters of individuals across the species range. Unrooted genealogical relationships between sequences were estimated using statistical parsimony in TCS 1.21 (Clement *et al*. 2000). Each haplotype was given a code (Fig. 3) and included once in subsequent phylogenetic analyses. For outgroup taxa in phylogenetic analyses, we included all 11 *Malurus* species for which ND2 sequences were available in GenBank: *M. lamberti* (AY488326), *M. cyaneus* (EU534191), *M. splendens* (EU144301), *M. alboscapulatus* (JN598704), *M. amabilis* (JN614694), *M. elegans* (GU825876), *M. grayi* (JN598688), *M. leucopterus* (GU825875), *M. melanocephalus* (GU825874), *M. cyanocephalus* (JN598690), and *M. pulcherrimus* (JQ027484). The dataset was aligned using Geneious Pro 4.6.4 (Drummond *et al*. 2010).

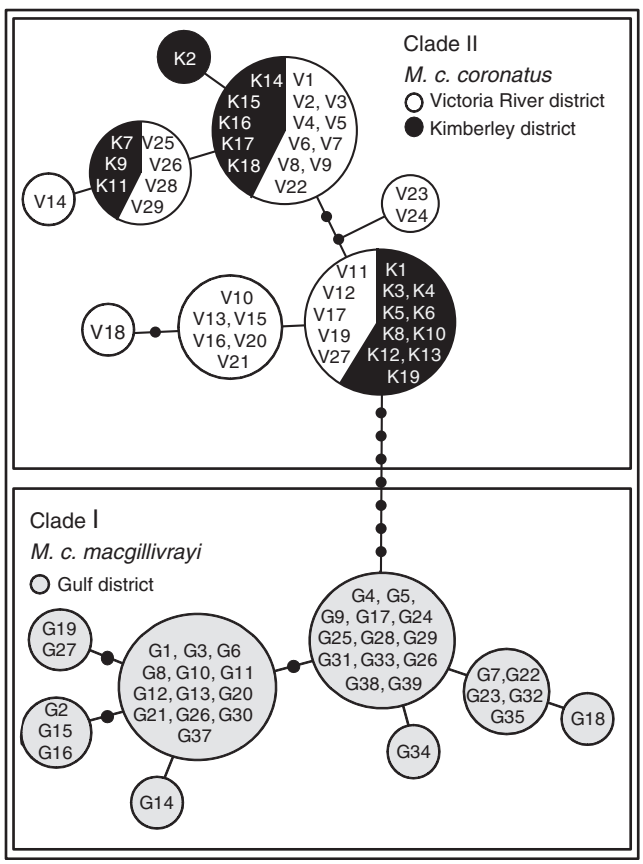

Fig. 3. Unrooted haplotype network of *Malurus coronatus* ND2 sequences. The first letter in the sample codes represents the district from which the birds were sampled (G = Gulf; K = Kimberley; V = Victoria River) (see Table 1). Each haplotype is given a unique code: W1–W8 are in the western subspecies (*M. c. coronatus*) and E1–E8 are in the eastern subspecies (*M. c. macgillivrayi*).

PartitionFinder (Lanfear *et al*. 2012) was used to choose the most appropriate partitioning scheme and the most appropriate model of DNA sequence evolution for each partition. To do this, we defined each codon position in ND2 as an initial data block, and performed an exhaustive search of all possible partitioning schemes ('search = all'), using the models of molecular evolution implemented in MrBayes 3.1.2 ('models = MrBayes'), and using the Bayesian Information Criterion ('model_selection = BIC') to choose partitioning schemes and models of molecular evolution. This method compares all possible combinations of codon positions to find the partitioning scheme and models of molecular evolution that are most appropriate for the data. The optimal partitioning scheme was to treat each codon position separately, using an HKY+G model for the first codon position, an HKY+I model for the second codon position, and a GTR model for the third codon position.

Bayesian phylogenetic analyses were performed using MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001), with the partitioning scheme and models of molecular evolution selected in PartitionFinder. The Bayesian Markov Chain Monte Carlo (MCMC) search was started with random trees and run for 100 million generations using one cold and three heated chains across two independent runs. Samples were taken from the MCMC every 10 000 generations and Tracer ver. 1.5 (Rambaut and Drummond 2007) was used to check for stationarity of results. As calculation of posterior probabilities can be influenced by the starting point of the MCMC (Ronquist *et al*. 2009) the first 1 000 000 generations of the analysis were discarded as burn-in. We used Tracer to check that the effective sample sizes of all parameters was sufficient (i.e. >200).

In addition to the Bayesian analyses, we conducted maximum-likelihood (ML) phylogenetic analyses using RaxML ver. 7.2 (Stamatakis 2006). ML analyses were conducted using the partitioning scheme selected in PartitionFinder, with a separate GTR+G model of molecular evolution applied to each partition. The ML tree was calculated using 100 search replicates, with 1000 bootstrap replicates performed on resampled datasets.

Population genetic analysis

We used population genetic analyses to investigate the likely impact that the recent extirpation of purple-crowned fairy-wrens on the Ord River system (Skroblin and Legge 2010) may have on connectivity between remnant populations. For this analysis we partition the distribution of *M. coronatus* into three districts: the western Kimberley and Victoria River populations of *M. c. coronatus*, and the Gulf populations of *M. c. macgillivrayi.* We included these three districts in our analyses as the Victoria River population is now similarly isolated from the Gulf populations of *M. c. macgillivrayi* as it is from *M. c. coronatus* in the western Kimberley (Fig. 1). We used sequences from all 87 individuals in an analysis of molecular variance (AMOVA: Excoffier *et al*. 1992) to estimate partitioning of variance between populations in the three districts. AMOVA was implemented with 999 permutations for significance testing in GenAlEx 6.4. (Peakall and Smouse 2006). Pairwise ΦPT values (fraction of the total

variance that is among districts) were calculated to estimate divergence between the three districts.

Morphological sampling

Morphological measures were taken from live purple-crowned fairy-wrens at a total of 41 sites across the species range (Fig. 2). In the Kimberley region 159 males and 129 females were sampled from 30 sites across five catchments. In the Victoria River district 34 males and 18 females were sampled from three sites, whereas in the Gulf 51 males and 41 females were measured at eight sites across six rivers. Sex of birds was determined by plumage. Vernier callipers were used to measure tarsus length, head–bill length (from the back of the skull to the tip of the beak) and bill length, to the nearest 0.1 mm. Weight was measured to the nearest 0.1 g using a spring-loaded Pesola balance, and a butted ruler was used to measure tail length and wing length (maximum chord). To avoid pseudoreplication, morphological traits were averaged across individuals of the same sex within a territory. The final dataset consisted of average body measurements from 188 male and 163 female territories. We tested for normality, homogeneity of variances and multicollinearity (correlation between independent traits) before commencement of analysis using GENSTAT 11.1 (VSN International).

Analysis of morphology

To test for an effect of district on overall morphology for each sex, multivariate analysis of variance (MANOVA) was performed, using a Wilks' Lambda test to investigate significance of among-district differences. As morphology may vary in response to latitudinal changes (Meiri and Dayan 2003), a confounding effect of latitude was tested for each separate morphological trait for each sex using analysis of covariance (ANCOVA) before morphology was compared between districts. Latitudinal effects were tested for in two ways. First, as the three districts from which birds were sampled (Kimberley, Victoria, Gulf) differed significantly in latitude (ANOVA: $F_{2,185} = 58$, $P < 0.001$), we examined the effect of latitude on the common-slope of each morphological trait across the districts. Second, as birds were sampled from territories occurring over a large latitudinal range (–15.61 to –18.29 Decimal Degrees), we tested for differential effects of latitude on each morphological trait within a district. In both cases a covariate of latitude was used in ANCOVA. To assess whether trait morphology varied across the three districts we employed three methods dependant on whether an effect of latitude on the trait was evident: (1) ANOVA was used when no effect of latitude was evident, (2) Linear Models, with latitude as a covariate, were used if an effect of latitude was evident on the common-slope of the trait, and (3) ANCOVA, with latitude as the covariate, was employed if the effect of latitude varied between districts. All analyses were grouped by district (as the independent level of replication) and site nested within district.

Results

Partial ND2 sequences (960 bp) were obtained for 87 individuals. Base frequencies were representative of avian mitochondrial DNA (A = 0.29, T = 0.25, G = 0.10, C = 0.34) (Joseph and Wilke 2006; Kearns *et al*. 2009). Neither multiple peaks nor mismatch in overlapping sequences were found. Translation into amino acids did not reveal any internal stop codons and tests for neutrality were not significant. This suggests that the sequences were mitochondrial in origin rather than nuclear copies (numts) of ND2 sequences (Zhang and Hewitt 1996; Sorenson and Quinn 1998).

Haplotype diversity

The two subspecies of *M. coronatus* were each found to contain a unique set of ND2 haplotypes that were separated by eight mutational steps (Fig. 3). A total of 16 haplotypes were contained within the 87 individuals sampled, and haplotype diversity was similar for the eastern and western populations. The eastern polytomy ($n = 38$; 8 haplotypes) contained shallow genetic structure (Fig. 3). Two internal haplotypes (separated by two mutational steps) were common and each shared by 13 individuals. The three haplotypes radiating from each internal haplotype were no more than two mutational steps divergent.

The western clade ($n = 48$, 8 haplotypes) was similarly structured, with two internal haplotypes that were both shared by 15 individuals and separated by three mutational steps (Fig. 3). Three of the eight haplotypes in the western clade were shared between individuals from the western Kimberley and Victoria River. The haplotype most closely joined to the eastern polytomy was identified in 10 individuals from the Kimberley and five individuals from Victoria River. The second large internal haplotype was conversely shared by 10 individuals from Victoria River and five from the Kimberley. Of the remaining six haplotypes in the clade, one was shared (3 Kimberley, 4 Victoria River), one was unique to the Kimberley, and four were unique to the Victoria River.

Phylogenetic analyses

ML, Bayesian analysis, and statistical parsimony all identified two groups that are consistent with an east/west subspecies division within *M. coronatus* (Figs 3, 4). There was strong support for the hypothesis that the eastern and western subspecies formed separate clades on the tree, as all Bayesian trees in the posterior sample contained the western clade as monophyletic (Baysian Posterior Probability of 1.0) with ML bootstrap support of 0.90 (Fig. 4). Individuals from the western clade were separated by eight fixed genetic differences from the eastern sequences (Fig. 3). There was little support for monophyly of the eastern clade due to variability in the rooting position of the outgroups. The root was positioned within the eastern clade in at least 50% of the trees and in the remainder of the trees it occurred between the two clades (Fig. 4). There was no evidence of further phylogenetic divisions other than between *M. c. coronatus* and *M. c. macgillivrayi*.

Population genetics

The results of AMOVA indicated that most haplotype variation occurs between the three districts rather than between individuals within the same district (Table 2). Divergence was detected between the Gulf (*M. c. macgillivrayi*) and each of the two districts containing *M. c. coronatus* (Kimberley and Victoria River). However there was no variance detected between populations of *M. c. coronatus* in Kimberley and Victoria River districts (Table 3), indicating that they belong to an interconnected population.

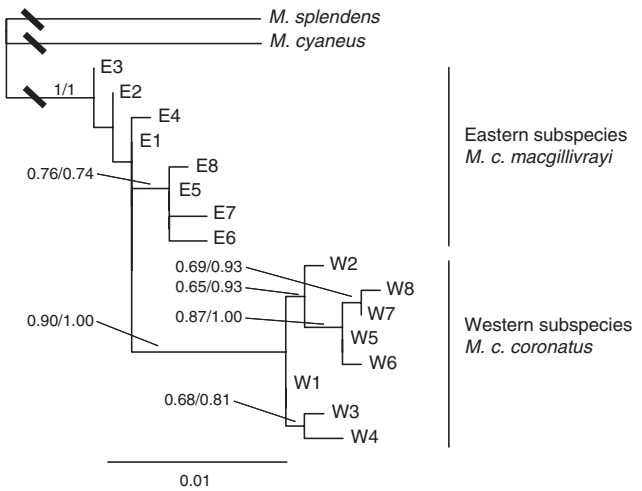

Fig. 4. The maximum-likelihood (ML) tree of *Malurus coronatus* ND2 haplotypes. For each node with bootstrap support >50% the ML bootstrap value is shown followed by Bayesian Posterior Probabilities. The sample codes represent the individual haplotypes (Fig. 2). Haplotypes beginning with an (E) are from the eastern subspecies (*M. c. macgillivrayi*), and those beginning with a (W) are from the western subspecies (*M. c. coronatus*). Phylogenetic analyses included all *Malurus* as outgroups (see Methods); however, to improve clarity of the figure, only two outgroups are presented and branches with a diagonal bar are truncated.

Morphology

Adult *M. coronatus* were sexually dimorphic (Fig. 5). Males were heavier than females (ANOVA: $F_{1,343} = 65.05$, $P < 0.001$) and possessed longer bills (ANOVA: $F_{1,350} = 31.28$, $P < 0.001$), larger head–bill length (ANOVA: $F_{1,350} = 171.34$, $P < 0.001$), larger wings (ANOVA: $F_{1,350} = 247.22$, $P < 0.001$), greater tarsus length (ANOVA: $F_{1,350} = 152.73$, $P < 0.001$) and longer tails (ANOVA: $F_{1,347} = 45.38$, $P < 0.001$).

The morphology of male (MANOVA: $F_{12,268} = 6.41$, $P < 0.001$) and female (MANOVA: $F_{12,242} = 5.07$, $P < 0.001$) purple-crowned fairy-wrens varied between the Kimberley, Victoria River and Gulf districts (Fig. 5). Multicollinearity was not a problem for MANOVA as all morphological traits were correlated less than $r = 0.6$. A significant effect of latitude was identified on the common-slope of male head–bill length ($F_{1,186} = 6.52$, $P = 0.011$), female tarsus ($F_{1,161} = 6.68$, $P = 0.011$) and female wing length ($F_{1,161} = 6.62$, $P = 0.011$), while the effect of latitude on male wing length differed within the three districts ($F_{1,184} = 4.25$, $P = 0.041$). Confounding effects of latitude were controlled for in the results presented below.

Although eastern purple-crowned fairy-wrens weighed less (ANOVA, females: $F_{2,127} = 26.93$, $P < 0.001$; males: $F_{2,143} = 6.37$, $P = 0.002$) and tended to be smaller than the western subspecies (Fig. 5), we discovered incremental changes in body proportions between the three districts rather than a consistent scaling in body size between the subspecies (Fig. 5). Males of the eastern subspecies had smaller bills (ANOVA: $F_{2,149} = 9.02$, $P < 0.001$), shorter wings (ANCOVA: $F_{2,149} = 18.92$, $P < 0.001$) and longer tails (ANOVA: $F_{2,146} = 12.83$, $P < 0.001$) than their western counterparts. However, male head–bill size differed between the three districts (GLM: $F_{2,148} = 6.02$, $P = 0.003$): males in the Gulf were comparably smaller than those in the Victoria River district ($t_{148} = 2.16$, $P = 0.032$), yet not significantly different from those in the Kimberley ($t_{148} = 0.66$, $P = 0.512$). There was no overall difference in male tarsus length among districts (ANOVA: $F_{2,149} = 2.73$, $P = 0.069$). Nevertheless, males in the Gulf had longer tarsi than those in the Kimberley (Contrast: $F_{1,187} = 5.08$, $P = 0.026$), even though there was no discernible difference between males in the Gulf and Victoria River (Contrast: $F_{1,187} = 3.07$, $P = 0.082$). Females of the eastern subspecies had shorter wings (GLM: $F_{2,127} = 16.49$, $P < 0.001$) and longer tails (ANOVA: $F_{2,128} = 9.49$, $P < 0.001$) than those of the western subspecies. Bill length decreased incrementally from west to east (ANOVA: $F_{2,128} = 3.48$, $P = 0.034$), with females in the Kimberley having larger bills than those in the Victoria River (Contrast: $F_{1,128} = 5.67$, $P = 0.019$). There was no discernible difference in female head–bill size (ANOVA: $F_{2,128} = 0.77$, $P = 0.465$) or tarsus length (GLM: $F_{2,127} = 2.62$, $P = 0.077$) between the districts.

Discussion

This study investigated the broad-scale population structure of *M. coronatus* to aid in determining management priorities and strategies for effective conservation. We identified two phylogenetic clusters across the range of *M. coronatus* (Figs 3, 4), which corresponded with the two recognised subspecies (Schodde 1982). The genetic divergence between these subspecies is consistent with isolation by a natural barrier to gene-flow, and supports their separate management (DEWHA 2009; Garnett *et al*. 2011). The lack of genetic structure for ND2 within each of the two subspecies suggests that females were historically dispersing between the waterways in both the eastern and western section of the species range. The extirpation of purple-crowned fairy-wrens from the Ord River system (Skroblin and Legge 2010), is likely to disrupt dynamics in the remnant Victoria River and western Kimberley populations of *M. c. coronatus*, which although slightly morphological divergent (Fig. 5), were highly connected by gene-flow over recent evolutionary time (Table 3).

Genetic divisions within M. coronatus

Our phylogenetic analyses revealed two clusters of ND2 sequences within *M. coronatus* that correspond with the

Table 2. Partitioning of genetic variation between and within the Kimberley, Victoria River and Gulf districts using AMOVA

Source of variation	d.f.	s.s	m.s.	Variance	% Var.	ϕ Statistic	Probability
Between districts	2	202.42	101.21	3.59	77%	0.772	0.001
Within districts	84	89.31	1.06	1.06	23%		
Total	86	291.72		4.66	100%		

Table 3. Pair-wise ϕPT differences between the Kimberley, Victoria River and Gulf districts using AMOVA

ϕPT is the fraction of the total variance that is among populations; it is shown below the diagonal. Probability values are calculated using 999 random permutations of ϕPT; they are shown above the diagonal (**, significant ϕPT differences, $P < 0.001$)

	Kimberley	Victoria River	Gulf
Kimberley	–	0.321	<0.001**
Victoria River	0.000	–	<0.001**
Gulf	0.827	0.813	–

phenotypically defined western *M. c. coronatus* and eastern *M. c. macgillivrayi* (Figs 3, 4). The western subspecies forms a separate branch within the eastern subspecies, suggesting that *M. coronatus* may have originally occurred within the eastern portion of its range before colonising the west. The lack of mitochondrial haplotype sharing between the two subspecies indicates that there has been little or no successful migration of females between the two districts. Populations of *M. c. coronatus* on the Victoria River are separated by ~300 km from populations of *M. c. macgillivrayi* in the Gulf (Fig. 1). This barrier comprises semiarid uplands that lack permanent water (Ford 1978), and thus the dense river-fringing vegetation that *M. coronatus* requires (Rowley 1993; van Doorn 2007; Skroblin and Legge 2011). The northern portion of this barrier contains the headwaters of the Daly River catchment. Although *M. coronatus* has never been recorded within the Daly River catchment (Rowley 1993; Barrett *et al*. 2003), these waterways provide a likely route for previous connectivity between the subspecies. The Daly River drainage has been identified as a biogeographic barrier for other species in the monsoonal tropics (summarised in Eldridge *et al*. 2012). There was no evidence, however, that the Ord arid intrusion,

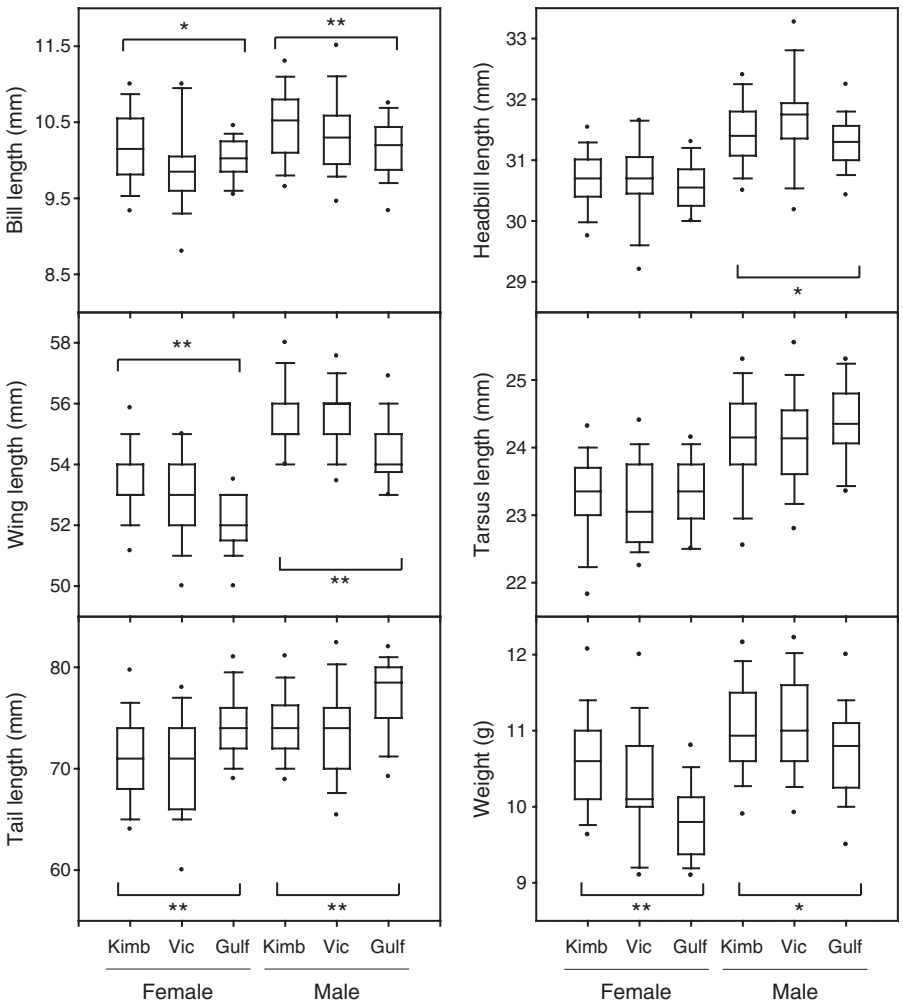

Fig. 5. Morphological variation in purple-crowned fairy-wrens sampled from the Kimberley (Kimb), Victoria River (Vic) and Gulf districts. Boxes denote mean and standard deviations, bars represent 95% confidence intervals and dots denote outliers. Asterisks indicate significant between-district variation of morphological trait for given sex: *, $P < 0.05$; **, $P < 0.001$ from ANOVA across the three districts. Significant between-district contrasts are given in the text.

another major barrier in northern Australia (Bowman *et al*. 2010), influenced gene-flow between the western Kimberley and Victoria River populations (Figs 3, 4).

The confirmation of a natural barrier to dispersal between the eastern and western populations of *M. coronatus* verifies the delineation of conservation management between the two subspecies (Rowley 1993; Garnett *et al*. 2011). As this study utilised a neutral marker, the signal of genetic divergence between the subspecies does not reveal evolutionarily relevant differences among populations (Halliburton 2004; Zink 2005). Likewise, the morphological change from east to west and decoupling of morphological body proportions (Fig. 5), although possibly an adaptive response to local habitat structure or environmental conditions, may be a consequence of genetic drift. As a precaution, however, we advocate that prospective management actions, such as translocations and captive-breeding programs, should maintain division between the eastern and western subspecies to preserve the integrity of potential adaptive divergence (Crandall *et al*. 2000; Moritz 2002).

Connectivity within subspecies

Although dispersal is prevented between *M. c. coronatus* and *M. c. macgillivrayi*, the lack of genetic structure for ND2 within each of these subspecies (Figs 3, 4), suggests that females of both subspecies have been undertaking long-distance dispersal between the waterways that were sampled. The connectivity within *M. c. coronatus* was such that Victoria River and western Kimberley, which are now isolated by more than 200 km (Fig. 1), show no divergence in the frequency of haplotypes (Table 3). This finding is unexpected, as it is incongruent with behavioural observations which suggest that this small passerine has limited dispersal capabilities (Rowley and Russell 1993) and the subsequent prediction that separate catchments contain isolated populations (Rowley 1993). A discrepancy between behavioural and genetic inferences of dispersal is not uncommon (e.g. Fedy *et al*. 2008; Howeth *et al*. 2008), and occasional dispersal between catchments would be sufficient to prevent genetic divergence of neutral markers (Mills and Allendorf 1996). The slight morphological variation between fairy-wrens in the Kimberley and Victoria River districts (Fig. 5) suggests that, rather than belonging to a panmictic population, these districts contain separate subpopulations of *M. c. coronatus* that are linked by gene flow. Fine-scale patterns of gene flow within the subspecies could be better investigated using more variable genetic markers, for instance microsatellites (Selkoe and Toonen 2006; Diniz-Filho *et al*. 2008).

Impact of declines on connectivity

It is likely that natural patterns of dispersal within *M. c. coronatus* and *M. c. macgillivrayi* have been disrupted by the widespread degradation of riparian habitat that has occurred since pastoralism began in northern Australia around the turn of the 20th century (Rowley 1993; National Land and Resources Audit 2002). Degradation and fragmentation of habitat may isolate populations and place them at heightened risk of extinction from interacting genetic, demographic and environmental effects (Pimm *et al*. 1988; Holsinger 2000; Ray 2001; Spielman *et al*. 2004), and also prevent recolonisation following extinction events (Fahrig and Merriam 1994). As female purple-crowned fairy-wrens undertake long-distance and between-river dispersal, decreasing connectivity between patches of habitat and associated increases in hostile matrix may further worsen the mortality of dispersing individuals (Brooker and Brooker 2001).

Of particular concern is the impact that contraction of *M. c. coronatus* (Smith and Johnstone 1977; Rowley 1993; Skroblin and Legge 2010) will have on connectivity between remnant populations of this declining subspecies. The extirpation of purple-crowned fairy-wrens from the Ord River system (Skroblin and Legge 2010) is likely to severely disrupt population dynamics within *M. c. coronatus*. Prior to anthropogenic degradation, populations across the range of *M. c. coronatus* were well connected by intercatchment dispersal. Following the decline on the Ord River, the Victoria River population of *M. c. coronatus* is now isolated by a similar geographic distance from extant populations of *M. c. coronatus* in the western Kimberly as from populations of *M. c. macgillivrayi* in the Gulf (Fig. 1). Although the Victoria River population is genetically indistinguishable (Table 3) and only slightly morphologically divergent (Fig. 5) from *M. c. coronatus* in the western Kimberley, it is likely that it will diverge from the other remnant populations of *M. c. coronatus* if connectivity is not restored.

Management directives

The priority for conservation management of *M. coronatus* must be the preservation of quality habitat and decreasing the risk of further population declines. As *M. c. coronatus* and *M. c. macgillivrayi* are isolated by a natural barrier to dispersal, management of these subspecies should be undertaken independently and the division between the subspecies maintained during interventions such as translocations and captive-breeding. Active conservation is more urgent for the Endangered *M. c. coronatus*, while the status of the eastern subspecies requires monitoring. It is likely that dispersal between waterways occupied by *M. c. coronatus*, and thus population dynamics, have been disrupted by wide-scale habitat degradation. As over-grazing and fire are the major threats for purple-crowned fairy-wren habitat (Rowley 1993; van Doorn 2007; Skroblin and Legge 2011), it is important that management actions reduce grazing pressure and the threat of fire in areas where quality habitat occurs. The conservation management of *M. c. coronatus* could therefore be further improved by information on the current extent, quality and arrangement of remaining habitat (Prugh *et al*. 2008), and the influence that habitat arrangement has on contemporary dispersal patterns (Fahrig and Merriam 1985; Holsinger 2000; Cox and Engstrom 2001). Conservation efforts will be best prioritised once the size and isolation of populations, and hence localised extinction risk (Pimm *et al*. 1988; Berger 1990), is known. Restoring connectivity between populations that have been isolated by habitat degradation is an important future conservation action.

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