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Placing the Fijian Honeyeaters within the meliphagid radiation: implications for origins and conservation

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Abstract. Understanding the evolutionary relationships of threatened species provides an important framework for making decisions about their conservation. However, unrecognised problems with the underlying phylogenetic analyses may bias the decision-making process. Recent phylogenetic studies have improved our understanding of Meliphagidae, but also indicate discordance between molecular datasets. Here, we examine the causes of this discordance using maximum likelihood tree-building and network analyses of identically sampled datasets for four genetic loci. Our results suggest that while we can be reasonably confident of relationships within species groups, discordance within and between molecular datasets tends to obscure relationships towards the base of the meliphagid tree. This ongoing uncertainty likely reflects differences in the sampling of markers and taxa between previously published analyses. To avoid the problems of conflicting data we used divergence time analyses of only the most densely sampled marker, NADH-ubiquinone oxidoreductase chain 2, to investigate the age and origins of the Fijian Meliphagidae. Our analyses suggest two temporally distinct colonisations of the Fijian archipelago. The large-bodied honeyeaters arrived ~15.6 million years ago, subsequently diversifying and spreading to Tonga and Samoa. In contrast, *Myzomela* appears to have arrived within the last 5.0 million years. The phylogenetic results therefore imply that conserving the evolutionary diversity of Meliphagidae in Polynesia requires that effort be spread across both the currently recognised taxa and geographical range.

Additional keywords: Austro-Pacific, conflicting data, divergence time analysis, Meliphagidae, phylogenetic analysis.

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Introduction

The Fijian archipelago is isolated in the Pacific Ocean. Its island neighbours include Tonga and Samoa to the east, but the closest continental landmass is Australia some 3000 km to the west. Fiji comprises over 330 islands ranging from four large volcanic islands with rugged relief (e.g. Viti Levu, Vanua Levu, Taveuni and Kadavu) to smaller low-lying limestone islands (e.g. Kabara, Ogea, Vatulee and Vulaga). The archipelago, which is part of the Polynesia–Micronesia biodiversity hotspot, has a diverse biota that remains underexplored, particularly in terms of its evolutionary origins and affinities (Irestedt *et al.* 2008; Keppel *et al.* 2009, 2011; Olson *et al.* 2010). The origins of the Fijian biota have been controversial (Ash 1992). The original landmass was linked to Gondwana, but the modern islands reflect volcanic activity that began 50–34 million years ago (Green and Cullen 1973; Neall and Trewick 2008). Thus, as for other landmasses in the Pacific (i.e. New Caledonia and New Zealand), the extant Fijian biota may be explained by either ancient presence or recent arrival by dispersal. Recent arrival in Fiji has been suggested for birds (Driskell and Christidis 2004), invertebrates (Sarnat and Moreau 2011; Strandberg and Johanson 2011) and plants (Ghazanfar *et al.* 2001).

A total of 170 bird species belonging to 43 families are recorded from Fiji (Lepage and Warnier 2014). Among them is the highly diverse Austro-Pacific family Meliphagidae, which comprises ~180 species occurring predominantly in Australia

and New Guinea. Seventeen species are recorded from the islands of Micronesia and Polynesia (Driskell and Christidis 2004; Gardner *et al.* 2010; Andersen *et al.* 2014). In Fiji, the family is represented by five phenotypically and ecologically distinctive species (Watling 2001). Three are large-bodied species that primarily occur in forested areas. *Foulehaio carunculatus* and *Gymnozyma viridis* have broadly overlapping geographical distributions, both occurring on the islands of Viti Levu, Vanua Levu, and Taveuni, with *F. carunculatus* also reaching the Lau Archipelago, Tonga and Samoa. The third species, *Xanthotis provocator*, is restricted to the island of Kadavu. Two smaller *Myzomela* species occupy a range of habitats in the Fijian lowlands. *Myzomela jugularis* is relatively common in Fiji whereas *M. chermesina* is restricted to the northern islands of Rotuma.

Over the last 15 years phylogenetic analyses of nuclear and mitochondrial DNA sequences have greatly improved our understanding of the evolutionary relationships and taxonomy of Meliphagidae (e.g. Cracraft and Feinstein 2000; Barker *et al.* 2004; Driskell and Christidis 2004; Norman *et al.* 2007; Nyári and Joseph 2011; Andersen *et al.* 2014; Joseph *et al.* 2014). The study of Andersen *et al.* (2014) is of particular interest because it focuses on the origins of Pacific Island honeyeaters. Generally, this study suggests that the western Pacific meliphagid fauna reflects multiple dispersals from Austro-Papuan sources. However, the large-bodied forest-dwelling species formed a clade, suggesting that it represents a Pacific Island radiation (Andersen *et al.* 2014). Despite these advances there are outstanding issues. For example, while previous analyses have consistently resolved species groups within Meliphagidae, relationships between these groupings are unstable. This is particularly concerning because although issues associated with combining conflicting data are widely acknowledged, trees based on concatenated data have been assumed to provide the best estimate of meliphagid phylogeny. Yet even with combined data, deeper-level relationships remain uncertain. For example, the placement of *Myzomela* and its relatives differs between the analyses of Andersen *et al.* (2014) and those of Driskell and Christidis (2004), Gardner *et al.* (2010), and Joseph *et al.* (2014). The differences between recovered relationships have been attributed to poor sequence alignment (Gardner *et al.* 2010), contrasting sampling (Andersen *et al.* 2014), and stochasticity (Joseph *et al.* 2014). This uncertainty obviously limits our understanding of evolutionary relationships within the Meliphagidae and, given the increasing importance of evolutionary data in threatened species conservation, may also bias conservation decision-making.

Here we report phylogenetic analyses aimed at (1) evaluating the causes of discordance between gene trees for different DNA loci, and (2) understanding the origins of the Fijian meliphagid fauna. For the former we compare datasets with identical taxon sampling for mitochondrial cytochrome *b* (cytB), NADH-ubiquinone oxidoreductase chain 2 (ND2), and 12S rDNA (12S rDNA) loci as well as the fifth intron of the nuclear encoded β-Fibrinogen gene (Fib5). We then use ND2 sequences for an expanded sample of Meliphagidae to establish a temporal framework for understanding the assembly of the Fijian meliphagid fauna. We also discuss our results in the context of setting conservation priorities for the group.

Materials and methods

Sample collection, DNA extraction, locus amplification and sequencing

Blood or tissue samples were obtained from *Xanthotis provocator* as well as three non-Fijian meliphagids (*Lichenostomus flavescens*, *Melithreptus albogularis*, and *Myzomela cardinalis*) that were kindly provided by W. Boles (The Australian Museum). Collection details for all four accessions are provided in Table S1 (available online as supplementary material to this paper).

Total genomic DNA was extracted using either a salting-out procedure (Sunnucks and Hales 1996) or the DNeasy Blood and Tissue kit (Qiagen). In both cases we made minor modifications to the original procedures. For example, when using the DNeasy kit cell lysis was allowed to continue until it was visually apparent that all cells had lysed. Amplification of target loci was carried out in 30 μL reaction volumes using 1 μL of a 1 : 10 dilution of the extracted DNA solution. Typically, the same PCR mix consisting of 1 × Red Hot reaction buffer IV, 0.133 mM of each dNTP, 2.5 mM $MgCl_2$, 10 pmol of each amplification primer, and 1.5U of Red Hot DNA Polymerase (Thermo Scientific) was used for all markers. However, amplification from some templates required the addition of bovine serum albumin (New England BioLabs) to a final concentration of 0.66 mg mL^{-1}. Amplification of the ND2, cytB, 12S rDNA, and Fib5 loci used the primers L5206/H6313 (Kirchman *et al.* 2001), L15191/H15916 (Lanyon and Hall 1994), L1276/H1811 (Driskell and Christidis 2004), and FIB5/FIB6 (Marini and Hackett 2002), respectively. Thermocycling consisted of an initial 30 s denaturation at 95°C, followed by 30 cycles of 95°C for 30 s (denaturation), 52–63°C for 30 s (annealing), and 72°C for 60 s (extension) with a final 10 min extension at 72°C.

Typically, a SAP/Exonuclease I (Thermo Scientific) procedure was used to purify amplified fragments, although where necessary a Gel Purification kit (Qiagen) was used to isolate and purify the band of interest. Purified fragments were then sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and amplification primers. The resulting fragments were analysed on an ABI 3100 Genetic Analyzer (Applied Biosystems) at the Massey University Genome Service. ChromasPro (Technelysium) was used to evaluate sequence quality and to assemble contigs from forward and reverse sequences.

Phylogenetic trees and networks

To examine discordance between DNA sequence datasets, we compiled matrices containing the same 61 taxa for ND2, cytB, 12S rDNA and Fib5 by combining our newly generated sequences with published sequences obtained from GenBank (see Table S1 for the list of included accessions). Initial multiple sequence alignments were performed using MUSCLE (Edgar 2004) and edited manually to remove gapped positions. A series of combined datasets were constructed by concatenating individual datasets in all possible pairwise combinations.

The Akaike Information Criterion, as implemented in jModelTest2 (Guindon and Gascuel 2003; Darriba *et al.* 2012), was used to estimate best-fit nucleotide substitution models for each of the individual and combined matrices. Using best-fit models

Table 1. A comparison of the data matrices in terms of best-fit nucleotide substitution models and levels of bootstrap support for clades containing Fijian representatives

Dataset	Aligned length (nt)	Best-fit substitution model			Maximum likelihood bootstrapping support for reference clades (%)	
		Model	Proportion of invariable sites	Gamma shape parameter	*Myzomela–C. niger*	*Foulehaio–Xanthotis*
Individual genes						
12S rDNA	394	TIM2ef+I+G	0.4690	0.317	–	–
cytB	637	TVM+I+G	0.5080	0.502	16(ND2 resolution)	43(ND2 resolution)
Fib5	350	TPM2uf+G	0.5067	1.132	86(Fib5 resolution)	–
ND2	948	GTR+I+G	0.3620	0.6340	38(ND2 resolution)	86(ND2 resolution)
Concatenated genes						
ND2/12S rDNA	1342	GTR+I+G	0.4540	0.6280	53(ND2 resolution)	71(ND2 resolution)
ND2/cytB	1585	GTR+I+G	0.4140	0.5580	33(ND2 resolution)	99(ND2 resolution)
ND2/Fib5	1298	GTR+I+G	0.3470	0.5870	57(Fib5 resolution)	68(ND2 resolution)
cytB/12S rDNA	1031	GTR+I+G	0.5590	0.5460	35(ND2 resolution)	26(ND2 resolution)
cytB/Fib5	987	TVM+I+G	0.4030	0.5230	82(Fib5 resolution)	–
12S rDNA/Fib5	744	TPM2uf+G	0.4020	0.5720	79(Fib5 resolution)	–

(Table 1) and PhyML (Guindon *et al.* 2010), we then estimated maximum likelihood (ML) trees and conducted ML bootstrapping (500 replicates) for each matrix. To further examine the underlying structure of the data we constructed split networks using observed distances in NeighbourNet (Huson and Bryant 2006).

Divergence time estimation

To avoid problems associated with combining conflicting data we used a dataset composed solely of ND2 sequences for divergence time analyses. This dataset was compiled by adding an additional 58 previously published sequences, obtained from GenBank, to our 61-taxon dataset (see Table S1 for the list of included accessions).

Divergence time analyses were carried out using BEAST 1.8.1 (Drummond *et al.* 2012). Two constraints were applied. The first was on the age of the split between Maluridae and the clade containing Meliphagidae and Pardalotidae, which corresponds to the root of our tree. Specifically, based on Barker *et al.* (2004) and Cracraft and Barker (2009), we applied a uniform prior with upper and lower bounds of 58 and 37 million years (Ma), respectively, to this node. A uniform probability distribution was used because the prior is based on results from several analyses and it was therefore difficult to justify any of the alternatives. The second constraint was on the mean nucleotide substitution rate. Pacheco *et al.* (2011) report that the range of ND2 substitution rates for Passeriformes was 0.0031–0.0148 substitutions per site per year. These data were used to define a uniform prior as we lacked information on the distribution of rates within them.

A pair of BEAST runs, each 2.0×10^7 generations in length and sampled every 500 generations, were conducted. Each run used a ML topology (estimated using PhyML) as a start tree, an uncorrelated log-normal model of rate evolution (Drummond *et al.* 2006), a GTR+I+G model of sequence evolution (determined using the BIC as implemented in jModelTest2), and a Yule speciation model (Yule 1925). An appropriate burn-in was estimated using the average standard deviation of split frequencies (i.e. <0.01) with convergence and stationarity evaluated on the basis of estimated sample sizes (i.e. >200) and potential scale reduction factors (i.e. ~ 1.0). Parameter estimates, divergence time estimates, and trees were combined using LogCombiner 1.8.1. The combined results were examined using Tracer 1.5 and TreeAnnotator 1.8.1.

Results

Phylogenetic trees and networks

For each of the individual and concatenated 61-taxon matrices jModelTest2 indicated that a general time-reversible, or closely related submodel, was the best-fit nucleotide substitution model (Table 1). In general, best-fit models were simpler for shorter datasets (e.g. 12S rDNA, Fib5), presumably because the total number of substitutions was also smaller for these (Table 1). Results from jModelTest2 also suggested that, with the exception of Fib5, our datasets were characterised by substantial positional rate heterogeneity. This was indicated by estimates of the shape parameter being less than 1.0 for all datasets except Fib5 (Table 1).

PhyML trees for individual datasets differed both in terms of the recovered relationships and support for them (Fig. 1). As a basis for comparison we considered only clades containing Fijian representatives and used the ML tree for ND2 (Fig. 1*a*) as a reference topology since this marker appears in all recent broad-scale phylogenetic analyses of the group (Driskell and Christidis 2004; Gardner *et al.* 2010; Andersen *et al.* 2014; Joseph *et al.* 2014). In our ND2 topology the pairing of *F. carunculatus and X. provocator* was strongly supported (i.e. bootstrap value $>80\%$) as sister to a clade containing representatives of *Certhionyx*, *Lichmera*, *Phylidonyris* and *Trichodere*. In contrast, although recovered in the optimal ML tree, there was $<50\%$ bootstrap support (BS) for the *Myzomela* plus *Certhionyx niger* clade falling sister to that including members of *Grantiella*, *Philemon*, *Plectorhyncha* and *Xanthomyza*. The cytB tree was broadly similar to that found using ND2. The same three primary clades were recovered and although relationships within these vary, the differences were not well supported by ML

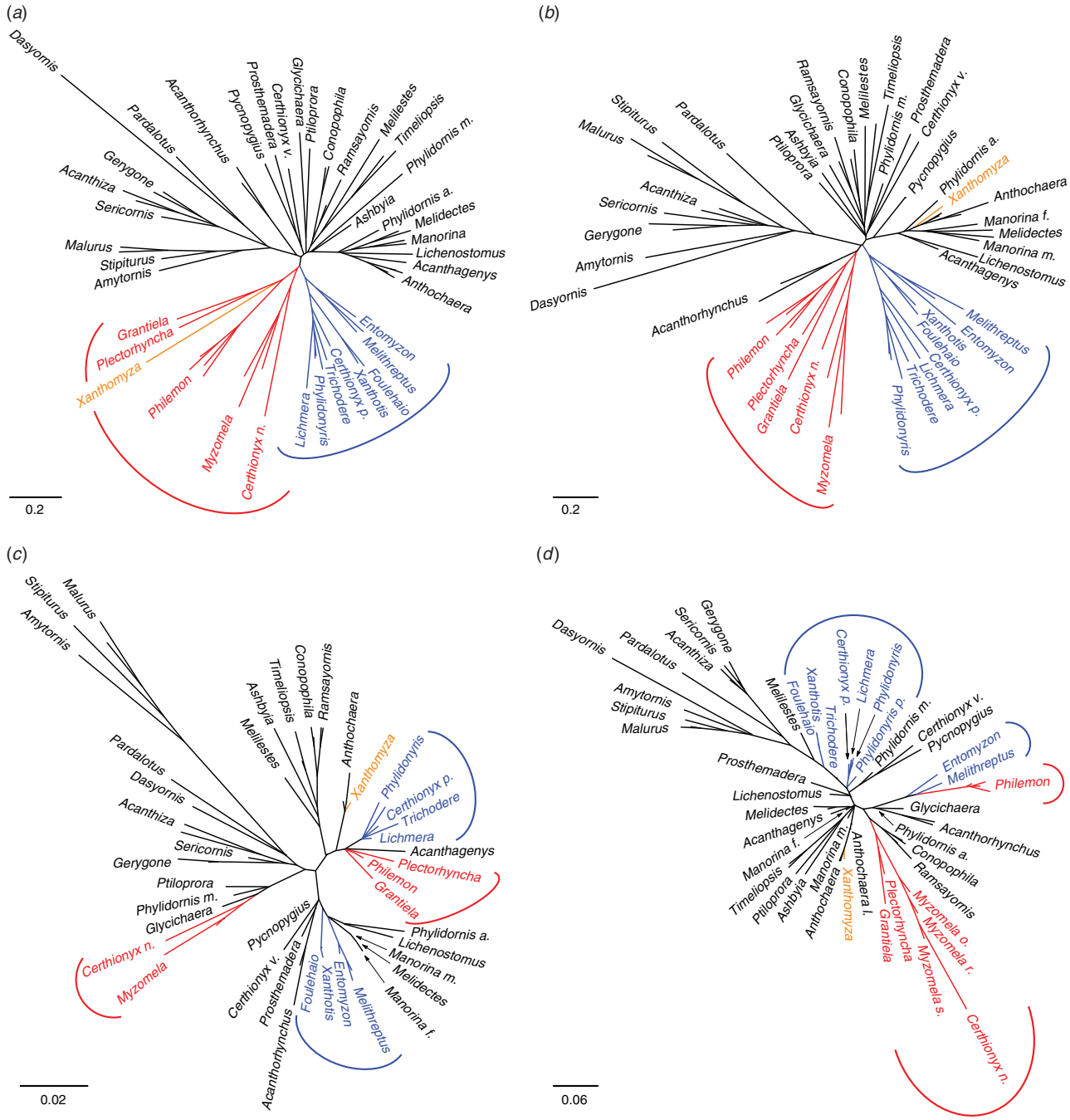

Fig. 1. Phylogenies for Meliphagidae and its relatives based on maximum likelihood analyses of identically sampled 61-taxon single-locus matrices. (*a*) tree based on ND2 sequences; (*b*) tree based on cytB sequences; (*c*) tree based on Fib5 sequences; (*d*) tree based on 12S sequences. For clarity, genus names are given when the genus is represented by a single species or if all (or most) sampled members form a clade; where a genus is not monophyletic the first letter of the species epithet is given to distinguish it from other members of the genus (see Table S1 for list of included species). The two reference clades (and their relatives), as described in the text, are highlighted in red (*Myzomela* plus *Certhionyx niger*) and blue (*Foulehaio–Xanthotis*) with *Xanthomyza* highlighted in orange to illustrate positional instability.

bootstrapping (i.e. <50% BS). The *Foulehaio–Xanthotis* and *Myzomela–C. niger* lineages also retained the same wider relationships, although both these relationships receive <50% BS (Table 1). The *Foulehaio–Xanthotis* and *Myzomela–C. niger* clades were also recovered in the 12S and Fib5 trees (Fig. 1*e* and 1*d*, respectively). However, their wider relationships differed from those based on mitochondrial genes. For example, in the optimal Fib5 tree the pairing of *Myzomela–C. niger* was nested within a clade containing *Glycichaera*, *Phylidornis melanops* and *Ptiloprora*; this relationship was strongly supported (Table 1).

NeighbourNet analyses of individual matrices suggested that limited bootstrap support in phyML analyses were, at least in part, due to internal conflict. That is, different positions within the sequences supported contrasting relationships. For ND2 and cytB this conflict was largely restricted to internal relationships; networks were more box-like for these, with distal relationships being more tree-like (not shown). The greater extent of box-like relationships among taxa in analyses of Fib5 and 12S rDNA suggests higher levels of conflict for these markers.

PhyML trees from combined analyses differed from each other and from those based on individual markers to varying degrees (Fig. 2). When ND2 was combined with any other marker we recovered the same wider relationships for the *Foulehaio–Xanthotis* lineage as obtained when ND2 was analysed alone (Fig. 2*a–c*). This resolution was also found in analyses of combined cytB–12S (Fig. 2*d*) matrices but not for either cytB–Fib5 or 12S–Fib5 (Fig. 2*e*,*f*). These relationships received varying levels of support from ML bootstrapping. The situation was a similar for the *Myzomela–C. niger* lineage. The relationship recovered using ND2 alone was found in combined analyses of ND2–cytB (Fig. 2*a*), ND2–12S (Fig. 2*b*), and cytB–12S (Fig. 2*d*); again there was also instability in the position of *Xanthomyza*. In the remaining analyses the relationship suggested by Fib5 alone was recovered, often with moderate bootstrap support (Table 1).

Divergence time estimation

Our BEAST analysis of an expanded ND2 dataset provided a temporal framework for Meliphagidae (Fig. 3). The extant crown is suggested to be 34.1 Ma old (95% highest posterior density (HPD) 26.7–44.7 Ma) with many of the major lineages having arisen within the subsequent 10 Ma and most of the generic-level diversity being established before 10 Ma ago.

In this analysis, the large-bodied Fijian species (*Foulehaio*, *Gymnomyza* and *Xanthotis*) form a clade that diverged from its sister 15.6 Ma ago (95% HPD 10.9–21.7 Ma). Within this clade the Fijian endemic *G. viridis* is the sister to the remaining taxa, diverging 13.5 Ma ago (95% HPD 9.5–18.9 Ma), *Xanthotis provocator* is next to diverge, ~8.8 Ma ago (95% HPD 6.0–12.3 Ma). Finally, *G. samoensis* and *F. carunculatus* split from one another 7.3 Ma ago (95% HPD 5.0–10.5 Ma). Within *Foulehaio* three subclades correspond to the recognised subspecies. Based on the 95% HPDs for the divergence events these lineages originated 1.5–9.5 Ma ago. Sampling within the Polynesian lineages remains limited but crown group diversity within each appears to be less than 1.2 Ma old. A possible exception is *G. viridis*, where current sampling suggests that crown group diversity could be as much as 2.3 Ma (95% HPD 1.2–3.7 Ma) old.

The other lineage with Fijian representatives is *Myzomela*. In this case, arrival in Fiji appeared to be more recent, with the sampled Fijian species, *M. jugularis*, splitting away from the remaining representatives of *Myzomela* 4.5 Ma ago (95% HPD 2.7–6.8 Ma).

Discussion

Evaluating molecular markers for inferring relationships within the honeyeater clade

Our analyses suggest that currently available molecular datasets offer limited resolution of evolutionary relationships within Meliphagidae. For lineages represented in Fiji we recover contrasting results. For both the *Foulehaio–Xanthotis* and *Myzomela–C. niger* lineages the relationships suggested by ND2 and cytB both (Fig. 1*a*, *b*) differ from those suggested by Fib5 and 12S (Fig. 1*c*, *d*). The uncertainty we found in the placement of *Myzomela* mirrors previous results (Driskell and Christidis 2004; Gardner *et al.* 2010; Andersen *et al.* 2014; Joseph *et al.* 2014). Combining these data did not improve the stability of the suggested relationships. For *Foulehaio–Xanthotis* the resolution suggested by ND2 was preferred whenever it was included in an analysis (Figs 2*a–c*). In contrast, the Fib5 resolution was favoured for the *Myzomela–C. niger* lineage (Fig. 2*b*, *e*, *f*). When neither of these two genes was included the ND2 resolution was preferred for both clades (Fig. 2*d*). Moreover, relative to cytB alone, support for the wider relationships of *Myzomela–C. niger* and *Foulehaio–Xanthotis* increased and decreased, respectively, in this analysis (Table 1). This implies a mixture of mutual support and conflict between datasets.

Our trees and networks included the same taxa, allowing us to directly compare them. The distal portions of our trees and networks were generally well resolved and moderately to strongly supported. In contrast, for more internal portions, relationships were often poorly supported by bootstrapping and appeared box-like in split networks. These observations may be explained, at least in part, by the pattern of evolution in the Meliphagidae. Our analyses and those of previous phylogenetic studies suggest that early diversification occurred over a short period. Problems associated with reconstructing evolutionary relationships in groups that have experienced rapid species radiations are well documented (Whitfield and Lockhart 2007). In our study the problems appeared to be particularly pronounced for Fib5 and 12S rDNA. Although all the loci had broadly similar proportions of variable sites, the absolute numbers of such sites was smaller for these two markers because they were shorter (Table 1). Additionally, we suspect that taxon sampling was a confounding factor. Most obviously in our analyses was instability in the position of *Xanthomyza*. In the ND2 tree *Xanthomyza* fell within the sister clade of *Myzomela–C. niger* (Fig. 1*a*), but in preliminary analyses on expanded ND2 datasets (97–130 taxa) and final BEAST analyses (Fig. 3) *Xanthomyza* was associated with sampled representatives of *Anthochaera.* This latter placement was also recovered in analyses of the other single-locus datasets (Fig. 1*b–d*). Inconsistency in the placement of *Xanthomyza* based on ND2 may be explained by changes to model parameter estimates as a result of increased taxon sampling. In any case, it is apparent from our analyses that deeper-level relationships in Meliphagidae will be difficult to resolve without additional datasets.

Previous molecular phylogenetic analyses of Meliphagidae have assumed that concatenated datasets provide the best estimate of relationships (Driskell and Christidis 2004; Gardner *et al.* 2010; Andersen *et al.* 2014; Joseph *et al.* 2014). However, given the observed conflict between datasets the assumption made when combining data (i.e. that all sampled loci share the same underlying gene tree) needs to be treated cautiously. This is particularly so if, as results to date suggest, diversification involved species radiations. In this case, incomplete lineage sorting could have led to incompatibility between genes trees and the underlying species tree (Whitfield and Lockhart 2007;

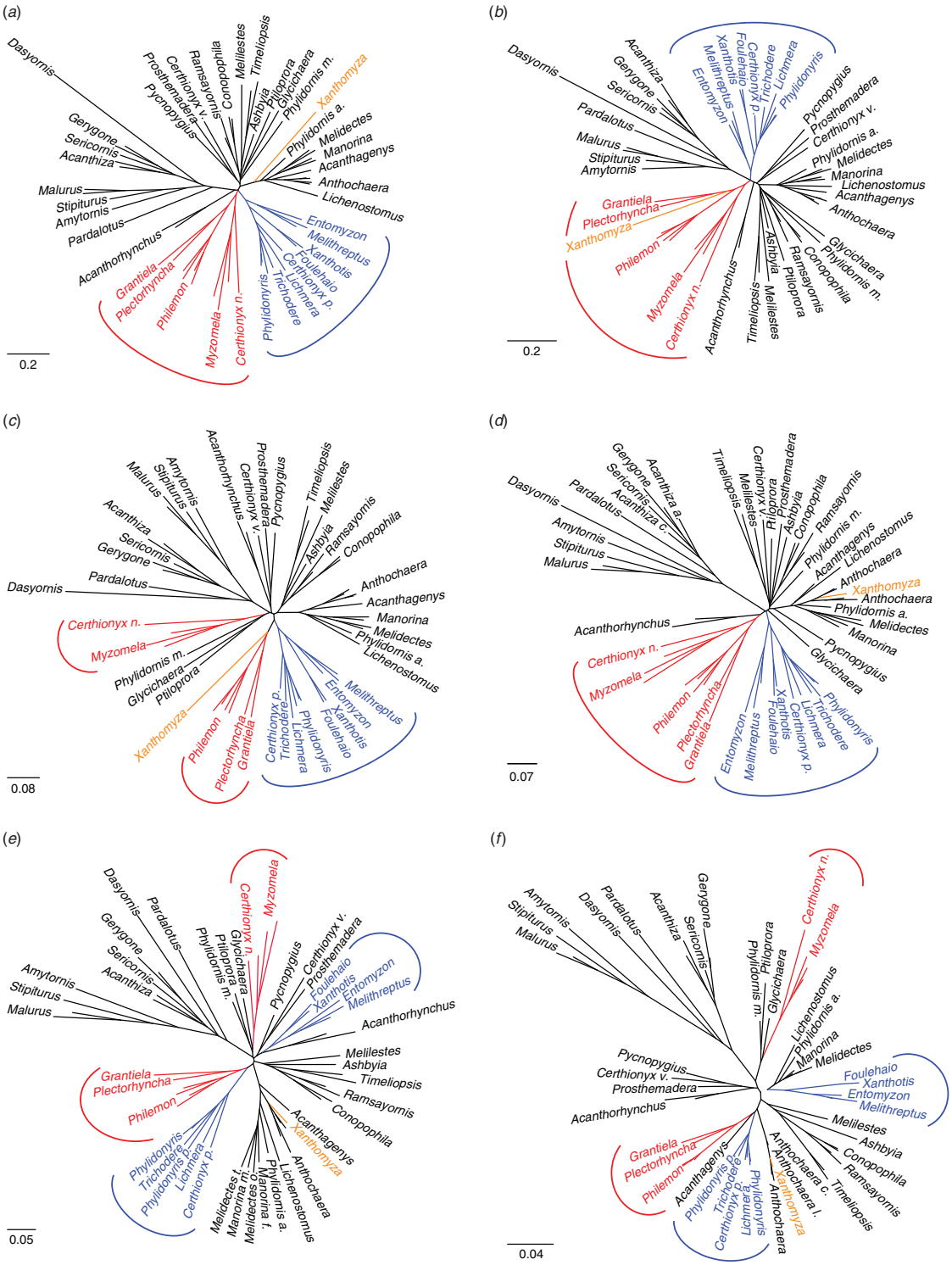

Fig. 2. Phylogenies for Meliphagidae and its relatives based on maximum likelihood analyses of identically sampled 61-taxon two-locus matrices. (*a*) tree based on ND2 and cytB sequences; (*b*) tree based on ND2 and 12S sequences; (*c*) tree based on ND2 and Fib5 sequences; (*d*) tree based on cytB and 12S sequences; (*e*) tree based on cytB and Fib5 sequences; (*f*) tree based on 12S and Fib5 sequences. For clarity, genus names are given when the genus is represented by a single species or where all (or most) sampled members form a clade; where a genus is not monophyletic the first letter of the species epithet is given to distinguish it from other members of the genus (see Table S1 for list of included species). The two reference clades (and their relatives), as described in the text, are highlighted in red (*Myzomela* plus *Certhionyx niger*) and blue (*Foulehaio–Xanthotis*) with *Xanthomyza* highlighted in orange to illustrate positional instability.

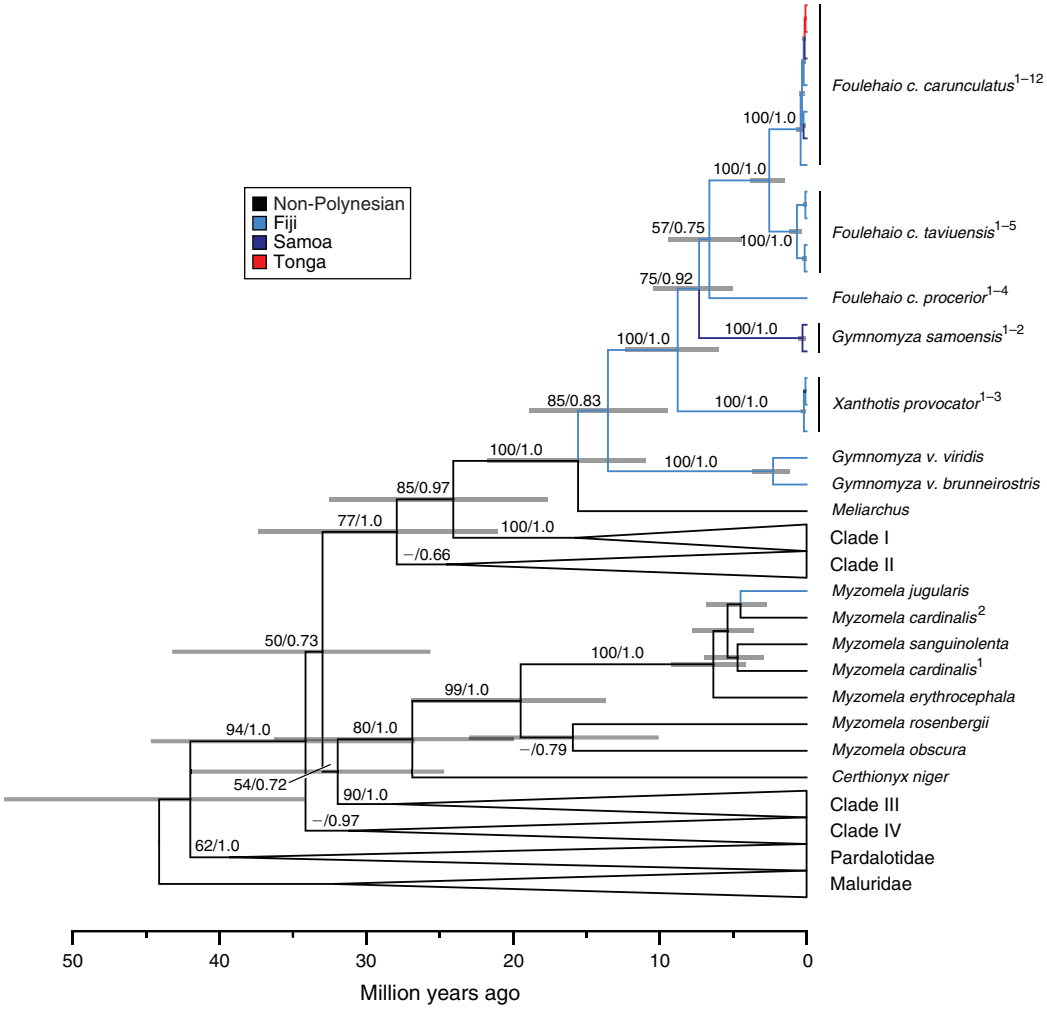

Fig. 3. The maximum clade credibility tree from BEAST analyses of ND2 sequences for Meliphagidae and relatives. Branch lengths are proportional to time with grey bars indicating 95% highest posterior densities on node ages. Genus, species, and, where appropriate, subspecies epithets are given for groups with Fijian representatives; resolution for clades not represented in Fiji is collapsed. Clade I contains species of *Certhionyx*, *Lichmera*, *Phylidonyris* and *Trichodere*; Clade II contains *Entomyzon*, *Lichenostomus* and *Melithreptus*; Clade III contains *Philemon* and *Xanthotis*; Clade IV includes representatives of 22 genera including *Certhionyx*, *Lichenostomus* and *Phylidonyris* (see Table S1 for list of included species; full tree available on request). For groups with Fijian representatives, branches are coloured on the basis of maximum parsimony inference of ancestral character states. Support values are given for branches. The first number is the maximum likelihood bootstrap support (estimated using phyML), the second is the posterior probability; a dash indicates that the bootstrap value was below 50%. For clarity, values are omitted for relationships within subspecies of *Foulehaio*.

Joly *et al.* 2009). While conflict between the datasets has previously been downplayed (e.g. Joseph *et al.* 2014), our analyses suggest that data conflict limits our ability to resolve evolutionary relationships within the honeyeater clade.

A tentative sequence for the assembly of the Fijian honeyeater fauna

Our divergence time analysis provides a temporal framework for understanding the origins of the Fijian honeyeaters. However, we remain cautious about the absolute timing of events for several reasons. First, our estimates are based on ND2 alone. Whilst avoiding problems associated with combining conflicting data, use of single markers can limit our ability to resolve relationships and widen confidence limits around divergence time estimates. Second, there is internal conflict in the ND2 matrix. Since BEAST evaluates topology and node age simultaneously we therefore expect estimation of both to have been impacted. Third, the paucity of the fossil record for Meliphagidae (Boles 2005) means we lack strong temporal constraints. Use of additional constraints may help to refine age estimates. Despite these limitations we consider our analysis to be a useful framework for inference because these issues are likely to have greater impact at deeper nodes and those relevant to the assembly of the Fijian honeyeater fauna are primarily distal (Fig. 3).

Our BEAST analysis suggests that the earliest divergences within Meliphagidae occurred during the Oligocene (34.1 Ma

ago, 95% HPD 26.7–44.7 Ma). Joseph *et al.* (2014) suggested that these divergences occurred more recently and we suspect that this difference reflects the calibration scheme used. Specifically, Joseph *et al.* (2014) used only rate estimates whereas we also place a constraint on the possible age of the root. Our result is consistent with Boles' (2005) suggestion that although the earliest meliphagid fossils are of Miocene age the group is likely to have arisen earlier. Our analysis also suggests that the contemporary Fijian honeyeater fauna is the result of at least two colonisation events (Fig. 3). The first, at the base of the lineage that currently consists of the large-bodied species, occurred during the early to middle Miocene (15.6 Ma ago, 95% HPD 10.9–21.7 Ma); *Myzomela* arrived more recently, perhaps during the early Pliocene (4.5 Ma ago, 95% HPD 2.7–6.8 Ma). As sampling is still somewhat limited we cannot entirely rule out additional arrivals in Fiji (e.g. additional arrivals of *Myzomela* or back dispersal of *Foulehaio* from Samoa or Tonga). That said, we think large numbers of additional dispersal events are unlikely given that few meliphagid lineages successfully colonised Polynesia.

Our analyses and those of Andersen *et al.* (2014) are consistent both in terms of topology and levels of support for relationships in clades that include Polynesian representatives. Both studies suggest that the large-bodied honeyeater lineage arrived first in Fiji, where it diversified and from where it has dispersed to other Polynesian islands. Diversification began during the Miocene (13.5 Ma ago, 95% HPD 9.5–18.9 Ma), with the youngest of the main lineages – *Foulehaio c. carunculatus* and *Foulehaio c. taviuensis* as well as subspecies within *Gymnomyza viridis* – arising during the Pliocene (2.3–2.5 Ma ago, 95% HPD 1.2–3.9 Ma). The combination of evolutionary relationships, divergence times, and geographical distributions for the main lineages strongly suggests that their origins often reflect dispersal. Although dispersal itself can be inferred with confidence, for some of the events we must remain cautious about the details. For example, although it seems likely that dispersal played a role in the formation of subspecies within *Gymnomyza viridis* we do not know on which of the islands (i.e. Viti Levu, Vanua Levu or Taveuni) the species originated and we therefore cannot confidently discuss dispersal direction. In contrast, that the Samoan and Tongan representatives are nested well within an otherwise Fijian radiation strongly suggests these are derived from Fijian ancestors. It appears *G. samoensis* arrived in Samoa 7.3 Ma ago (95% HPD 5.0–10.5 Ma) whereas the spread of *Foulehaio c. carunculatus* to Samoa and Tonga has occurred within the last million years. In the latter case it remains unclear whether Samoa and Tonga were colonised independently or via sequential eastward dispersal. In any case, we suspect the spread of these birds to other Polynesian island archipelagos is linked to habitat availability. For example, populations of *Foulehaio* on Fiji and Tonga occupy similar habitats (Watling 2001).

Conservation implications

The idea of prioritising conservation efforts on the basis of the contribution that a species or taxon makes in terms of evolutionary diversity was suggested more than two decades ago (May 1990; Vane-Wright *et al.* 1991; Faith 1992). The value of this approach is now well established (Winter *et al.* 2013) and the metrics for evaluating evolutionary diversity are now being applied in practical settings (Bennett *et al.* 2014; Jetz *et al.* 2014). Despite the advantages of phylogeny-based metrics for evaluating biological diversity, if our ability to confidently reconstruct the underlying phylogeny is compromised then estimates of evolutionary diversity and hence conservation decision-making may be biased. For Meliphagidae as a whole we would be cautious about setting conservation priorities based on currently available phylogenies. Trees inferred from different datasets suggest differing broad-level relationships and, at least potentially, contrasting estimates of evolutionary diversity. At this point it is difficult to confidently determine which, if any, of the topologies corresponds to the species tree and a cautious approach should be adopted when using the data to inform conservation decision-making.

Although broad-level relationships remain uncertain, the overall similarity of the present analyses and those of Andersen *et al.* (2014) with respect to results for the Polynesian species allows us to make some general observations for these. Results to date indicate considerable evolutionary diversity within Polynesia; conserving this will require efforts to be spread across taxa. For example, conserving the evolutionary diversity of *Foulehaio* requires each of the recognised subspecies to be maintained, which given the distributions of these taxa implies that areas of suitable habitat will need to be protected throughout Fiji. In contrast, in Samoa it may be appropriate to focus conservation efforts on *Gymnomyza samoensis* because, in evolutionary terms, this species is more distinct from Fijian *Gymnomyza* than are Samoan populations of *Foulehaio*. For *Myzomela* sampling remains limited and it is difficult to evaluate the evolutionary distinctiveness of the two Fijian species. However, that both are currently recognised as endemics suggests some degree of difference from related forms.

Many Pacific Island habitats are threatened by human activity and biodiversity loss is anticipated (Steadman and Martin 2003). For the Meliphagidae, forest clearance is of particular concern, although the impacts of changing land use are likely to vary between species. Our findings are, however, preliminary and further work aimed at understanding the evolution and biogeography of this group is needed to better inform conservation decision-making. In particular, studies focussed on population dynamics are needed to evaluate the connectivity of populations. These will be critical for determining where, and of what size, preservation areas need to be if we are to ensure long-term survival of these species.

Future Prospects

The advantages and disadvantages of using multiple genetic loci to examine evolutionary relationships are widely recognised. Although several molecular datasets are available for Meliphagidae, our analyses suggest that they provide limited phylogenetic resolution. Clearly, we need to investigate further genetic loci if we are to overcome topological uncertainty and refine the temporal framework of honeyeater evolution.

Use of next-generation sequencing methods has dramatically increased the ease with which genetic data can be generated (McCormack *et al.* 2013; Seo *et al.* 2014), suggesting that it should be possible to generate the data needed to resolve the

meliphagid tree. Increasingly, researchers are looking to nuclear loci as a data source for resolving the relationships of taxa that arose during species radiations (Murphy *et al.* 2007; Xi *et al.* 2014). These may be particularly useful for evaluating the histories of *Myzomela* and *Foulehaio* in Fiji and nearby archipelagos. In contrast, complete mitochondrial genome sequences may be useful for resolving uncertainty at deeper nodes in the meliphagid tree; this has been the case for mammalian (Ye *et al.* 2004; Chan *et al.* 2010) and frog (Xia *et al.* 2014) phylogenies. Another potential advantage of complete mitochondrial genomes is that using such long sequences is likely to increase the precision of divergence time estimates. This will be important if we are to understand the origins of the Polynesian lineages and the drivers that underpinned their diversification in the region.

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