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Disparate origins for endemic bird taxa from the 'Gondwana Rainforests' of Central Eastern Australia

KIEREN J. MITCHELL[1,*], ANDREW F. HUGALL[2], HOLLY HEINIGER[1], LEO JOSEPH[3] and PAUL M. OLIVER[4,5,*]

[1]*ARC Centre of Excellence for Australian Biodiversity and Heritage (CABAH), School of Biological Sciences, University of Adelaide, North Terrace Campus, Adelaide, SA 5005, Australia*
[2]*Department of Sciences, Museums Victoria, Melbourne, VIC 3001, Australia*
[3]*Australian National Wildlife Collection, CSIRO National Research Collections Australia, GPO Box 1700, Canberra, ACT 2601, Australia*
[4]*Centre for Planetary Health and Food Security, Griffith University, 170 Kessels Road, Nathan, QLD 4111, Australia*
[5]*Biodiversity and Geosciences Program, Queensland Museum, South Brisbane, QLD 4101, Australia*

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Subtropical and temperate rainforests of Central Eastern Australia are some of the largest remaining fragments of their kind globally. The biota of these rainforests appears to comprise two broad biogeographical elements: a more ancient (Miocene or older) and typically upland temperate ('Gondwanan') element and a younger (Plio-Pleistocene) lowland tropical element. We present the first phylogenetic synthesis of the spatiotemporal origins for the eight bird taxa endemic to Central Eastern Australian Rainforests. At least five of these eight focal taxa show Plio-Pleistocene divergences from their respective northern sister taxa, consistent with origins driven by recent expansion and contraction of lowland rainforest. In contrast, two more strictly upland species, the rufous scrub-bird (*Atrichornis rufescens*) and the logrunner (*Orthonyx temminckii*), diverged from their nearest living relatives during the Miocene, suggesting potentially longer histories of persistence and more temperate origins. Finally, we did not recover reciprocal monophyly in mitogenomes from the two extant lyrebirds, Albert's lyrebird (*Menura alberti*) and the superb lyrebird (*Menura novaehollandiae*). The disparate divergence ages recovered among all eight taxa are consistent with the biota of the Central Eastern Australian Rainforests comprising isolates either of younger age and tropical lowland origins or of older age and temperate upland origins.

ADDITIONAL KEYWORDS: biodiversity hotspot – Gondwana Rainforests – habitat fragmentation – mitochondrial genomes – phylogeography – phylogenetics.

INTRODUCTION

Since the Miocene climatic optimum, rainforests in Australia have contracted and fragmented (Bryant & Krosch, 2016), and Plio-Pleistocene glacial cycles have driven further expansion and retraction of rainforest habitats (e.g. Hugall *et al.*, 2002). Today, the rainforests of Australia are concentrated in < 1% of the land area of the continent, mainly along its eastern seaboard. They nonetheless host exceptional species richness and phylogenetic endemism (Stork *et al.*, 2008; Byrne *et al.*, 2011). The tropical rainforests in the Wet Tropics region of north-east Queensland (Fig. 1) have been recognized as a natural laboratory for understanding the processes underpinning isolation, persistence and speciation in rainforest biotas (e.g. Singhal *et al.*, 2018). However, fewer studies have focused on the subtropical Central Eastern Australian Rainforests (CEAR) in south-eastern Queensland and north-eastern New South Wales (Hugall *et al.*, 2003). These borderlands are home to some of the largest remaining areas of subtropical and temperate rainforest in the world, and they too host a distinct assemblage of endemic species (Weber *et al.*, 2014).

*Corresponding authors. E-mail: kieren.mitchell@adelaide.edu.au; p.oliver@griffith.edu.au

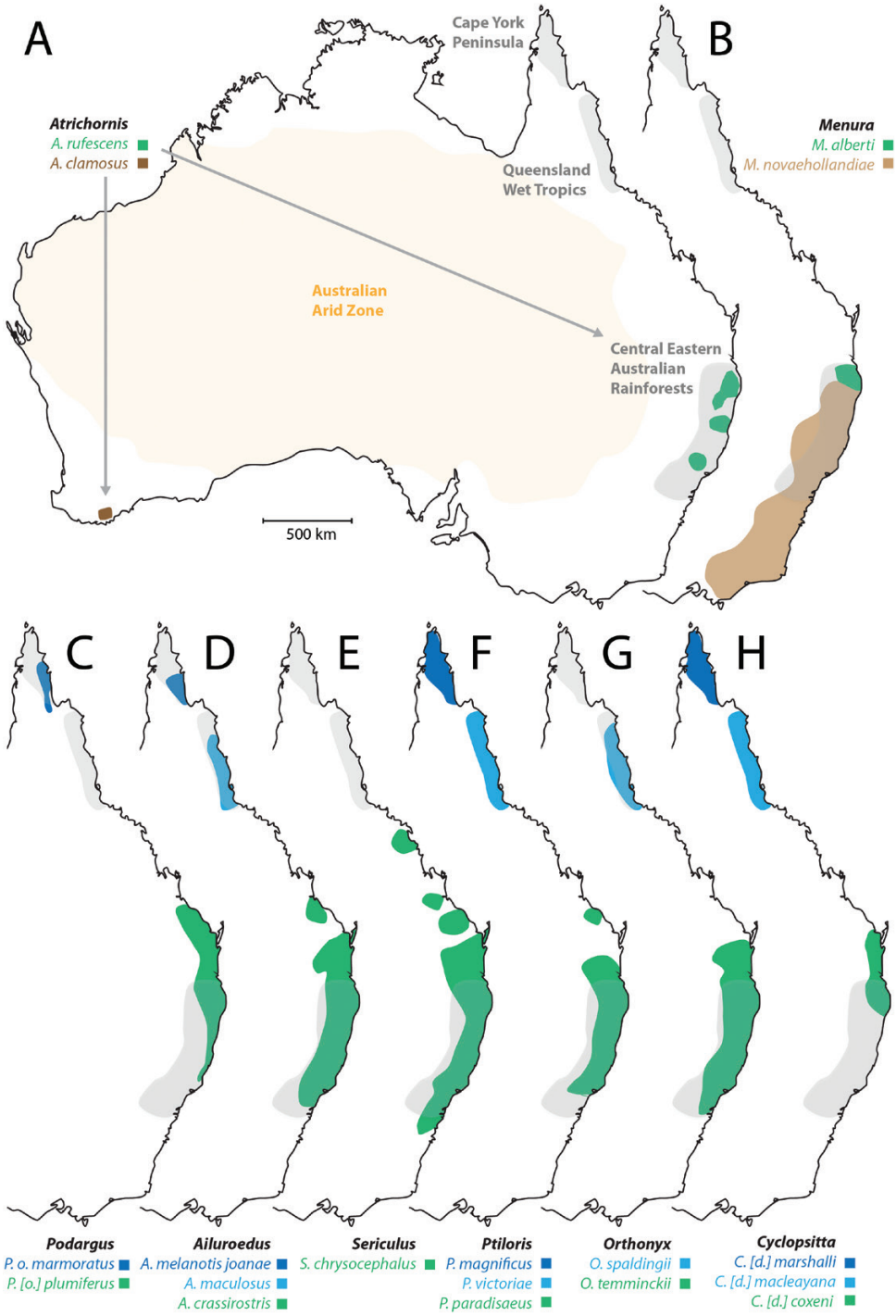

Figure 1. Distribution of major remaining patches of Australian rainforest [the Cape York Peninsula; Queensland Wet Tropics; and Central Eastern Australian Rainforests (CEAR)], the Australian Arid Zone, and the current range of our CEAR focal taxa and their nearest Australian relatives. A, rufous scrub-bird (*Atrichornis rufescens*). B, Albert's lyrebird (*Menura alberti*). C, plumed frogmouth (*Podargus [ocellatus] plumiferus*). D, spotted catbird (*Ailuroedus crassirostris*). E, regent bowerbird (*Sericulus chrysocephalus*). F, paradise riflebird (*Ptiloris paradisaeus*). G, logrunner (*Orthonyx temminckii*). H, Coxen's fig-parrot (*Cyclopsitta [diophthalma] coxeni*).

The dynamics and history of diversification of organisms from the CEAR are more poorly understood than those of the Wet Tropics rainforest further north. Understanding these issues for all rainforests and their associated biotas is a central focus in Australian biogeography (Byrne *et al.*, 2011; Bryant & Krosch, 2016). Two broad biogeographical elements have previously been identified in Australian rainforest biota. One element comprises deeply divergent relict lineages, which often date to the Miocene (e.g. Hugall *et al.*, 2008; Skinner *et al.*, 2013; Skipwith *et al.*, 2019; Tallowin *et al.*, 2020) or even earlier (Ponniah & Hughes, 2004; Rix & Harvey, 2012; Moreau *et al.*, 2015). Many but not all of this first group are descended from predominantly temperate and/or mesic Australian lineages. They often have long evolutionary histories in Southern Hemisphere (Gondwanan) landmasses (Sniderman & Jordan, 2011; Yap *et al.*, 2018), and biogeographical affinities with montane New Guinean rainforests have also been noted (Schodde & Calaby, 1972; Rix & Harvey, 2010). The second biogeographical element comprises groups for which genetic and fossil data suggest much more recent connectivity across eastern Australia. They are often associated with range expansion and contraction during the Plio-Pleistocene (Nicholls & Austin, 2005; Macqueen *et al.*, 2011). This group is therefore often linked to recent colonization from tropical regions to the north (e.g. Queensland Wet Tropics and/or New Guinea; see Irestedt *et al.*, 2017; Ericson *et al.*, 2020). These younger lineages typically inhabit lowland and upland rainforest dominated by floristic lineages that have colonized Australia from Malesia (Yap *et al.*, 2018; Kooyman *et al.*, 2019; Brambach *et al.*, 2020; Joyce *et al.*, 2020).

The prevalence of the first group of lineages in the CEAR has led to popularization of the term 'Gondwana Rainforests' (e.g. DAWE, 2020; Kooyman *et al.*, 2020; UNESCO, 2020). However, despite the use of that term in the names of UNESCO World Heritage and Australian National Heritage sites, we favour the name Central Eastern Australian Rainforests here for two reasons: rainforests with numerous Gondwanan-derived lineages occur across eastern Australia, and many species inhabiting these rainforests occur further north and south than those officially dedicated areas. For the latter reason, earlier literature referred to these 'Gondwana Rainforests' as the Gympie–Illawarra block (Schodde & Calaby, 1972). Recently, bushfires of unprecedented extent have threatened these rainforests, exacerbating the impacts of anthropogenic land clearance and invasive species on biodiversity (Hines *et al.*, 2020; Kooyman *et al.*, 2020). This highlights the importance and urgency of understanding the history of the biodiversity of these rainforests and the sensitivity of their ecosystems to climatic change.

To address these biogeographical and conservation-related issues, we have focused on the eight bird taxa that are endemic or nearly endemic to the CEAR (Fig. 1A–H; whether some of these taxa are species or subspecies is debated; see Table 1). To date, the phylogenetic divergences of only four of these endemic birds have been studied closely with molecular data (Norman *et al.*, 2002; Irestedt *et al.*, 2016, 2017; Ericson *et al.*, 2020). Six of the eight endemic birds clearly have closest relatives in rainforest habitats in north Queensland and/or New Guinea (Table 1; Norman *et al.*, 2002; Irestedt *et al.*, 2016, 2017; Menkhorst *et al.*, 2017; Ericson *et al.*, 2020). The other two, although unquestionably distinct species in highly divergent relict genera of passerines, have different patterns of relationships. The first, Albert's lyrebird (*Menura alberti*), has one of the most restricted ranges of any Australian bird, being confined to the Queensland–New South Wales border region (Fig. 1B). It is parapatric with respect to its morphologically distinctive sister species and only extant congener, the superb lyrebird (*Menura novaehollandiae*) (Higgins *et al.*, 2001; Menkhorst *et al.*, 2017). The second species, the rufous scrub-bird (*Atrichornis rufescens*), is separated by > 3000 km from its only other extant congener, the noisy scrub-bird (*Atrichornis clamosus*) from Western Australia (Fig. 1A). Obviously, these birds have different spatial histories, but the temporal patterns of their divergences remain unknown.

Recent advances in high-throughput DNA sequencing and the use of hybridization enrichment have made it feasible and cost-effective to retrieve genetic information from challenging substrates, such as ancient bone and museum skins, from birds (e.g. Mitchell *et al.*, 2014a, b; Scofield *et al.*, 2017; Wood *et al.*, 2017; Boast *et al.*, 2019; Cole *et al.*, 2019; Irestedt *et al.*, 2019; Oliver *et al.*, 2020). These are the only types of specimens available for many birds from the CEAR. In the present study, we used these methods to generate new genetic data that, when combined with published resources, allowed us to perform a taxonomically comprehensive comparative analysis of all eight birds endemic to the CEAR. Our primary aim was to test whether each taxon is either an older (e.g. Miocene) or younger (Plio-Pleistocene) rainforest element. Secondarily, and in light of the answer to the first question, we sought to determine whether each lineage originated in temperate or tropical habitats or *in situ* in the subtropical region. We thus provide the first comparative phylogenetic approach to understanding the origin of these unique avifaunal elements of the iconic subtropical CEAR.

Table 1. Summary of species, specimens and literature examined

Species/subspecies	Range	Sample type	Specimen
Superb lyrebird *Menura novaehollandiae*	SEA	L	All ANWC: B43600; B42766; B44814; B20296; B46196; B42001
Albert's lyrebird *Menura alberti*	CEAR	L	All ANWC: B47109; B47080; B47113
Noisy scrub-bird *Atrichornis clamosus*	WA	T	NMV 55127;
Rufous scrub-bird *Atrichornis rufescens*	CEAR	T	NMV 55794; QM O.18498
Double-eyed fig-parrot *Cyclopsitta d. diophthalma*	NG	T	All ANWC: B26206; B02046; B02306
Double-eyed (Coxen's) fig-parrot *Cyclopsitta [d.] coxeni*	CEAR	T	Both QM: O.13369; O.13368
Double-eyed (Marshall's) fig-parrot *Cyclopsitta [d.] marshalli*	CYP	L	ANWC B43108
Double-eyed (red-browed) fig-parrot *Cyclopsitta [d.] macleayana*	WT	L	ANWC B34867; QM A013531
Dusky-cheeked fig-parrot *Cyclopsitta melanogenia*	NG	L	ANWC: B56211
Marbled frogmouth *Podargus ocellatus marmoratus*	CYP	L	Both ANWC B57048; B57049
Marbled (plumed) frogmouth *Podargus [ocellatus] plumiferus*	CEAR	L	NMV Z41825 (liver) = ANWC B39287 (voucher)
Tawny frogmouth *Podargus strigoides*	WA, SE QLD	L	QM A013570; ANWC B34665
Papuan frogmouth *Podargus papuensis*	CYP	L	QM A005242; ANWC B42882
Logrunner *Orthonyx temminckii*	CEAR	P	Norman *et al.* (2002)
Paradise riflebird *Ptiloris paradisaeus*	CEAR	P	Irestedt *et al.* (2017)
Regent bowerbird *Sericulus chrysocephalus*	CEAR	P	Ericson *et al.* (2020)
Green catbird *Ailuroedus crassirostris*	CEAR	P	Ericson *et al.* (2020); Irestedt *et al.* (2016)

Scientific nomenclature follows Schodde & Mason (1997, 1999) except for Irestedt *et al.*, (2016) for catbirds; square brackets in scientific names indicate ongoing debate and uncertainty about species rank of the indicated taxon, but prevalent usage is indicated (see Dickinson & Remsen, 2013; Dickinson & Christidis, 2014; del Hoyo & Collar, 2014; Clements *et al.*, 2019; Gill *et al.*, 2020). Vernacular English names associated with well-marked subspecies of contentious species rank are included in parentheses. Abbreviations: ANWC, Australian National Wildlife Collection, Canberra; CEAR, Central Eastern Australian Rainforests, including but not restricted to the popularly termed Gondwana Rainforests; CYP, Cape York Peninsula; L, liver; NG, New Guinea; NMV, Museum Victoria, Melbourne; P, published source as indicated; QM, Queensland Museum; SEA, south-east Australia; SE QLD, south-east Queensland non-rainforest; T, toepad; WA, Western Australia; WT, Wet Tropics Rainforests of north-eastern Australia.

MATERIAL AND METHODS

Sampling

Liver and/or toepad samples for four of our focal taxa and relevant outgroups were obtained from the Australian National Wildlife Collection (ANWC), Museums Victoria (NMV) and Queensland Museum (QM), including multiple geographically distant samples where possible (N = 28; Table 1). DNA extraction and library preparation were performed on the toepad samples at the University of Adelaide in a clean-room laboratory dedicated to molecular analysis of low-biomass museum specimens, whereas the tissue samples were processed at the South Australian Regional Facility for Molecular Ecology and Evolution (SARFMEE).

Among our remaining four focal taxa, phylogenetic and molecular dating results have already been published for three (Table 2): the paradise riflebird (*Ptiloris paradisaeus*; Irestedt *et al.*, 2017), green catbird (*Ailuroedus crassirostris*; Irestedt *et al.*, 2016; Ericson *et al.*, 2020) and regent bowerbird (*Sericulus chrysocephalus*; Ericson *et al.*, 2020). For the remaining taxon, the logrunner (*Orthonyx temminckii*), genetic data have been published (Norman *et al.*, 2002) but not included in a time-calibrated phylogenetic analysis. We downloaded Norman *et al.*'s (2002) *Orthonyx* data and analysed them alongside the new data produced as part of the present study.

DNA extraction, library preparation and sequencing

DNA was extracted from all samples using a QIAGEN (Hilden, Germany) DNeasy Blood & Tissue kit following the manufacturer's protocol. DNA from the liver samples was then fragmented to 200–300 bp using a focused ultrasonicator (Covaris: Woburn, Massachusetts, USA). Extracts from all samples were

converted into DNA sequencing libraries following the protocol of Meyer & Kircher (2010), with each adapter (5′ and 3′) modified to contain a unique 7mer barcode sequence. Libraries created from the toepad samples included an additional partial uracil–DNA–glycosylase treatment (Rohland *et al.*, 2015).

At the end of the library preparation protocol, each library was amplified using polymerase chain reaction (PCR) in eight separate reactions to reduce amplification bias. Each reaction contained 1× PCR buffer, 2 mM $MgSO_4$, 1 mM dNTPs, 0.4 mM primer, 0.5 U Platinum *Taq* High Fidelity (ThermoFisher: Waltham, Massachusetts, USA) and 3 μL DNA library template. Reactions were subjected to the following thermocycling regimen: 94 °C for 2 min; 12 or 16 cycles (for tissues or toepads, respectively) of 94 °C for 30 s, 60 °C for 30 s and 68 °C for 40 s; and a final extension of 68 °C for 10 min. Individual PCR products for each library were pooled after amplification, and purified using 1.8× AxyPrep magnetic beads (Axygen: Blackburn, Victoria, Australia).

Libraries generated from the scrub-bird and fig-parrot toepad samples were enriched for bird mitochondrial DNA using hybridization enrichment with the RNA probes described by Mitchell *et al.* (2014b) following the protocol outlined by Cole *et al.* (2019). All libraries were then subjected to a final round of PCR in five separate reactions using fusion primers to add full-length indexed Illumina (San Diego, California, USA) sequencing adapters for sequencing (Meyer & Kircher, 2010). Each reaction contained 1× PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 0.5 mM primer, 1.25 U AmpliTaq (ThermoFisher) Gold and 2 μL DNA library template. Reactions were subjected to the following thermocycling regimen: 94 °C for 6 min; seven or 15 cycles (for tissues or toepads, respectively) of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s; and a final extension of 72 °C for 10 min.

All libraries were pooled and sequenced together on an Illumina NextSeq using a 150-cycle high-output sequencing kit (in 2 × 75 paired-end mode). Raw reads were demultiplexed using 'sabre' (http://github.com/najoshi/sabre) according to their unique 7mer barcode combinations (one nucleotide mismatch allowed). Using AdapterRemoval v.2.1.2 (Schubert *et al.*, 2016), we trimmed low-quality bases (Phred score <20; -minquality 4), merged paired-end reads (minimum overlap = 11 nt) and discarded merged reads < 30 bp (-minlength 30). Read quality was visualized using fastQC v.0.10.1 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) before and after trimming to make sure the trimming was efficient.

Mapping and mitochondrial genome assembly

To create the final mitochondrial consensus sequences used for all downstream analyses, all merged reads for each library were mapped against an appropriate

Table 2. Summary of divergence times between our eight Central Eastern Australian Rainforest focal taxa and their nearest living relatives based on published literature (where available) and the results of our analyses

Central Eastern Australian Rainforest focal taxon	Sister taxon/clade	Mean time to most recent common ancestor (95% HPD); millions of years ago (Mya)	Source
Cyclopsitta [d.] coxeni	*Cyclopsitta [d.] macleayana* [WT]	0.61 (0.29–0.94)	This study
Podargus [o.] plumiferus	*Podargus ocellatus marmoratus* [CYP]	0.66 (0.3–1.1)	This study
Ailuroedus crassirostris	*Ailuroedus maculosus* [WT]	1.78 (0.92–2.83)	Ericson *et al.* (2020)
Ptiloris paradisaeus	*Ptiloris victoriae* [WT]	1.08	Irestedt *et al.* (2017)
Sericulus chrysocephalus	*Sericulus bakeri* [NG] + *Sericulus aureus* [NG] + *Sericulus ardens* [NG]	3.30 (2.06–4.72)	Ericson *et al.* (2020)
Orthonyx temminckii	*Orthonyx spaldingii* [WT] + *Orthonyx novaeguineae* [NG]	9.47 (7.33–11.75)	This study
Atrichornis rufescens	*Atrichornis clamosus* [WA]	11.1 (8.4–14.1)	This study
Menura alberti	*Menura novaehollandiae* [SEA]	Equivocal; see text	This study

Abbreviations: CYP, Cape York Peninsula; HPD, highest posterior density; NG, New Guinea; SEA, south-east Australia; SE QLD, south-east Queensland non-rainforest; WA, Western Australia; WT, Wet Tropics Rainforests of north-eastern Australia.

reference using BWA v.1.7.8 (Li & Durbin, 2009; aln -t 8 -l 1024 -n 0.04 -o 2). In some cases no appropriate reference was publicly available; therefore, we initially constructed a draft reference using the iterative mapping feature of the GENEIOUS READ MAPPER (Kearse *et al.*, 2016) in GENEIOUS v.9.1.6 (https://www.geneious.com; for details, see Supporting Information, Table S1). After mapping with BWA, reads with a mapping quality Phred score > 30 were selected and retained using the SAMTOOLS v.1.4 (Li *et al.*, 2009) view command (-q 30), and duplicate reads were discarded using 'FilterUniqueSAMCons.py' (Kircher 2012). The only exception to this was the mitochondrial genome sequence we assembled from the previously published shotgun sequencing data from a superb lyrebird specimen of unknown provenance (UWBM 76638; SRX1458178; Moyle *et al.*, 2016), for which reads were mapped using BBMAP v.36.92 (Bushnell, 2014) and then filtered using SAMTOOLS v.1.3.1 (Li *et al.* 2009). A final 75% majority consensus sequence was then generated for each library and checked by eye in GENEIOUS calling nucleotides for sites with a minimum depth-of-coverage of 3× for frogmouths, fig-parrots and scrub-birds or 2× for lyrebirds (for details, see Supporting Information, Table S2).

PHYLOGENETIC ANALYSES AND INTRA-TAXON GENETIC DIVERSITY

We aligned our new frogmouth sequences with existing mitochondrial genome sequences from *Podargus ocellatus* (MT180468 and MT180469), *Podargus strigoides* (MT180466), *Podargus papuensis* (MT180467), *Batrachostomus cornutus* (MT180470) and *Rigidipenna inexpectata* (MT180465) using the MUSCLE algorithm (Edgar, 2004) as implemented in GENEIOUS. Likewise, we aligned our new *Cyclopsitta* mitochondrial genome sequences with 47 previously published parrot mitochondrial genome and *CytB* sequences. The 12 mitochondrial H-strand protein-coding genes were checked by eye and extracted from these two alignments for downstream analysis. After establishing the optimal partitioning schemes and nucleotide substitution models (Supporting Information, Table S3) using PARTITIONFINDER v.1.1.1 (Lanfear *et al.*, 2012), we created separate maximum likelihood trees for the frogmouths and fig-parrots using IQ-TREE v.1.6.11 (Nguyen *et al.*, 2015). In each analysis, we partitioned our data such that each partition had its own evolutionary rate but all partitions contributed to the branch lengths of a single best tree (-spp; Chernomor *et al.*, 2016), and we assessed topological support using 1000 ultrafast bootstrap replicates (resampling within partitions; Hoang *et al.*, 2017).

We aligned our newly assembled lyrebird sequences with the previously published superb lyrebird sequence (AY542313) using the MUSCLE algorithm as implemented in GENEIOUS. We used POPART (Leigh, 2015) to create a median-joining network from this alignment (Bandelt, 1999). To test the reciprocal monophyly of the two lyrebird species, we also aligned the lyrebird sequences with the mitochondrial genome of *Prosthemadera novaeseelandiae* (NC_029144), a passerine outgroup. We then extracted the 12 mitochondrial H-strand protein-coding genes from this alignment and performed a phylogenetic analysis using RAXML v.8.2.0 (Stamatakis, 2014), comprising a maximum likelihood search for the best-scoring tree from 1000 rapid bootstrap replicates.

We also added our new lyrebird and scrub-bird data, and previously published sequence data for three species of *Orthonyx*, to a modified version of a previously published Passeriformes supermatrix (see Hugall & Stuart-Fox, 2012). In addition to the sequences we added, we subsampled down to representatives for each passerine family (with Passerida reduced further to only four representatives) and 13 loci (five nuclear protein coding, three nuclear introns and five mitochondrial protein coding). The final alignment amounted to 13 361 nucleotides (8574 nuclear and 4787 mitochondrial) for 74 taxa (including four outgroups) and contained 29.1% missing data (Supporting Information, Tables S4 and S5). We created a maximum likelihood tree from this alignment using the ultrafast bootstrap consensus in IQ-TREE (Hoang *et al.*, 2017), based on a partitioning scheme and substitution models (Supporting Information, Table S3) determined by MODELFINDER (as implemented in IQ-TREE; Kalyaanamoorthy *et al.*, 2017).

MOLECULAR DATING ANALYSES

We performed individual molecular dating analyses corresponding to three different alignments, as follows.

*Australasian frogmouths (*Podargus *spp.)*

We subsampled the alignment used previously for our IQ-TREE analysis to retain only single representatives of each *Podargus* subspecies (removing samples with the highest number of missing data). The optimal partitioning scheme and nucleotide substitution models for this subsampled alignment were established using PARTITIONFINDER. We then co-estimated the phylogeny and node ages using BEAST v.1.8.4 (Drummond & Rambaut, 2007), with a birth–death tree prior and a single lognormal relaxed clock model (including a rate multiplier parameter

for each partition). We constrained the crown age of *Podargus* using a normal distribution (mean = 9.60 Mya; standard deviation = 1.449 Mya) to match the mean (9.60 Mya) and 95% highest posterior density (95% HPD; 6.76–12.71 Mya) for this node estimated by Oliver *et al.* (2020). We ran three independent Markov chain Monte Carlo (MCMC) chains of 1 000 000 generations each, sampling every 1000 generations. Convergence of parameter values was monitored using TRACER v.1.6 (Rambaut & Drummond, 2014), ensuring an Effective Sample Size (ESS) > 200. The first 10% of sampled trees from each chain was discarded as burn-in. We combined the remaining trees and generated a final maximum clade credibility tree.

*Fig-parrots (*Cyclopsitta *spp.)*

We subsampled the alignment used previously for our IQ-TREE analysis to retain only single representatives of each *Cyclopsitta diophthalma* subspecies studied (removing samples with the highest number of missing data). The optimal partitioning scheme and nucleotide substitution models for this subsampled alignment were established using PARTITIONFINDER. We then co-estimated the phylogeny and node ages using BEAST, with a birth–death tree prior and a single lognormal relaxed clock model (including a rate multiplier parameter for each partition). Following the results of Schweizer *et al.* (2011), we enforced the reciprocal monophyly of the clade comprising *Cyclopsitta* and *Psittaculirostris* and the clade comprising the remaining samples, and we constrained the age of the root of the tree according to a normal distribution (mean = 18.6 Mya; standard deviation = 3.5 Mya). Otherwise, this analysis was performed as described above for our frogmouth alignment.

*Lyrebirds (*Menura *spp.), scrub-birds (*Atrichornis *spp.) and logrunners (*Orthonyx *spp.)*

We estimated divergence times for passerines using the same modified supermatrix described above, because it: (1) provided a backbone of slowly evolving nuclear genes that are less affected by issues of saturation than a mitochondrial-only dataset; (2) included a range of passerine taxa, facilitating dating calibration; and (3) contained enough tips to properly inform sequence evolution and tree prior models. Molecular dating was performed using BEAST2 v.2.4.2 (Bouckaert *et al.*, 2014), with a lognormal relaxed clock model and Yule tree prior (both using 1/X parameterization), and the molecular clock was calibrated by constraining the age of six nodes in accordance with the recent literature on Neoaves and Passeriformes (Supporting Information, Table S6). Briefly, three deep Neoaves and Passeriformes secondary calibrations were drawn from Prum *et al.* (2015), and three fossil calibrations were implemented following Jønsson *et al.* (2016) and Oliveros *et al.* (2019). Two independent MCMC chains of 50 000 000 generations were run using BEAST2, sampling every 5000 generations. Convergence of parameter values was monitored using TRACER, ensuring ESS > 200. The first 10% of sampled trees from each chain was discarded as burn-in. We combined the remaining trees and generated a final maximum clade credibility tree.

DATA AVAILABILITY

Mitochondrial consensus sequences produced as part of this study are available on GenBank (MW883515-MW883541). Consensus sequences, demultiplexed sequencing reads, and phylogenetic analysis files are available through figshare (DOI: 10.25909/c.5405865).

RESULTS

AUSTRALASIAN FROGMOUTHS (*PODARGUS* SPP.)

Samples of *Podargus ocellatus* from across New Guinea and eastern Australia were reciprocally monophyletic with respect to other *Podargus* species [bootstrap support (BS) = 100%; Fig. 2A; Supporting Information, Fig. S1]. Within *Podargus ocellatus*, the sample from New Guinea (representing *Podargus o. ocellatus*) was sister to the remaining samples (BS = 100%), which in turn comprised two reciprocally monophyletic clades corresponding to the plumed frogmouth (*Podargus [o.] plumiferus*) from the CEAR and *Podargus o. marmoratus* from Cape York Peninsula. Molecular dating analyses suggested a mean divergence age of 0.66 millions of years ago (Mya; 95% Highest Posterior Density [HPD] = 0.3–1.1 Mya; Fig. 3; Table 2; Supporting Information, Fig. S2). The New Guinean *Podargus o. ocellatus* sample was highly differentiated from Australian samples, having a mean node age of 5.71 Mya (95% HPD = 3.22–8.29 Mya). *Podargus strigoides* samples were not monophyletic, with our results weakly supporting one Western Australian sample (ANWC B34665) as sister to all other *Podargus strigoides* and *Podargus papuensis* samples combined (BS = 53%). More comprehensive sampling, including nuclear DNA of *Podargus strigoides* from its continent-wide distribution, will be necessary to resolve relationships within and between these two species.

FIG-PARROTS (*CYCLOPSITTA* SPP.)

In all our analyses, *C. diophthalma* and *Psittaculirostris* formed a strongly supported clade, with the dusky-cheeked fig-parrot (*Cyclopsitta melanogenia*) being their sister (Fig. 2B; Supporting

Information, Fig. S3). Non-monophyly of *Cyclopsitta* is consistent with previously proposed genus-level reclassification of the dusky-cheeked fig-parrot and its close relatives (e.g. Schnitker, 2014), highlighting the need for a detailed taxonomic reassessment of the group. A close relationship between the two *C. [d.] coxeni* samples was strongly supported (BS = 100%), as was a clade comprising *C. [d.] coxeni* and *C. [d.] macleayana*, which was sister to *C. [d.] marshalli*. Our molecular dating analysis suggested that the mean age of the divergence between *C. [d.] coxeni* and *C. [d.] macleayana* was 0.61 Mya (95% HPD = 0.29–0.94 Mya; Fig. 3; Table 2; Supporting Information, Fig. S4).

Scrub-birds (*Atrichornis* spp.), lyrebirds (*Menura* spp.) and logrunners (*Orthonyx* spp.)

Overall, the phylogeny (Fig. 4A) and node ages (Fig. 3) we estimated for passerines were consistent with previous studies (e.g. Hugall & Stuart-Fox, 2012; Oliveros *et al.*, 2019; Supporting Information, Fig. S5). *Atrichornis* and *Menura* formed a strongly supported clade (BS = 100%) that was sister to all other extant oscines (Passeri) and of considerable age (i.e. 36.9 Mya, 95% HPD = 34.0–39.8 Mya). *Atrichornis* and *Menura* themselves were monophyletic (BS = 100%) with respect to each other and diverged ~28.7 Mya (95% HPD = 25.6–29.9 Mya), the two scrub-bird species having diverged during the mid to late Miocene ~11.1 Mya (95% HPD = 8.4–14.1 Mya; Table 2). In contrast, the two lyrebird species appeared paraphyletic for mitochondrial DNA. The haplotype network showed that *M. alberti* samples fell within the greater diversity of *M. novaehollandiae* (Fig. 4B). For example, one *M. alberti* haplotype (ANWC B47113) was separated from an *M. novaehollandiae* haplotype by as few as eight substitutions, whereas some other *M. novaehollandiae* haplotypes were separated by > 50 substitutions. Consistent with this finding, phylogenetic analysis of

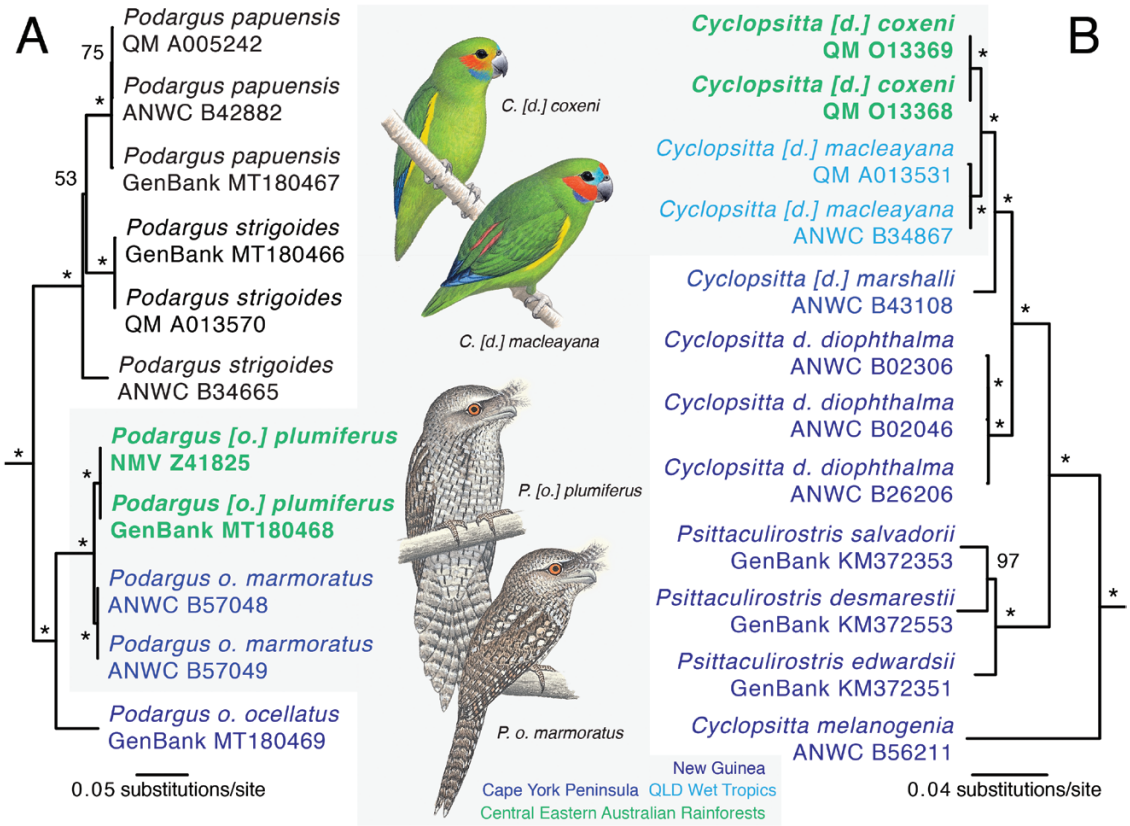

Figure 2. Maximum likelihood phylogenies for frogmouths (A) and fig-parrots (B), pruned to display focal genera (*Podargus* and *Cyclopsitta/Psittaculirostris*, respectively). Full phylogenies with all outgroup taxa are displayed in the Supporting Information (Figs S1, S3). Values associated with branches are bootstrap support percentages (branches with 100% support are marked with an asterisk). Samples belonging to focal taxa are coloured according to their distribution. Central Eastern Australian Rainforest taxa are given in bold text. Artwork © Menkhorst *et al.* (2017) with permission from CSIRO Publishing. This material is not published under an open access licence and cannot be reproduced without permission.

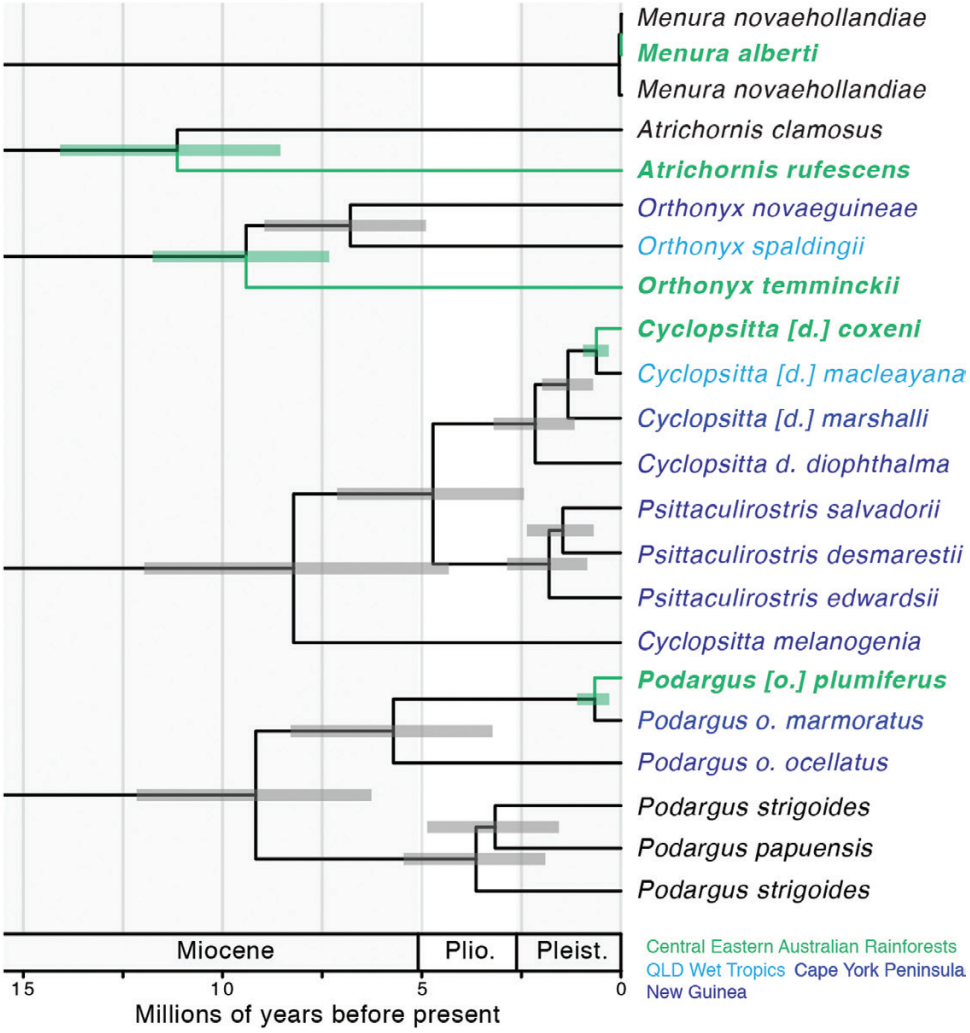

Figure 3. Time-calibrated phylogenies for our focal taxa. Full phylogenies with all outgroups are displayed in the Supporting Information (Figs S2, S4, S5) and tree files are available through figshare (DOI: 10.25909/c.5405865). Node heights represent mean estimated node ages, and bars associated with nodes represent the 95% highest posterior density (HPD) for node age estimates (for focal taxon age values, Table 2). Samples belonging to focal taxa are coloured according to their distribution. Central Eastern Australian Rainforest taxa are highlighted with bold text and green branches and node bars.

all our lyrebird samples resulted in a phylogeny that was largely unresolved (many nodes with BS < 90%; Supporting Information, Fig. S6).

Phylogenetic analyses of the logrunner (*O. temminckii*) strongly supported it as the sister taxon to the clade comprising both the chowchilla (*Orthonyx spaldingii*) from the Australian Wet Tropics and the New Guinea logrunner (*O. novaeguineae*). The split between *O. temminckii* and other congeners was estimated to have occurred in the late Miocene ~9.47 Mya (95% HPD = 7.33–11.75 Mya; Table 2), over a similar time frame to the split between the two scrub-bird species.

DISCUSSION

We present the first comparative analysis of patterns of divergence among eight birds that are endemic to the CEAR (Fig. 1; Table 1) and add to the body of similar data for many other groups (see Bryant & Krosch, 2016: fig. 3). Mitochondrial data for seven of the eight species (i.e. all except the Albert's lyrebird) are broadly consistent with the previously proposed dichotomy between older, Miocene upland temperate lineages and more recent, Plio-Pleistocene lowland tropical lineages (Sniderman & Jordan, 2011). Two species, the logrunner and the rufous scrub-bird, clearly fall within

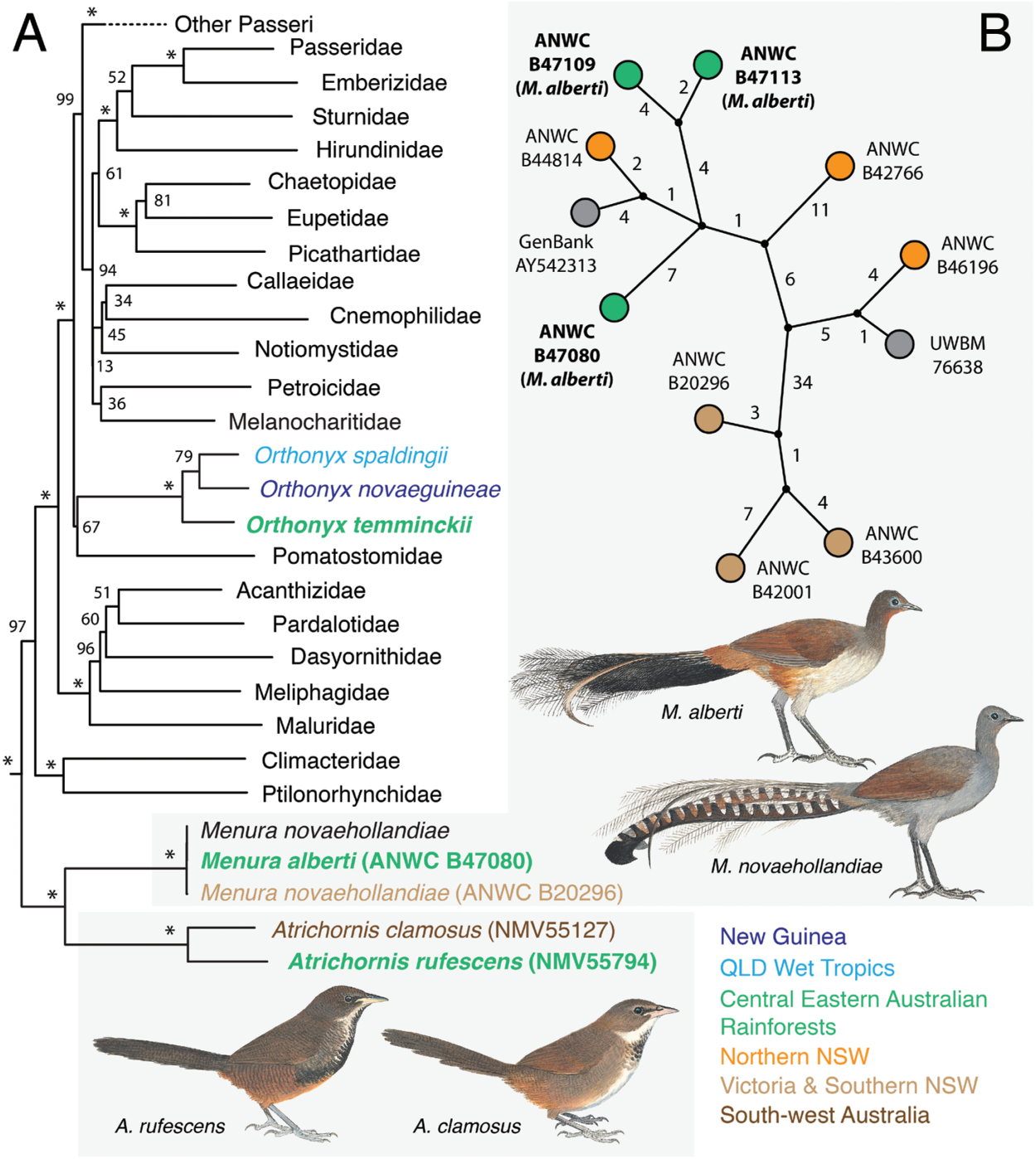

Figure 4. A, maximum likelihood phylogeny for passerines based on a modified version of the supermatrix published by Hugall & Stuart-Fox (2012), including a subset of our new lyrebird and scrub-bird mitochondrial data, in addition to previously published data for three *Orthonyx* species (coloured according to distribution). Values associated with branches are bootstrap support percentages (branches with 100% support are marked with an asterisk). Central Eastern Australian Rainforest taxa are shown in bold text. B, median-joining haplotype network for all lyrebird mitochondrial genome sequences (Albert's lyrebird specimens labelled with bold text). Values associated with branches are the number of substitutions separating observed haplotypes and unobserved intermediate haplotypes (small black nodes). Samples belonging to focal taxa are coloured according to their distribution (or coloured grey where information is not available). Artwork © Menkhorst *et al.* (2017) with permission from CSIRO Publishing. This material is not published under an open access licence and cannot be reproduced without permission.

the group of older divergences. Although the remaining five taxa fall into the second group of more recent divergences, there is some variation in their age.

Our new genetic data show that Coxen's fig-parrot (*C. [d.] coxeni*) and the plumed frogmouth (*Podargus [o.] plumiferus*), both of which are endemic to CEAR, have relatively shallow, Pleistocene divergences from their more northern sister taxa at 0.61 and 0.66 Mya (Table 2; Figure 3), respectively. We suggest that their isolation was driven by Pleistocene cycles of rainforest expansion and contraction. Both taxa are nested within genera that primarily inhabit tropical rainforest habitats (Figure 2), whether upland or lowland, to the north in Queensland's Wet Tropics, Cape York Peninsula or New Guinea. Notably, both are also phenotypically divergent, being substantially larger in size than members of their sister lineages, and thus represent possible examples of Bergmann's rule. In addition, both are currently separated by large geographical distances from their nearest relatives (Higgins, 1999). The geographical disjunction is particularly striking in the case of the frogmouths. The CEAR plumed (*Podargus [o.] plumiferus*) and Cape York Peninsula (*Podargus o. ocellatus*) isolates are separated by > 1000 km, and there are no populations in the intervening Wet Tropics. Published data for the CEAR endemic green catbird and paradise riflebird show similarly shallow divergences from closely related taxa occurring further north. Divergences of these two species from their closest relatives, however, are possibly slightly older than in the frogmouths and fig-parrots (Table 2). Finally among the five younger bird lineages, the divergence of the regent bowerbird from its extant *Sericulus* congeners appears the oldest, with published data indicating an age of > ~3 Mya (Table 2). Nonetheless, the distribution of *Sericulus* spp. across subtropical rainforest of eastern Australia, and lowland and lower montane rainforest in New Guinea, again points to a predominantly Plio-Pleistocene history of range contraction and expansion.

Taken together, the results for the five most recently diverging taxa highlight that although some components of the CEAR avifauna are relictual and diverged before the Plio-Pleistocene, most endemics are likely to stem from dynamic recent histories of Plio-Pleistocene range contraction. Indeed, fossil and genetic data provide complementary evidence of recent range contraction, isolation and fragmentation across central Queensland in rainforest or wet forest vertebrates (Nicholls & Austin, 2005; Hocknull *et al.*, 2007; Macqueen *et al.*, 2010; Irestedt *et al.*, 2017) and plants (Sniderman & Jordan, 2011). Fig-parrots and catbirds are more diverse in New Guinea than in Australia. This suggests that New Guinea, in addition to acting as a refuge for Australian rainforest lineages (Schodde & Calaby, 1972; Brambach *et al.*, 2020), might also have functioned as a source of dispersal during phases of lowland tropical rainforest expansion in Australia throughout the Plio-Pleistocene. We predict that future analyses of other less phenotypically distinct subspecies of birds with ranges including but less strictly confined to the CEAR will further reinforce this inference of Plio-Pleistocene connectivity between the major tropical and subtropical rainforest blocs in eastern Australia.

In contrast, only two of the eight endemic bird species in our study present phylogenetic patterns that point unequivocally to deeper (Miocene) divergence of taxa now endemic to the CEAR. The rufous scrub-bird and its only extant congener, the noisy scrub-bird from Western Australia, diverged during the late Miocene (Table 2), before several major cooling and drying cycles in Australia (Byrne *et al.*, 2008; Sniderman *et al.*, 2016; Andrae *et al.*, 2018), and the two taxa are currently separated by > 3000 km. The rufous scrub-bird is primarily restricted to upland heath and rainforest associations in the CEAR, but is typically recorded > 600 m above sea level. The noisy scrub-bird is confined to small relict patches of temperate mesic heath in far south-west Western Australia (Garnett & Dutson, 2010). Given that both scrub-bird species occur in habitats away from rainforest, their distribution might be limited primarily by aspects of habitat structure, climate and/or frequency of fire. Our new divergence date estimates for all logrunners (*Orthonyx* spp.) likewise indicate that the species endemic to the CEAR, *O. temminckii*, diverged from its sister lineage (i.e. the clade of two species from the Wet Tropics and montane New Guinea) in the late Miocene (Table 2). The depth of genetic divergences shown by scrub-birds and logrunners strongly indicates that they are Miocene relict elements of more temperate 'Gondwanan' rainforest ecosystems, mirroring patterns in other taxa, such as lizards (Skinner *et al.*, 2013; Tallowin *et al.*, 2020), crayfish (Ponniah & Hughes, 2004), snails (Hugall *et al.*, 2003) and spiders (Rix & Harvey, 2012).

Finally, our mitogenomic results for the two lyrebird species present another, entirely unexpected pattern. The age of the clade comprising the lyrebirds and scrub-birds, dating to before the Miocene (28.7 Mya; Fig. 3; Oliveros *et al.*, 2019), indicates that both genera are strikingly relictual and ancient components of the Australia avifauna. Indeed, Early Miocene fossil lyrebirds have been described further west in northern Australia (Boles, 1995). However, our observation of negligible mitochondrial differences between the two lyrebird species conflicts with the dramatic phenotypic differences between the species (Figure 4). We note that all our Albert's lyrebird samples were from males, which are phenotypically most distinct, suggesting that our unusual results are unlikely to be driven by

sample misidentification as has been reported for some field observations (Higgins *et al.*, 2001). Thus, although the genus *Menura* is clearly relictual and ancient, our data so far shed little light on the evolutionary origin of Albert's lyrebird. Its position in the historical biogeography of the CEAR remains enigmatic. Future studies will need to use nuclear genomic data to test alternative explanations for our observations, such as incomplete lineage sorting and recent speciation or fixation of an introgressed mitochondrial lineage following hybridization. As observed in other birds from Australia and New Guinea, species boundaries can be porous and allow for one-way flow of certain genetic markers (Joseph *et al.*, 2019a, b), making this a plausible hypothesis for our unexpected mitochondrial results.

Based on our analyses to date, we advocate no taxonomic changes from prevalent usage among the eight endemic birds of the CEAR. The taxonomic distinctiveness of the rufous scrub-bird, in particular, is unequivocal. Miocene divergence between the two extant species of *Atrichornis*, coupled with the Oligocene origins of the genus (Oliveros *et al.*, 2019), emphasizes the conservation importance of both the rufous and noisy scrub-birds. They are arguably the most endangered and evolutionarily distinctive relict taxa in the entire Australian avifauna. Although having merit as an interim measure, prevalent usage, however, maintains some ongoing inconsistency in recognizing subspecies or species rank among CEAR isolates. For example, despite splitting from their respective sister lineages over a comparable time frame, the paradise riflebird (*Ptiloris paradisaeus*) is ranked as a species, whereas the plumed frogmouth (*Podargus o. plumiferus*) is ranked as a subspecies (Table 1). In any case, both the plumed frogmouth and Coxen's fig-parrot are disjunct southern isolates deserving of recognition (Higgins, 1999), possibly as evolutionarily significant units (*sensu* Moritz, 1994). However, we suggest that the existing subspecies designations are still the most appropriate and will remain so at least until more extensive taxon and population sampling, including sampling of the nuclear genome, can broaden our perspective on their respective histories. There is further justification for maintaining prevalent usage of these two taxa at subspecies rank. Recognition at species rank would render their respective closest relatives in northern Australia and New Guinea paraphyletic and require more extensive taxonomic revisions. Those revisions, in turn, need to be based on more extensive population and nucleotide sampling that would involve extensive sampling of museum skins.

Finally, we highlight how our study relates to current understanding of the molecular phylogeography and divergence times in other elements of the CEAR biota. Figure 3 of Bryant & Krosch (2016) summarizes divergence times among a range of animal and plant taxa from eastern Australian mesic habitats. In their review, divergence times involving three biogeographical barriers pertinent to the isolation of CEAR taxa (Brisbane Valley Barrier, St Lawrence Gap and Burdekin Gap) fell broadly into the two main categories of divergence times that we have used to structure our study, either Plio-Pleistocene or at least Miocene. Notably, just as the rufous scrub-bird and logrunner in our study were exceptional among our focal bird lineages in having a Miocene origin, so too do other (but notably few) elements of the eastern Australian mesic biota reviewed by Bryant & Krosch (2016). We suggest that these results further emphasize the value of the framework we have adopted as a useful approach to understanding the history of the CEAR biota.

Conclusion

Our findings further illustrate the complex history and varying composition of all the Australian rainforest biota. Our results are consistent with the existence of two broad historical elements in the endemic bird fauna of the CEAR, and particularly highlight the prevalence of more recent divergences among lowland biogeographical elements in shaping the modern endemism of the ecosystem. Our results regarding fig-parrots and frogmouths point to Pleistocene connectivity among the CEAR habitats (see also Joseph *et al.*, 1993), and potentially also New Guinea to the north (Schweizer *et al.*, 2015). At least for birds, older relicts having Miocene affinities are a less dominant component of the endemic biota. We also highlighted the unexpected and possibly more complex, multifaceted origins and/or demographic history of Albert's lyrebird from the CEAR. Across all taxa, however, there remains a striking signal of relictual diversity regardless of the timescale of isolation. This pattern highlights the overall sensitivity of rainforest systems to climatic shifts and associated habitat contractions. It underscores the looming conservation issues posed by the combination of a legacy of past clearance and an impending future of unprecedented rapid climatic change. These challenges have been brought into sharp relief by recent fires that decimated large areas of habitat for many of our focal species (Boer *et al.*, 2020; Kooyman *et al.*, 2020; Ward *et al.*, 2020).

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REFERENCES

Andrae JW, **McInerney FA**, **Polissar PJ**, **Sniderman JMK**, **Howard S**, **Hall PA**, **Phelps SR. 2018.** Initial expansion of C4 vegetation in Australia during the late Pliocene. *Geophysical Research Letters* **45:** 4831–4840.

Bandelt H, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* **16:**37–48.

Boast AP, **Chapman B**, **Herrera MB**, **Worthy TH**, **Scofield RP**, **Tennyson AJD**, **Houde P**, **Bunce M**, **Cooper A**, **Mitchell KJ. 2019.** Mitochondrial genomes from New Zealand's extinct adzebills (Aves: Aptornithidae: *Aptornis*) support a sister-taxon relationship with the Afro-Madagascan Sarothruridae. *Diversity* **11:** 24.

Boer MM, **Resco de Dios V**, **Bradstock RA. 2020.** Unprecedented burn area of Australian mega forest fires. *Nature Climate Change* **10:** 171–172.

Boles WE. 1995. A preliminary analysis of the Passeriformes from Riversleigh, northwestern Queensland, Australia, with the description of a new species of lyrebird. *Courier Forschungsinstitut Senckenberg* **181:** 163–170.

Bouckaert R, **Heled J**, **Kuhnert D**, **Vaughan T**, **Wu CH**, **Xie D**, **Suchard MA**, **Rambaut A**, **Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computing Biology* **10:** e1003537.

Brambach F, **Leuschner C**, **Tjoa A**, **Culmsee H. 2020.** Predominant colonization of Malesian mountains by Australian tree lineages. *Journal of Biogeography* **47:** 355–370.

Bryant L, **Krosch M. 2016.** Lines in the land: a review of evidence for eastern Australia's major biogeographical barriers to closed forest taxa. *Biological Journal of the Linnean Society* **119:** 238–264.

Bushnell B. 2014. *BBMap: a fast, accurate, splice-aware aligner*. USA. Available at: https://www.osti.gov/servlets/purl/1241166.

Byrne M, **Steane DA**, **Joseph L**, **Yeates DK**, **Jordan GJ**, **Crayn D**, **Aplin K**, **Cantrill DJ**, **Cook LG**, **Crisp MD**, **Keogh JS**, **Melville J**, **Moritz C**, **Porch N**, **Sniderman JMK**, **Sunnucks P**, **Weston PH. 2011.** Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* **38:** 1635–1656.

Byrne M, **Yeates DK**, **Joseph L**, **Kearney M**, **Bowler J**, **Williams MAJ**, **Cooper S**, **Donnellan SC**, **Keogh JS**, **Leys R**, **Melville J**, **Murphy DJ**, **Porch N**, **Wyrwoll KH. 2008.** Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* **17:** 4398–4417.

Chernomor O, **von Haeseler A**, **Minh BQ. 2016.** Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* **65:** 997–1008.

Clements JF, **Schulenberg TS**, **Iliff MJ**, **Billerman SM**, **Fredericks TA**, **Sullivan BL**, **Wood CL. 2019.** *The eBird/Clements checklist of birds of the world: v2019*. Ithaca, NY: Cornell Laboratory of Ornithology.

Cole TL, **Ksepka DT**, **Mitchell KJ**, **Tennyson AJD**, **Thomas DB**, **Pan H**, **Zhang G**, **Rawlence NJ**, **Wood JR**, **Bover P**, **Bouzat JL**, **Cooper A**, **Fiddaman SR**, **Hart T**, **Miller G**, **Ryan PG**, **Shepherd LD**, **Wilmshurst JM**, **Waters JM. 2019.** Mitogenomes uncover extinct penguin taxa and reveal island formation as a key driver of speciation. *Molecular Biology and Evolution* **36:** 784–797.

DAWE. 2020. *World Heritage Places - Gondwana Rainforests of Australia.* Canberra: Department of Agriculture, Water and the Environment. Available at: http://www.environment.gov.au/heritage/places/world/gondwana

Dickinson EC, **Christidis L. 2014.** *The Howard and Moore complete checklist of the birds of the world, Vol. 2: Passerines, 4th edn*. Eastbourne: Aves Press.

Dickinson EC, **Remsen JV. 2013.** *The Howard and Moore complete checklist of the birds of the world, Vol. 1: Non-passerines, 4th edn*. Eastbourne: Aves Press.

Drummond AJ, **Rambaut A. 2007.** BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7** : 214.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5:** 113.

Ericson PGP, **Irestedt M**, **Nylander JAA**, **Christidis L**, **Joseph L**, **Qu Y. 2020.** Parallel evolution of bower-building behavior in two groups of bowerbirds suggested by phylogenomics. *Systematic Biology* **69:** 820–829.

Garnett ST, **Dutson G. 2010.** *The action plan for Australian birds*. Collingwood: CSIRO Publishing.

Gill F, **Donsker D**, **Rasmussen P. 2020.** *IOC World Bird list (v 10.2)*. Available at: http://www.worldbirdnames.org/

Higgins PJ. 1999. *Handbook of Australian, New Zealand and Antarctic birds, Vol. 4: parrots to dollarbird*. Melbourne: Oxford University Press.

Higgins PJ, **Peter JM**, **Steele WK. 2001.** *Handbook of Australian, New Zeland and Antarctic birds, Vol. 5: tyrant flycatchers to chats*. Melbourne: Oxford University Press.

Hines HB, **Brook M**, **Wilson J**, **McDonald WJF**, **Hargreaves J. 2020.** The extent and severity of the Mackay Highlands 2018 wildfires and the potential impact on natural values, particularly in the mesic forests of the Eungella-Crediton area. *Proceedings of the Royal Society of Queensland* **125:** 139–157.

Hoang DT, **Chernomor O**, **von Haeseler A**, **Minh BQ**, **Vinh LS. 2017.** UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35:** 518–522.

Hocknull SA, **Zhao J**, **Feng Y**, **Webb GE. 2007.** Responses of Quaternary rainforest vertebrates to climate change in Australia. *Earth and Planetary Science Letters* **264:** 317–331.

del Hoyo J, **Collar N. 2014.** *HBW and Birdlife International illustrated checklist of the birds of the world, Vol. 1, Non-passerines*. Barcelona: Lynx edicions.

Hugall A, **Moritz C**, **Moussalli A**, **Stanisic J. 2002.** Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). *Proceedings of the National Academy of Sciences of the United States of America* **99:** 6112–6117.

Hugall AF, **Foster R**, **Hutchinson M**, **Lee MSY. 2008.** Phylogeny of Australasian agamid lizards based on nuclear and mitochondrial genes: implications for morphological evolution and biogeography. *Biological Journal of the Linnean Society* **93:** 343–358.

Hugall AF, **Stanisic J**, **Moritz C. 2003.** The case of the Sphaerospira lineage and history of Queensland rainforests. In: Lydeard C, Lindberg D, eds. *Molecular systematics and phylogeography of mollusks*. Washington: Smithsonian Institution Press, 270–301.

Hugall AF, **Stuart-Fox D. 2012.** Accelerated speciation in colour-polymorphic birds. *Nature* **485:** 631–634.

Irestedt M, **Batalha-Filho H**, **Ericson PGP**, **Christidis L**, **Schodde R. 2017.** Phylogeny, biogeography and taxonomic consequences in a bird-of-paradise species complex, *Lophorina–Ptiloris* (Aves: Paradisaeidae). *Zoological Journal of the Linnean Society* **181:** 439–470.

Irestedt M, **Batalha-Filho H**, **Roselaar CS**, **Christidis L**, **Ericson PGP. 2016.** Contrasting phylogeographic signatures in two Australo-Papuan bowerbird species complexes (Aves: *Ailuroedus*). *Zoologica Scripta* **45:** 365–379.

Irestedt M, **Ericson PGP**, **Johansson US**, **Oliver P**, **Joseph L**, **Blom MPK. 2019.** No signs of genetic erosion in a 19th century genome of the extinct paradise parrot (*Psephotellus pulcherrimus*). *Diversity* **11:** 58.

Jønsson KA, **Fabre P-H**, **Kennedy JD**, **Holt BG**, **Borregaard MK**, **Rahbek C**, **Fjeldså J. 2016.** A supermatrix phylogeny of corvoid passerine birds (Aves: Corvides). *Molecular Phylogenetics and Evolution* **94:** 87–94.

Joseph L, **Dolman G**, **Iova B**, **Jønsson K**, **Campbell CD**, **Mason I**, **Drew A. 2019a**. Aberrantly plumaged orioles from the Trans-Fly savannas of New Guinea and their ecological and evolutionary significance. *Emu - Austral Ornithology* **119:** 264–273.

Joseph L, **Drew A**, **Mason IJ**, **Peters JL. 2019b.** Introgression between non-sister species of honeyeaters (Aves: Meliphagidae) several million years after speciation. *Biological Journal of the Linnean Society* **128:** 583–591.

Joseph L, **Moritz C**, **Hugall AF. 1993.** A mitochondrial DNA perspective on the historical biogeography of middle eastern Queensland rainforest birds. *Memoirs of the Queensland Museum* **34:** 201–214.

Joyce EM, **Thiele KR**, **Ferry Slik JW**, **Crayn DM. 2020.** Plants will cross the lines: climate and available land mass are the major determinants of phytogeographical patterns in the Sunda–Sahul Convergence Zone. *Biological Journal of the Linnean Society* **132:** 374–387.

Kalyaanamoorthy S, **Minh BQ**, **Wong TKF**, **von Haeseler A**, **Jermiin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14:** 587–589.

Kearse M, **Sturrock S**, **Meintjes P. 2016.** *The Geneious 6.0.3 read mapper*. Available at: http://assets.geneious.com/documentation/geneious/GeneiousReadMapper.pdf

Kircher M. 2012. Analysis of high-throughput ancient DNA sequencing data. In: Shapiro B, Hofreiter M, eds. *Ancient DNA. Methods in molecular biology (methods and protocols), Vol 840*. Humana Press. https://doi.org/10.1007/978-1-61779-516-9_23

Kooyman RM, **Morley RJ**, **Crayn DM**, **Joyce EM**, **Rossetto M**, **Slik JWF**, **Strijk JS**, **Su T**, **Yap J-YS**, **Wilf P. 2019.** Origins and assembly of Malesian rainforests. *Annual Review of Ecology, Evolution, and Systematics* **50:** 119–143.

Kooyman RM, **Watson J**, **Wilf P. 2020.** Protect Australia's Gondwana Rainforests. *Science* **367:** 1083.

Lanfear R, **Calcott B**, **Ho SYW**, **Guindon S. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution. *Molecular Biology and Evolution* **29:** 1537–1719.

Leigh, JW, Bryant D. 2015. PopART: Full-feature software for haplotype network construction. *Methods Ecol Evol* **6:** 1110–1116.

Li H, **Durbin R. 2009.** Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25:** 1754–1760.

Li H, **Handsaker B**, **Wysoker A**, **Fennell T**, **Ruan J**, **Homer N**, **Marth G**, **Abecasis G**, **Durbin R**, 100 Genome Project Data Processing Subgroup. **2009.** The Sequence Alignment/Map (SAM) format and SAMtools. *Bioinformatics* **25:** 2078–2079.

Macqueen P, **Goldizen AW**, **Austin JJ**, **Seddon JM. 2011.** Phylogeography of the pademelons (Marsupialia: Macropodidae: *Thylogale*) in New Guinea reflects both geological and climatic events during the Plio-Pleistocene. *Journal of Biogeography* **38:** 1732–1747.

Macqueen P, **Seddon JM**, **Austin JJ**, **Hamilton S**, **Goldizen AW. 2010.** Phylogenetics of the pademelons (Macropodidae: *Thylogale*) and historical biogeography of the Australo-Papuan region. *Molecular Phylogenetics and Evolution* **57:** 1134–1148.

Menkhorst P, **Rogers D**, **Clarke D**, **Davies J**, **Marsack P**, **Franklin K. 2017.** *The Australian bird guide*. Clayton South: CSIRO Publishing.

Meyer M, **Kircher M. 2010.** Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols* **2010:** pdb.prot5448.

Mitchell KJ, **Llamas B**, **Soubrier J**, **Rawlence NJ**, **Worthy TH**, **Wood J**, **Lee MSY**, **Cooper A. 2014a.** Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution. *Science* **344:** 898–900.

Mitchell KJ, **Wood JR**, **Scofield RP**, **Llamas B**, **Cooper A. 2014b.** Ancient mitochondrial genome reveals unsuspected taxonomic affinity of the extinct Chatham duck (*Pachyanas chathamica*) and resolves divergence times for New Zealand and sub-Antarctic brown teals. *Molecular Phylogenetics and Evolution* **70:** 420–428.

Moreau CS, **Hugall AF**, **McDonald KR**, **Jamieson BGM**, **Moritz C. 2015.** An ancient divide in a contiguous rainforest: endemic earthworms in the Australian Wet Tropics. *PLoS ONE* **10:** e0136943.

Moritz C. 1994. Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology & Evolution* **9:** 373–375.

Moyle RG, **Oliveros CH**, **Andersen MJ**, **Hosner PA**, **Benz BW**, **Manthey JD**, **Travers SL**, **Brown RM**, **Faircloth BC. 2016.** Tectonic collision and uplift of Wallacea triggered the global songbird radiation. *Nature Communications* **7:** 12709.

Nguyen LT, **Schmidt HA**, **von Haeseler A**, **Minh BQ. 2015.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32:** 268–274.

Nicholls JA, **Austin JJ. 2005.** Phylogeography of an east Australian wet-forest bird, the satin bowerbird (*Ptilonorhynchus violaceus*), derived from mtDNA, and its relationship to morphology. *Molecular Ecology* **14:** 1485–1496.

Norman JA, **Christidis L**, **Joseph L**, **Slikas B**, **Alpers D. 2002.** Unravelling a biogeographical knot: origin of the 'leapfrog' distribution pattern of Australo-Papuan sooty owls (Strigiformes) and logrunners (Passeriformes). *Proceedings of the Royal Society B: Biological Sciences* **269:** 2127–2133.

Oliver PM, **Heiniger H**, **Hugall AF**, **Joseph L**, **Mitchell KJ. 2020.** Oligocene divergence of frogmouth birds (Podargidae) across Wallace's Line. *Biology Letters* **16:** 20200040.

Oliveros CH, **Field DJ**, **Ksepka DT**, **Barker FK**, **Aleixo A**, **Andersen MJ**, **Alström P**, **Benz BW**, **Braun EL**, **Braun MJ**, **Bravo GA**, **Brumfield RT**, **Chesser RT**, **Claramunt S**, **Cracraft J**, **Cuervo AM**, **Derryberry EP**, **Glenn TC**, **Harvey MG**, **Hosner PA**, **Joseph L**, **Kimball RT**, **Mack AL**, **Miskelly CM**, **Peterson AT**, **Robbins MB**, **Sheldon FH**, **Silveira LF**, **Smith BT**, **White ND**, **Moyle RG**, **Faircloth BC. 2019.** Earth history and the passerine superradiation. *Proceedings of the National Academy of Sciences of the United States of America* **116:** 7916–7925.

Ponniah M, **Hughes JM. 2004.** The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. *Evolution* **58:** 1073–1085.

Prum RO, **Berv JS**, **Dornburg A**, **Field DJ**, **Townsend JP**, **Lemmon EM**, **Lemmon AR. 2015.** A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature* **526:** 569–573.

Rambaut A, **Drummond AJ. 2014.** *Tracer v1.6*. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Rix M, **Harvey M. 2010.** The spider family Micropholcommatidae (Arachnida: Araneae: Araneoidea): a relimitation and revision at the generic level. *ZooKeys* **36:** 1–321.

Rix MG, **Harvey MS. 2012.** Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: evidence for Miocene speciation within Tertiary refugia. *Molecular Phylogenetics and Evolution* **62:** 375–396.

Rohland N, **Harney E**, **Mallick S**, **Nordenfelt S**, **Reich D. 2015.** Partial uracil–DNA–glycosylase treatment for screening of ancient DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370:** 20130624.

Schnitker H. 2014. Erkenntnisse zur Systematik der Orangebrust-Feigenpapageien *Opopsitta gulielmitertii*. *Gesellschaft für Tropenornithologie e.V. Rastede* **18:**13–32.

Schodde R, **Calaby JH. 1972.** The biogeography of Australo-Papuan bird and mammal faunas in relation to the Torres Strait. In: Walker D, ed. *Bridge and barrier: the natural and cultural history of Torres Strait.* Canberra: Australian National University, 257–306.

Schodde R, **Mason IJ. 1997**. *Aves. (Columbidae to Coraciidae)*. *Zoological Catalogue of Australia*. Vol 37.2. Melbourne: CSIRO Publishing.

Schodde R, **Mason IJ. 1999.** *The directory of Australian birds: passerines*. Melbourne: CSIRO Publishing.

Schubert M, **Lindgreen S**, **Orlando L. 2016.** AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes* **9:** 88.

Schweizer M, **Seehausen O**, **Hertwig ST. 2011.** Macroevolutionary patterns in the diversification of parrots: effects of climate change, geological events and key innovations. *Journal of Biogeography* **38:** 2176–2194.

Schweizer M, **Wright TF**, **Peñalba JV**, **Schirtzinger EE**, **Joseph L. 2015.** Molecular phylogenetics suggests a New Guinean origin and frequent episodes of founder-event speciation in the nectarivorous lories and lorikeets (Aves: Psittaciformes). *Molecular Phylogenetics and Evolution* **90:** 34–48.

Scofield RP, **Mitchell KJ**, **Wood JR**, **De Pietri VL**, **Jarvie S**, **Llamas B**, **Cooper A. 2017.** The origin and phylogenetic relationships of the New Zealand ravens. *Molecular Phylogenetics and Evolution* **106:** 136–143.

Singhal S, **Hoskin CJ**, **Couper P**, **Potter S**, **Moritz C. 2018.** A framework for resolving cryptic species: a case study from the lizards of the Australian wet tropics. *Systematic Biology* **67:** 1061–1075.

Skinner A, **Hutchinson MN**, **Lee MS. 2013.** Phylogeny and divergence times of Australian *Sphenomorphus* group skinks (Scincidae, Squamata). *Molecular Phylogenetics and Evolution* **69:** 906–918.

Skipwith PL, **Bi K**, **Oliver PM. 2019.** Relicts and radiations: phylogenomics of an Australasian lizard clade with east Gondwanan origins (Gekkota: Diplodactyloidea). *Molecular Phylogenetics and Evolution* **140:** 106589.

Sniderman JMK, Jordan GJ. 2011. Extent and timing of floristic exchange between Australian and Asian rain forests. *Journal of Biogeography* **38:** 1445–1455.

Sniderman JMK, Woodhead JD, Hellstrom J, Jordan GJ, Drysdale RN, Tyler JJ, Porch N. 2016. Pliocene reversal of late Neogene aridification. *Proceedings of the National Academy of Sciences of the United States of America* **113:** 1999–2004.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30:** 1312–1313.

Stork NE, Goosem S, Turton SM. 2008. Australian rainforests in a global context. In: Turton SM, ed. *Living in a dynamic tropical forest landscape*. Carlton: Blackwell Publishing, 4–20.

Tallowin OJS, Meiri S, Donnellan SC, Richards SJ, Austin CC, Oliver PM. 2020. The other side of the Sahulian coin: biogeography and evolution of Melanesian forest dragons (Agamidae). *Biological Journal of the Linnean Society* **129:** 99–113.

UNESCO. 2020. *Gondwana Rainforests of Australia*. Available at: https://whc.unesco.org/en/list/368/

Ward M, Tulloch AIT, Radford JQ, Williams BA, Reside AE, Macdonald SL, Mayfield HJ, Maron M, Possingham HP, Vine SJ, O'Connor JL, Massingham EJ, Greenville AC, Woinarski JCZ, Garnett ST, Lintermans M, Scheele BC, Carwardine J, Nimmo DG, Lindenmayer DB, Kooyman RM, Simmonds JS, Sonter LJ, Watson JEM. 2020. Impact of 2019–2020 mega-fires on Australian fauna habitat. *Nature Ecology & Evolution* **4:** 1321–1326.

Weber LC, VanDerWal J, Schmidt S, McDonald WJF, Shoo LP. 2014. Patterns of rain forest plant endemism in subtropical Australia relate to stable Mesic refugia and species dispersal limitations. *Journal of Biogeography* **41:** 222–238.

Wood JR, Mitchell KJ, Scofield RP, De Pietri VL, Rawlence NJ, Cooper A. 2017. Phylogenetic relationships and terrestrial adaptations of the extinct laughing owl, *Sceloglaux albifacies* (Aves: Strigidae). *Zoological Journal of the Linnean Society* **179:** 907–918.

Yap J-YS, Rossetto M, Costion C, Crayn D, Kooyman RM, Richardson J, Henry R. 2018. Filters of floristic exchange: How traits and climate shape the rain forest invasion of Sahul from Sunda. *Journal of Biogeography* **45:** 838–847.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Details of draft references constructed using GENEIOUS READ MAPPER.
Table S2. Details of mitochondrial genome consensus sequences generated from genetic data produced in this study.
Table S3. Partitions and substitution models used for phylogenetic analyses.
Table S4. Passerine family-level supermatrix data information.
Table S5. Passerine family-level supermatrix gene partition information.
Table S6. Node age constraints used for BEAST analysis of passerine supermatrix.
Figure S1. IQ-TREE results for frogmouths, with ultrafast (+NNI) bootstrap node support values (as percentages). The scale is in number of substitutions per site. Specimen numbers are given in bold for new sequences generated as part of this study (Table 1; Supporting Information, Table S2). GenBank accessions are provided for published data.
Figure S2. BEAST maximum clade credibility (MCC) tree for frogmouths, with 95% highest posterior density (95% HPD) bars for node ages, posterior probability node support values (above node bars) and mean node ages (below node bars). Specimen numbers are given in bold for new sequences generated as part of this study (Table 1; Supporting Information, Table S2). GenBank accessions are provided for published data.
Figure S3. IQ-TREE results for parrots, with ultrafast (+NNI) bootstrap node support values (as percentages). The scale is in number of substitutions per site. Specimen numbers are given in bold for new sequences generated as part of this study (Table 1; Supporting Information, Table S2). GenBank accessions are provided for published data.
Figure S4. BEAST maximum clade credibility (MCC) tree for parrots, with 95% highest posterior density (95% HPD) bars for node ages, posterior probability node support values (black) and mean node ages (blue). Specimen numbers are given in bold for new sequences generated as part of this study (Table 1; Supporting Information, Table S2). GenBank accessions are provided for published data.
Figure S5. Passerine family-level supermatrix phylogeny. Left, IQ-TREE results with ultrafast (+NNI) bootstrap node support values (as percentages). Right, BEAST maximum clade credibility (MCC) tree with posterior probability node support values and 95% HPD bars for node ages. Key *Menura*, *Atrichornis* and *Orthonyx* lineages are highlighted in purple. Taxon labels include information on the number of nuclear (n) and mitochondrial

(m) loci (Supporting Information, Table S4). Menuridae_8n5m is a composite of NCBI sequences for southern Australian *Menura novaehollandiae*.

Figure S6. RAxML results for lyrebirds, with bootstrap node support values (as percentages). The scale is in number of substitutions per site. Specimen numbers are given in bold for new sequences generated as part of this study (Table 1; Supporting Information, Table S2). GenBank/NCBI accessions are provided for published data.